

A Comprehensive Review on Application of Hyaluronic Acid Innovel Drug Delivery System

Priyanshi A. Ramteke, Raksha Laxman Mhetre, Shashikant N. Dhole, Nilesh S. Kulkarni

Department of Pharmaceutics, PES Modern College of Pharmacy, Affiliated to Savitribai Phule Pune University,
Pune, Maharashtra, India 412105.

Date of Submission: 04-02-2026

Date of Acceptance: 14-02-2026

ABSTRACT

Research indicates that Hyaluronic Acid(HA) has potential applications in immunomodulation, wound healing, targeted cancer therapy, and transdermal medication delivery. Through chemical conjugation and crosslinking, Hyaluronic Acid's characteristics can be changed to provide customized delivery systems with regulated drug release and improved therapeutic efficacy. The historical discovery, chemical structure, synthesis, and several uses of Hyaluronic acid are disguised in this paper. It also provides an overview of current studies that demonstrate the use of HA in sophisticated drug delivery systems, such as injectable hydrogels, nanoparticles, and microneedles. Formulations based on HA have the potential to overcome the difficulties involved in traditional drug delivery by providing tailored delivery to certain tissues, enhanced bioavailability, and decreased toxicity. To fully grasp the promise of HA in therapeutic applications, future research should concentrate on in vivo studies and clinical trials.

Keywords: Drug delivery systems, Hyaluronic acid, Hydrogels, Microneedles, Nanoparticles, Targeted delivery

I. INTRODUCTION

The compound hyaluronic acid name comes from the Greek word hyalos, which means "glass" and describes its physical characteristics[1]. N-acetylglucosamine and glucuronic acid are joined by glycosidic bonds to form this high molecular weight glycosaminoglycan. It can be found in sodium hyaluronate and is found in many soft connective tissues, such as the brain, muscles, lungs, skin, and kidneys[2,3]. A variety of HA-based biomedical products, including hydrogels, microneedles, and microspheres, have been produced because of its remarkable biocompatibility, biodegradability, lack

of toxicity, non-immunogenicity, and ability to connect with other substances[4].

DISCOVERY:

- The first study that can be referred to about HA dates from 1880: the French scientist Portes noted that mucin from vitreous body was different from other mucoids in cornea and cartilage and dubbed it "hyalomucine"[5,6].
- In 1934, Meyer and Palmer isolated HA, a new polysaccharide containing uronic acid and amino sugar, from the vitreous humor of cows. Later, it was separated from human umbilical cord, rooster comb, and streptococci.
- Meyer and Weissmann determined the molecular structure of HA in 1954 after extensive research into its physico-chemical characteristics began in the 1940s.
- The first pharmaceutical-grade HA was produced in 1979 when Balazs devised a technique to extract and purify HA from human umbilical cords and rooster combs. HA has grown to be a major product in ophthalmology because of its safety and corneal endothelium[7,8,9,10,11].
- The development of bacterial fermentation techniques for HA generation, as well as the identification and characterization of enzymes involved in HA metabolism, received a lot of interest in the 1990s and 2000s.

CHEMICAL STRUCTURE:

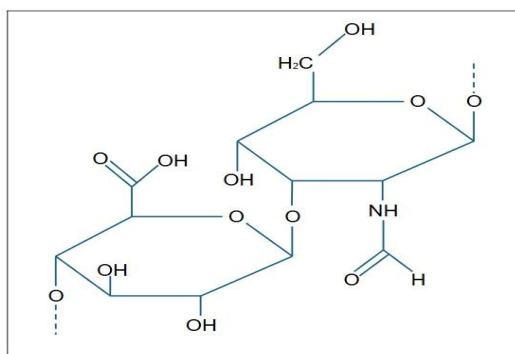


Fig. 1.1: Chemical structure of Hyaluronic acid

It is a linear, non-sulfated glycosaminoglycan with an energetically stable structure made up primarily of repeated units of N-acetylglucosamine and glucuronic acid connected by β -(1-4) and β -(1-3) glycosidic linkages [12,13]. Hyaluronic acid, with 25,000 disaccharide repetitions, can have sizes ranging from 5,000 to 20,000,000 Da in vivo, with human synovial fluid having an average molecular weight of 3-4 million Da. Silicon is also present in hyaluronic acid, with concentrations varying from 350 to 1,900 $\mu\text{g/g}$ based on the organism's location [14,15].

SYNTHESIS:

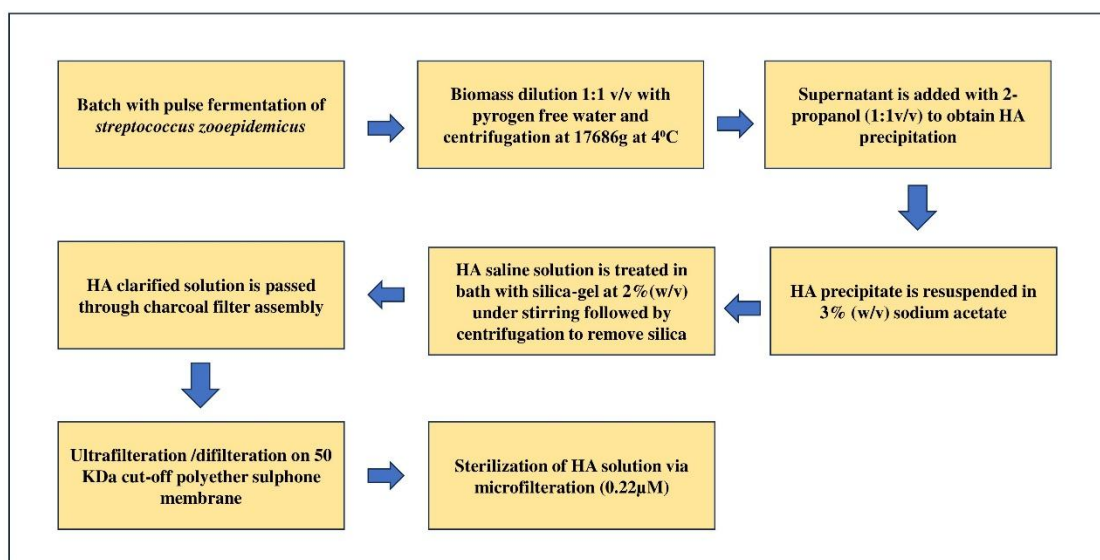


Fig. 1.2: Synthesis of Hyaluronic acid

FUNCTIONS:

It is essential for maintaining the elasto-viscosity of connective tissues, controlling tissue hydration and water transport, and promoting the supramolecular assembly of proteoglycans in the extracellular matrix. Additionally, HA participates in a wide range of receptor-mediated processes, including inflammation, tumor formation and metastasis, cell dissociation, mitosis, and migration[16,17].HA functions as a water-binding agent that lubricates moveable body components like muscles and joints by forming a hydrated gel when it is bound to water molecules. Numerous uses in the medical industry have resulted from HA's characteristics and capabilities[18].

Hyaluronic Acid in Nanoparticles:

Lisna Meylinaet al., investigated Using ionic gelation and scanning electron microscopy to analyze morphology, this work explores the utilization of hyaluronic acid-coated chitosan nanoparticles for targeted alpha mangostin delivery against MCF-7 breast cancer cells. The nanoparticles were roughly spherical, and the formulated AM-CS and AM-CS/HA1 had EE (%) and DL (%) of 88.325 ± 3.340 , 8.674 ± 0.018 , and 90.404 ± 2.161 , 8.514 ± 0.007 , respectively. Spectroscopy was used to determine the drug loading and entrapment efficiency of the nanoparticles. The average entrapment efficiency of the AM-CS and AM-CS/HA1 was $90.40\% \pm 0.161\%$ and $85.32\% \pm 0.40\%$, respectively. The chemical

interactions between raw materials and nanoparticles were investigated using Fourier-transform infrared spectroscopy analysis, and the crystallinity of AM-CS/HA was examined using X-ray diffraction. A crystalline pattern was revealed by the AM's strong multiple peaks at 2θ of 5.4° , 11.6° , and 13.3° [19,20,21]. and the CS had semi-crystalline patterns at the 10.4° , 19.7° , and 29.3° peaks [22,23,24]. The AM-CS/HA1 exhibited amorphous peaks similar to HA, with a clear glass transition peak at 85°C and an exothermic peak at 241°C . Differential scanning calorimetry revealed an endothermic phase at 177°C , chitosan DSC thermograms displaying glass transition patterns. The cytotoxic effects of AM and nanoparticles on MCF-7 cells were evaluated using the MTT assay and two-way ANOVA. The results showed promise for targeting Alpha Mangostin against breast cancer cells, however further in vivo research is required.

Yoshihiro Tokudome et al., studied used a polyion complex technique to assess the passive transport of HA nanoparticles into the skin of hairless mice. Among the analytical-grade reagents were squalane, PRT, and sodium HA. Zeta potential, particle diameter, and polydispersity index were measured in the results. The Belder and Wik method was used to synthesize fluorescence labeled HA (FL-HA) [25,26]. The effect of UV-B irradiation on the repair of epidermal membranes in male hairless mice was examined in this study. According to the researchers, UV-B irradiation might promote skin healing. Additionally, the study discovered that emulsions containing Pemulen TR-2® and squalane did not exhibit FL-HA origin fluorescence. According to the study, FL-HANP preserved FL-HA's characteristics while increasing its content. The HANP group's telomere width was considerably smaller than that of the control and non-particulate HA groups following UV-B exposure. This implies that polymeric HA can be synthesized into nanoparticles using the polyion complex approach, which could provide an injectable substitute.

Gurusamy Saravanakumar et al., examined Amphiphilic hyaluronic acid conjugates were created as a possible paclitaxel delivery mechanism for cancer treatment. To improve solubility, a hydrotropic oligomer was added to the conjugates. Their cytotoxicity, selective absorption by cancer cells, encapsulation, release, and nanoparticle production were all assessed in the study. A monomer of 2-(4-(Vinylbenzyloxy)-N,N-diethylnicotinamide) (VBODENA) was made

[27,28]. Using AET and free radical chain transfer telomerization of VBODENA, the study produced oligo (VBODENA)-NH₂. DS and ¹H NMR were used to confirm hydroHA conjugates, and morphological analysis was carried out. HydroHA conjugates' critical aggregation concentrations (CACs) were ascertained using the pyrene fluorescence technique [29]. PTX-containing HydroHA nanoparticles were made via a dialysis process. After dissolving hydroHA conjugates in DMF/H₂O, PTX was added. To create a white powder, the mixture was dialyzed, centrifuged, and filtered. HPLC was used to measure the PTX content, and a Spectra 100 UV-vis detector was used to detect it at 227 nm. As previously reported, the in vitro drug release profile of PTX from HydroHA-PTX nanoparticles was assessed using a 0.1 M sodium salicylate medium [30]. The concentration of PTX was measured by HPLC after HydroHA-PTX nanoparticles were agitated in a water bath and dissolved in PBS (pH = 7.4). Following cytotoxicity testing, self-assembling nanoparticles with sizes ranging from 274 to 356 nm were discovered. This outcome is in line with findings from other polymeric amphiphiles, including chitosan and bile acid-modified HA [31]. According to the study, HydroHA nanoparticles were spherical in shape and retained their physical integrity in PBS (pH = 7.4), indicating great stability. Their zeta potentials ranged from -23.82 to -22.14 mV. Likewise, self-assembled chitosan nanoparticles and polymeric micelles that use hydrotropic DENA as the hydrophobic component have demonstrated exceptional physical stability [32]. Because of their hydrophobic inner cores and negatively charged shells, which improve their integrity and stop inter-particle aggregation through electrostatic repulsion, HydroHA nanoparticles have a high stability. Using a fluorescence approach with pyrene as the fluorescent probe—which has been widely used to monitor the self-aggregation behavior of various surfactants and amphiphilic polymers—the aggregation behavior of HydroHA conjugates under aqueous circumstances was examined [33]. Encased in hydrophobic conjugates, hydroHA-PTX nanoparticles efficiently absorb up to 20.7% of hydrophobic medicine, control PTX release, and give off fluorescence signals on cancer cells.

Silvia Santos Pedrosa et al., investigated using disulfide bonds to improve stability and drug-loading capabilities, the study focuses on producing a thiolated HyA conjugate for nanoparticles. It evaluates the efficacy of drug loading with curcumin and simvastatin, showcasing the redox-sensitivity of

crosslinked nanogels. Scheme 1 shows the amphiphilic conjugate that was created by chemically grafting odium hyaluronate with a lengthy thiolated alkyl chain by the creation of amide bonds [34,35,36,37]. The size distribution and zeta potential of hydrophobic 11-amino-1-undecanethiol hydrochloride and a nanogel dispersion with DPDPB were investigated using AG 50W-X8 cation exchange resin. The 11 protons—with the number 2—that correspond to the peaks for hyaluronic acid utilized in the calculus are $d \frac{1}{4}$ 4.51 (G1), $d \frac{1}{4}$ 4.61 (N1), $d \frac{1}{4}$ 3.92 (N2), $d \frac{1}{4}$ 3.63 (G3), $d \frac{1}{4}$ 3.74–3.85 (N3, N6, G4, G5), and $d \frac{1}{4}$ 3.51–3.62 (N4, N5) [38]. By chemically altering a disaccharide unit, the study shows that AT can produce nanogels with a diameter of 91.85-0.410 nm that exhibit a yellow curcumin hue and self-assemble in water and DTT for up to six months. Simvastatin was loaded into a nanogel dispersion using an ethanolic stock solution, resulting in a final concentration of 71.7 mm

Hyaluronic Acid in Microneedles:

Dazhi Wang et al., studied and evaluated the mechanical properties of dissolving microneedles for skin medication delivery, examining pressurization strains and the relationship between medication loading and immunological responses. To confirm the findings, a nanoindentation device was employed. Ex vivo puncture investigations were conducted using porcine skin, which is frequently utilized as a model for human skin [39,40]. Using a vacuum drying process, the study investigates the effectiveness of a pig ear patch for piercing pigs and finds that faster, consistent OVA generates a greater immunological response. The dMNs' mechanical qualities might be diminished if they broke and changed from horizontal to convex if bubbles were present and the vacuum pressure was below ideal [41]. Pig ear nanoindentation production durations and distortion were decreased using conical MNs and higher drying temperatures, satisfying application requirements without causing any negative consequences. It stabilizes the solutions of macromolecules by forming hydrogen bonds with them [42]. The work employed a protein data library to run molecular docking simulations and find a hydrogen bond between HA and OVA using the Lamarckian Genetic Algorithm and Autodocking. A computer simulation method used in chemical research is called molecular dynamics. To determine the properties of molecular and atomic mobility [43]. OVA diffusion in HA-OVA =5:3 solutions was

simulated using Materials Studio 7.0 software, improving miscibility and diffusion characteristics. The immunological effect, release speed, and needle dispersion were all enhanced by OVA and low-molecular-weight HA.

Yuquan Chi et al., evaluated the effect of HA molecular weight on mechanical properties and rhodamine B distribution. The study used optical imaging and scanning electron microscopy to create dissolving microneedles for transdermal drug delivery utilizing molds, polydimethylsiloxane, rhodamine B, sodium hyaluronate, and ultrapure water. The mechanical properties of RhB-loaded HA-MNs were examined utilizing a compression test on a tensile testing machine, [44,45] The study investigated RhB-loaded patches on pig skin penetration and compared the forces of different HA-MNs at a 500 μ m compression distance. ImageJ software was used to examine the fluorescence intensity. Using a Franz diffusion cell technique, the efficacy of transdermal administration of RhB with HA-MNs was investigated. At different periods, samples were collected, and the transdermal diffusion area was approximately 2.2 cm². Using fluorescence spectrophotometry, the amount of RhB that was given was measured. An empirical Ritger-Peppas equation was used to calculate the transdermal release mechanism [46]. With an emphasis on the connection between HA molecular weight and MN transport efficiency, this work investigates the efficacy of transdermal RhB delivery utilizing HA-MNs on BALB/c nude mice. A one-way ANOVA was used to compare the two experimental groups, with a significance level of $P < 0.05$. According to the results, mechanical strength is essential for the best skin penetration. It was stated that approximately 0.058 N per needle, or 5.8 N per array (100 needles), was needed for a trustworthy skin penetration [47]. According to the study, the mechanical strength of various HA-MN kinds was more than the force required to pierce the skin of a pig cadaver. However, strength decreased as molecular weight rose. The most effective transdermal administration method for RhB was 74k-HA-MN. The exponential factors for 290k-HA-MN, 74k-HA-MN, and 10k-HA-MN were 0.4582, 0.8413, and 0.7726, respectively, underscoring the significance of molecular weights in transdermal administration.

Hyaluronic Acid in Hydrogels:

Yongsheng Gao et al., Hyaluronic acid hydrogels and nanocrystals have been combined to create an intra-articular drug delivery system for long-term inflammatory arthritis treatment that

improves solubility, cross-linkability, and biocompatibility. It was discovered that the ideal gelation time range was 1–10 minutes [48]. The work investigates how injectable hydrogel drug release behavior and the tunability of G' in recently created HA hydrogels are affected by cross-linking spots and chain entanglements. In order to evaluate the enhanced HA hydrogels' potential for drug delivery, the study assessed their mesh size and stability [49]. In an arthritic rat model, researchers created a hydrogel system with medication nanocrystals for intra-articular injection therapy of inflammatory arthritis, showing complete healing and articular abscess treatment.

Xu Yanget al., aimed in order to guarantee full wound filling, in-situ encapsulation of bioactive molecules, and adequate wound adherence, the project focuses on the development of HA-based injectable hydrogels as novel wound dressings and localized drug delivery systems. Like bodily fluids, injectable hydrogels are three-dimensional hydrophilic polymeric networks featuring sol-gel transitions [50,51]. They can be supplied directly to lesions and have mechanical qualities like viscoelastic liquid under particular stress ranges. Crosslinking techniques are the main source of mechanical strength, but viscoelasticity is also influenced by molecular weight, polymer architecture, chemical content, and the concentration of precursor aqueous solution [52,53]. While hydrogels based on hyaluronic acid promote healing and lessen inflammation, hydrogels such as hydroxyapatite and glycol chitosan are biocompatible and appropriate for injectable drugs and cell delivery systems. Chronic wounds, such as pressure ulcers, venous or arterial ulcers, and diabetic ulcers, [54,55] can result in endothelial cell membrane damage, decreased antimicrobial capacity, poor blood flow, chronic inflammation, and molecular malfunction [56]. Because of their enhanced drug concentration, low systemic toxicity, and biocompatibility, injectable hydrogels based on hyaluronic acid present interesting options for both drug delivery and wound diagnostics. For prolonged drug release with or without less systemic toxic side effects, they can be injected into tumor locations [57,58]. By strengthening the host immune system, cancerous cancer immunotherapy has demonstrated encouraging therapeutic results [59]. Although personalized cancer vaccines can produce strong antitumor immunity, their clinical use is restricted by their high cost and poor immunogenicity [60,61]. Natural hydrogels, such as polysaccharides, are being studied for their toxicity, degradability,

and bio-related functional characteristics, which make them perfect for wound care and medication delivery.

Dariush Nikjoo et al., Using a novel technique that combines chemical crosslinking and spray-drying, this study investigates the utilization of urea or glutaraldehyde-crosslinked hyaluronic acid hydrogels for controlled pulmonary drug delivery. To create HAGA hydrogel, HA and GA were combined in a 20 mL volume and agitated for 24 hours while the reaction was catalyzed by an acid ($\text{pH} = 2.6$) [62,63]. To examine the impact of crosslinking, the concentration of the crosslinker was varied from 1% to 16% v/v. The HAU hydrogel was utilized to create the gels, HAGA1–HAGA16, where the number denotes the crosslinker that was used [64]. Using HA and UR crosslinker, the study examined the effects of different UR concentrations on hydrogel crosslinking, determining the ideal conditions for hydrogel synthesis, and producing HAGA and HAU microparticles. To find the remaining weight, a fresh lysozyme solution was used. A modified Andersen Cascade Impactor was used to examine the formulations' aerodynamic characteristics (mACI) [65]. The study looks at how GA and UR affect the integrity and swelling of hydrogel films and finds that higher GA concentrations produce stiffer gels, whereas lower GA concentrations produce coherent gels. The concentration of GA determines its cytotoxicity; concentrations up to 8% are non-toxic [66,67]. After the hydrogels were purified, microparticles were made using HAGA8 hydrogel. Particle size was important for inhalation powders, and swelling was influenced by the UR concentration. The microparticles were 2.3 ± 1.1 to $3.2 \pm 2.9 \mu\text{m}$ in size, with a SPAN range of 1.2 to 3.5. Larger particles are typically produced by an increase in intake temperature because it speeds up liquid evaporation and causes shells to form earlier [68]. According to the study, spray drying is supported for hydrogel-based inhalable dry powders since crosslinked HA microparticles (HAGA and HAU) have a uniform size distribution and native HA microparticles have a multimodal distribution. The HA, HAGA, and HAU microparticles were found to have zeta potentials of $-25.5 \pm 8.5 \text{ mV}$, $-17.4 \pm 4.2 \text{ mV}$, and $-18.8 \pm 5.4 \text{ mV}$, respectively [69]. The study investigates the characteristics of microparticles using human adipose tissue (HA) as a biopolymer. The hyaluronic acid hydrogels, which were created via spray drying and chemical crosslinking, have smooth surfaces and spherical forms. Degradation stages and moisture content

were determined by TGA; the moisture content was 8.9%, 6.4%, and 3.9% (w/w). The microparticle architectures were unaffected by high temperatures, indicating that the aerodynamic size of the particles make continual release in the lung difficult. Swellable microparticles hold promise since they can be engineered to have respirable aerodynamic diameters while dry and can swell when deposited in the moist environment of the lungs [70]. Swelling speed, the preference for HAUR and natural particles for in vivo outcomes, and the impact of the lysozyme enzyme on biodegradation are all factors in understanding the pharmacological behavior of microparticles. Consequently, the inclusion of F-108 surfactant in the formulation sped up the biodegradation caused by lysozyme and, as a result, the release of the medication from the microparticles [71]. The aerosolization efficiency of dry powder formulations is critical for deeper lung areas. The fine particle fraction for HA, HAUR, and HAGA was determined using a modified ACI (mACI) with a 4.4 μm cut-off diameter the resulting mFPF was $7.2 \pm 3.0\%$, $9.5 \pm 3.2\%$, and $27.8 \pm 2.0\%$, respectively. Using a variety of methods, the study examined the fine particle fraction (mFPF) of HAGA microparticles. According to the study, HAGA microparticles exhibit a greater mFPF than HA and HAUR microparticles, as well as reversible water sorption, indicating improved aerosolization performance. The decrease in hydrophilic groups brought on by UR's and GA's chemical crosslinking is the reason for the decreased water absorption [72]. Water sorption and a weak contact between the adsorbent and the adsorbate are indicated by type IV isotherms [73,74]. Researchers demonstrated viability and thermal stability for prolonged pulmonary medication administration by using spray drying to produce inhalation powders from cross-linked hyaluronic acid.

Gangliang Huang et al., studied Despite issues with cytotoxicity, poor targeting, and low transfection rates, hyaluronic acid (HA) has promise as a drug delivery medium for tumor cells. Because HAP exhibited a high transfection rate in HepG2 cells and could circumvent the drawbacks of PEI nonspecific transfection, it could more successfully encourage cell uptake [75,76]. Gene delivery, tissue engineering, and regeneration all make use of hyaluronic acid. Its hydrogels offer continuous release and DNA protection, and the efficacy of transfection is increased by a gene delivery technique that encodes hyaluronan synthase two and hyaluronic acid films. The HAP conjugate was

created to target cells via LYVE-1 and deliver siRNA [77]. In biopharmaceutical research, hyaluronic acid is being investigated as a possible PEG replacement since it improves drug retention and reduces inflammation; however, cross-linked hydrogel networks impede its quick release. A new cross-linked hydrogel called erythropoietin (EPO) was made by changing the pKa between the amino group of the protein drug and the hydrazide group of hyaluronic acid-ADH [78]. By enhancing drug solubility, distribution, and half-life, hyaluronic acid-drug conjugates—a controlled release mechanism for protein therapeutics—show promise in tumor targeting and may even encourage the growth of malignant tissue. Several anticancer medications can be created by altering the main chain of hyaluronic acid [79]. In addition to outperforming paclitaxel-free drugs, HA-PTX, a pharmaceutical intended to increase cancer anticancer activity and decrease the toxicity of taxanes, also increased mice survival rates and suppressed growth on OSC-19 and HN5 cell lines. One popular treatment for solid tumors, cisplatin, has detrimental side effects. To boost the drug's concentration in lymphatic arteries, lower systemic toxicity, and prevent early tumor spreading, a novel delivery method combining cisplatin and hyaluronic acid has been created [80]. Through its amphiphilic HA-5 β cholic acid polymer, liver uptake, and tumor cell absorption, hyaluronic acid-cisplatin inhibits tumor growth, lessens kidney damage, and improves tumor targeting. Hyaluronic acid-coated liposomes can improve cancer cell targeting and increase treatment efficacy [81]. Better anticancer effects, longer circulation durations, lower IC50 values, higher drug concentrations, less toxicity, and affinity to cancer cells are all demonstrated by hyaluronic acid-loaded DOX liposomes. Clinical trials and adaptable synthesis methods, however, restrict its application.

Hyaluronic Acid in Nanogels:

NararatKotcharat et al., examined used TLR3 agonists for statistical analysis, EDC and NHS peptide coupling chemistry, and hyaluronic acid in nanogel formulations to improve adjuvant stability and resistance. As previously reported, HA-pNIPAM (abbreviated as HAg-pNI) grafted copolymer was synthesized to enable an efficient nucleic acid delivery of the nanocarrier [82,83,84]. HA-grafted pNIPAM nanogel carriers were made by researchers using poly(I:C), resulting in self-assembled nanogels with PDI values ranging from 0.37 to 0.66. According to the study, the spherical

particles in the nanogel formulation exhibit notable size fluctuations in the 300–1000 nm range as a result of increased poly(I:C) loading, with the goal of developing biodegradable nanogels for sophisticated uses of bioactive compounds.

Yuhuan Li et al., investigated Tacrolimus is administered to inflammatory joints via a novel treatment approach that employs hyaluronic acid-conjugated polyamino acid nanogels. After the medication was added to RAW 264.7 cells, its optical density was evaluated and its release properties were examined. Male DBA/1J mice that were eight weeks old were used to create the CIA mouse model [85,86]. According to a study, TAC targeted inflammatory cells in inflamed joints and decreased surface zeta potential when added to a nanogel. The immunosuppressant HA-NG/TAC shown increased efficacy over time, indicating possible RA therapy possibilities.

Hyaluronic Acid in Vaccine:

Rania Ibrahim Sheblet al., purposed of designing a rabies vaccine, the study assessed the inactivation capability of hydrogen peroxide, binary ethyleneimine, and β -propiolactone. The inactivated vaccinations exceeded WHO tolerance requirements of 3.75, 4.21, and 3.64 IU/mL, respectively, and successfully rendered the virus dormant within hours. The vaccine was contaminated with the virus, and immunity was assessed using an enzyme-linked immunosorbent test. A hollow fiber cartridge ultra-filtration technology helped to concentrate the viral suspension [87,88]. Swiss albino male mice were given the virus intracerebrally after it had been diluted in a salt solution and treated with inactivants. Mortality was tracked for 14 days. If every infected mouse survived for 28 days after intracerebral inoculation, the vaccine was deemed safe [89]. For research and development, VACSERA supplied BCG PPD and HA produced from *S. aureus*. Male Swiss albino mice were challenged with a viral standard, given injections of potential vaccines, and their mortality was noted. The NIH procedure was used to calculate the test vaccines' effective dose. In albino rats, the study also assessed humoral and cellular immunity. A light microscope attached to a digital camera was used to mount, cover, and inspect the slides [90]. Three separate tests were used for the studies, and the data was reported as mean \pm standard deviation. The Student t-test and one-way analysis of variance were used for analysis. According to the study, the inactivation potentials of β -propiolactone, hydrogen peroxide, and binary ethyleneimine were faster than BEI at

4.3, 3.2, and 2.5 log₁₀/hr post-treatment, respectively. Rabies vaccinations that used these inactivants were found to be efficacious, with no appreciable variations in ED₅₀. The reported ED₅₀ (4.21 \pm 0.03 IU/mL) for BEI inactivated vaccine was not significantly different from those of β PL (3.75 \pm 0.06 IU/mL) and H₂O₂ (3.64 \pm 0.15 IU/mL) inactivated vaccine candidates ($p > 0.05$). A study used direct ELISA to evaluate the immunogenicity of rabies vaccinations. Over time, anti-rabies IgG levels rose, peaking on the twenty-first day. IFN- γ levels were elevated by adjuvanted and unadjuvanted vaccinations. IFN- γ levels were considerably raised by the adjuvant hyaluronic acid (HA). (** $p < 0.01$) According to the study, rabies vaccinations containing hydrogen peroxide (HA) raised IL-5 levels. Furthermore, alum-treated kidney slices displayed increased connective tissue. Hydrogen peroxide can render the virus dormant without compromising the immune response. PPD derived from BCG and HA can boost immunity.

Hyaluronic Acid in Nanocapsules:

Juan I. Bussio et al., showed to assess protein composition using materials and solvents in order to combine transcutaneous vaccination with nanomedicine by employing hyaluronic acid nanocapsules to improve immune system engagement. The study team used solvent displacement techniques to create nanosystems [91,92,93]. Using Alamar blue, researchers assessed the morphology and adherence of Hyaluronic Acid Nanocapsules (HA-NCs) to OVA, a model antigen, as well as their effect on cell survival. They also looked at the stability of HA-NCs under physiological and storage settings. At 2:1 HA:antigen mass ratios, hyaluronic acid nanocapsules (HA-NCs) produced an OVA concentration of 0.25 mg/mL. Using the Western blot approach, the study examined how HA-NCs activated the complement cascade in vitro [94]. Veronal buffer and HA-NCs were employed to incubate human plasma, while dPBS and Cobra venom factor served as controls. The samples underwent electrophoresis, SDS-PAGE gelation, blocking, and incubation with mouse mAb and goat anti-mouse IgG. Pig skin from two to three-day-old pigs was employed in the study to forecast OVA penetration from HA-NCs [95]. PBS was utilized as the receptor solution, OVA-loaded HA-NCs or OVA in PBS was used as the donor solution, skin regions were chosen, hair was removed, and the area was washed and methanol-treated. Quantitative Western blot analysis was used to examine the samples [96].

According to a study looking at skin penetration metrics, chitosan nanocapsules—which are intended for transdermal antigen delivery—have the ability to stimulate the immune system. The group produced nanosystems with hydrodynamic diameter and surface charge that had formulations smaller than 100 nm. The polydispersity score was less than 0.25, indicating homogeneity. Even after 48 hours, the nanosystems displayed nanometric droplets and a steady particle population for at least 24 months. With a polydispersity index of 0.304, the formulation showed a homogeneous population [97]. Using Ovalbumin (OVA) as a model antigen that causes system aggregation, HA-NCs can be improved by varying their density, benzalkonium chloride concentration, or surfactant. The diameters (nm) of OVA loaded HA-NCs and OVA loaded HA-NCs (isolated) were 85 ± 4 , 95 ± 5 , and 93 ± 4 , respectively. Since larger particle sizes or bulk materials can enhance cytotoxicity when their

particle size is lowered, these systems' tiny size may be critical to cell survival [98]. The study discovered that complement activity strongly regulates inflammatory and immunological processes in the body, which are key components of the innate immune system. Three pathways—classical, lectin, and alternative—are used to activate it, and a common step is C3 degradation [99]. Since the complement cascade is essential to both innate and adaptive immunity as well as the antigen-specific response, it is a major focus of vaccine research. Like aluminum salts, HA-NCs have adjuvant qualities that recruit inflammatory cells, trigger inflammasome activation, and activate the complement cascade [100]. It is possible to optimize Hyaluronic Acid Nanocapsules (HA-NCs) for needle-free immunization, transdermal antigen delivery, and enhanced skin absorption and retention. They are perfect for Ovalbumin (OVA) as a model antigen and have greater cytotoxicity.

APPLICATIONS:

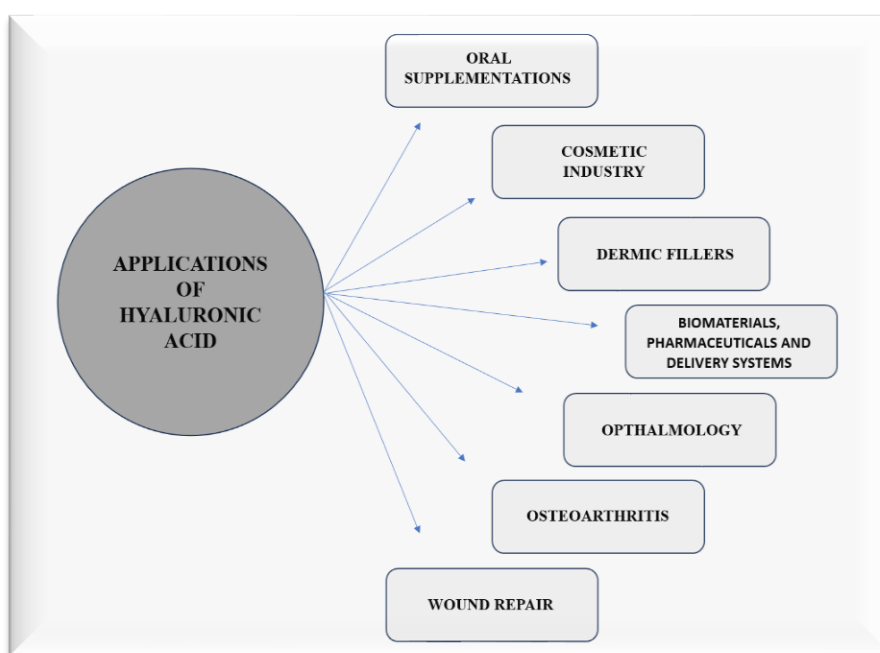


Fig. 1.3: Application of Hyaluronic acid

APPLICATIONS

1. Oral Supplementation: Because oral HA supplements can increase skin hydration, suppleness, wrinkle reduction, and skin renewal, they are well-liked for their anti-aging qualities [101,102].

2. Cosmetic Industry: Creams, serums, gels, lotions, intradermal filler injections, and facial fillers are just a few of the treatments that include HA for tissue augmentation, collagen activation, and cosmetic rejuvenation [103].

3. Dermic Filler: Because of their low cost and few side effects, HA-containing dermal fillers are widely

utilized as a non-invasive, non-permanent, and non-painless substitute for surgery[104,105].

4. Biomaterials, Pharmaceutical and Delivery Systems: In medicine, biomaterials like hyaluronic acid scaffolds are utilized for space-filling, muscle regeneration, bone and nerve tissue repair, wound healing, and cell transfer[106].

5. Ophthalmology: Because of its physical-chemical characteristics, hyaluronic acid is essential for ocular microsurgery, especially for anterior/posterior eye surgery, keratoplasty, cataract extraction, and vitreous-retinal surgery[107,108].

6. Osteoarthritis: By boosting synovial fluid viscoelasticity, reviving hyaluronic acid synthesis, halting degradation, and alleviating joint pain, hyaluronic acid is a successful treatment for osteoarthritis in the knee[109].

7. Wound Repair: Because it mediates several biological processes that are necessary for the healing process after injury, hyaluronic acid plays a critical role in wound healing[110].

ADVANTAGES:

1. Intense Hydration: The appearance of wrinkles and fine lines is lessened by this hydration.

2. Reduced Inflammation: It is helpful for dermatitis and eczema because it helps calm sensitive skin and lessen redness.

3. Wound Healing: HA contributes to tissue regeneration and repair, which speeds up wound healing and lessens scarring.

4. Joint Lubrication: It can lessen stiffness and pain in the joints caused by diseases like osteoarthritis.

II. CHALLENGES AND LIMITATIONS:

1. Potential degradation: According to research, nitroxide-containing materials or hyaluronidase inhibitors can be added to controlled release medications to prevent hyaluronic acid degradation[111].

2. Tumor microenvironment complexity: HA's complex function in both healthy and malignant tissues must be considered when using it as a cancer treatment[112].

3. Molecular Weight Considerations: The application of HA is greatly influenced by its molecular weight; large weights for drug delivery applications create protective barriers, whereas low weights encourage skin penetration[113].

Table 1: Patent of Hyaluronic acid granted to inventors

SR NO.	NAME	DOSAGE	ROUTE	COMPANY	PATENT NO.
1.	Acne patch	Transdermal patch	Topical	LEC TEC CORP	US6495158B1
2.	Sodium hyaluronate eye drop	Eye drop	Ophthalmic	Zhejiang Jianfeng Pharmaceutical Co., Ltd.	CN102697713B
3.	Cicatrizant hyadocolloidal patch containing HA and chondroitin sulphate	Transdermal patch	Topical	Comp IBSA Institute Biochemique S.A	EP1243260B1
4.	Allergan	Filler	Topical	Juvederm	US20090155314A1
5.	Hyaluronic acid eye drop	Eye drop	Ophthalmic	Sontern pharmaceutical	JP2530491B2
6.	Hyaluronic acid gel composition of dermatological use	Gel	Topical	Allergan Industries SAS Allergan Inc	US20110171286A1
7.	Synvisc	Injection	Intra articular	Sanofi	US-7,931,030-B2
8.	Hyaluronic acid formulation	Powdered	Topical	Jessup Donald W	WO2015061618A1
9.	Oral formulation	Capsule	Oral	Novozymes Biopharma DK A/S	EP2750665A1

	containing HA for sustained drug release				
--	--	--	--	--	--

III. DISCUSSION:

Hyaluronic acid (HA) is a biocompatible polymer widely used in drug delivery systems like nanoparticles, microneedles, hydrogels, nanogels, and vaccines. It enhances targeted delivery, improves drug stability, and enables sustained release, especially in cancer therapy and wound healing. HA also boosts immune response in vaccines. While promising, its applications require further in vivo and clinical validation.

In the upcoming time hyaluronic acid can be use in Advanced Nanoparticle Systems: For accurate medication delivery that reacts to stimuli like pH or enzymes, hybrid drug delivery systems combine HA with other nanomaterials.

Enhanced Targeted Delivery: Researchers are enhancing HA's targeting capabilities by modifying it with specific ligands, focusing on the CD44 receptor for accurate drug delivery in diseased tissues.

Smart Hydrogels: HA-based hydrogels, capable of responding to environmental changes, have the potential to revolutionize drug delivery for chronic diseases and promote tissue regeneration in tissue engineering.

Gene Therapy: HA is being explored as a carrier for gene therapy, potentially leading to new treatments for genetic disorders and cancer by delivering therapeutic genes to specific cells

IV. CONCLUSION:

Hyaluronic acid (HA) is a promising material in drug delivery systems due to its biocompatibility, biodegradability, and ability to target specific cells overexpressing the CD44 receptor. Despite challenges like molecular weight variability and modification complexity, ongoing research is focusing on advanced nanoparticle systems, smart hydrogels, and personalized drug delivery approaches. HA's role in drug delivery is evolving, potentially revolutionizing therapeutic strategies and improving patient outcomes.

Acknowledgement

I would like to express my sincere gratitude to all those who contributed to the successful completion of this work. I am deeply thankful to my guide Dr. Raksha L. Mhetre for their valuable guidance, continuous support, and constructive suggestions throughout the course of this study.

I also extend my sincere thanks to the Head of the Department and all faculty members for their encouragement and academic support. I am grateful to the laboratory and technical staff for their assistance during the experimental work.

Finally, I express my heartfelt thanks to my family and friends for their constant encouragement and moral support, which played a vital role in completing this work successfully.

Author contribution statement

Priyanshi Arvind Ramteke – Conceptualization, Methodology, Writing – Original Draft, Data Collection, Analysis, Writing Review & Editing.

Raksha Laxman Mhetre – Visualization, Supervision, Project Administration.

Nilesh Shrikant Kulkarni – Resources, Supervision.

Shashikant Nivrutti Dhole – Final approval of Manuscript.

Conflict of Interest

The authors do not have any conflict of interest.

Ethical Approval

This review did not involve human participation, animal subjects, or any material that requires ethical approval.

REFERENCE:

- [1]. Salwowska, N.M., Bebenek, K.A., Źądło, D.A. and Wcisło-Dziadecka, D.L., 2016. Physiochemical properties and application of hyaluronic acid: a systematic review. *Journal of cosmetic dermatology*, 15(4), pp.520-526.
- [2]. Salih, A.R.C., Farooqi, H.M.U., Amin, H., Karn, P.R., Meghani, N. and Nagendran, S., 2024. Hyaluronic acid: Comprehensive review of a multifunctional biopolymer. *Future Journal of Pharmaceutical Sciences*, 10(1), p.63.
- [3]. Necas, J.B.L.B.P., Bartosikova, L., Brauner, P. and Kolar, J.J.V.M., 2008. Hyaluronic acid (hyaluronan): a review. *Veterinarnimedicina*, 53(8), pp.397-411.

- [4]. Ye, H., Zhang, R., Zhang, C., Xia, Y. and Jin, L., 2024. Advances in hyaluronic acid: Bioactivity, complexed biomaterials and biological application: A review. *Asian Journal of Surgery*.
- [5]. Fallacara, A., Baldini, E., Manfredini, S. and Vertuani, S., 2018. Hyaluronic acid in the third millennium. *Polymers*, 10(7), p.701.
- [6]. Boeriu, C.G., Springer, J., Kooy, F.K., van den Broek, L.A. and Eggink, G., 2013. Production methods for hyaluronan. *International journal of carbohydrate chemistry*, 2013(1), p.624967.
- [7]. Miller, D., 1980. Use of Na-hyaluronate in anterior segment surgery. *Am Intraocular Implant Soc J*, 6, pp.342-343.
- [8]. Binkhorst, C.D., 1981. Advantages and disadvantages of intracameral Na-hyaluronate (Healon) in intraocular lens surgery. *Documenta Ophthalmologica*, 50, pp.233-235.
- [9]. Binkhorst, C.D., 1980. Inflammation and intraocular pressure after the use of Healon® in intraocular lens surgery. *Journal of Cataract & Refractive Surgery*, 6(4), pp.340-341.
- [10]. Percival, S.P.B., 1985. Results of a clinical trial of sodium hyaluronate in lens implantation surgery. *Journal of Cataract & Refractive Surgery*, 11(3), pp.257-259.
- [11]. Graue, E.L., Polack, F.M. and Balazs, E.A., 1980. The protective effect of Na-hyaluronate to corneal endothelium. *Experimental eye research*, 31(1), pp.119-127.
- [12]. Percival, P., 1981. Protective role of Healon during lens implantation. *Transactions of the Ophthalmological Societies of the United Kingdom*, 101(1), pp.77-78.
- [13]. Serra, M., Casas, A., Toubarro, D., Barros, A.N. and Teixeira, J.A., 2023. Microbial hyaluronic acid production: a review. *Molecules*, 28(5), p.2084.
- [14]. Zhai, P., Peng, X., Li, B., Liu, Y., Sun, H. and Li, X., 2020. The application of hyaluronic acid in bone regeneration. *International journal of biological macromolecules*, 151, pp.1224-1239.
- [15]. Schwarz, K., 1973. A bound form of silicon in glycosaminoglycans and polyuronides. *Proceedings of the National Academy of Sciences*, 70(5), pp.1608-1612.
- [16]. Salih, A.R.C., Farooqi, H.M.U., Amin, H., Karn, P.R., Meghani, N. and Nagendran, S., 2024. Hyaluronic acid: Comprehensive review of a multifunctional biopolymer. *Future Journal of Pharmaceutical Sciences*, 10(1), p.63.
- [17]. Salwowska, N.M., Bebenek, K.A., Żądło, D.A. and Wcisło-Dziadecka, D.L., 2016. Physicochemical properties and application of hyaluronic acid: a systematic review. *Journal of cosmetic dermatology*, 15(4), pp.520-526.
- [18]. Valachová, K., Volpi, N., Stern, R. and Soltes, L., 2016. Hyaluronan in medical practice. *Current medicinal chemistry*, 23(31), pp.3607-3617.
- [19]. Meylina, L., Muchtaridi, M., Joni, I.M., Elamin, K.M. and Wathoni, N., 2023. Hyaluronic acid-coated chitosan nanoparticles as an active targeted carrier of alpha mangostin for breast cancer cells. *Polymers*, 15(4), p.1025.
- [20]. Iqbal, A., Muhammad Shuib, N.A., Darnis, D.S., Miskam, M., Abdul Rahman, N.R. and Adam, F., 2018. Synthesis and characterisation of rice husk ash silica drug carrier for α -mangostin. *J. Phys. Sci*, 29, pp.95-107.
- [21]. Sriyanti, I., Edikresnha, D., Rahma, A., Munir, M.M., Rachmawati, H. and Khairurrijal, K., 2018. Mangosteen pericarp extract embedded in electrospun PVP nanofiber mats: Physicochemical properties and release mechanism of α -mangostin. *International journal of nanomedicine*, pp.4927-4941.
- [22]. Mulia, K., Rachman, D. and Krisanti, E.A., 2019, September. Preparation, characterization and release profile of chitosan alginate freeze dried matrices loaded with mangostins. In *Journal of Physics: Conference Series* (Vol. 1295, No. 1, p. 012009). IOP Publishing.
- [23]. Eddy, M., Tbib, B. and Khalil, E.H., 2020. A comparison of chitosan properties after extraction from shrimp shells by diluted and concentrated acids. *Heliyon*, 6(2).
- [24]. Podgorbunskikh, E., Kuskov, T., Rychkov, D., Lomovskii, O. and Bychkov, A., 2022. *Mechanical Amorphization of Chitosan with Different Molecular Weights*. *Polymers* 2022, 14, 4438 [online]
- [25]. Tokudome, Y., Komi, T., Omata, A. and Sekita, M., 2018. A new strategy for the passive skin delivery of nanoparticulate, high molecular weight hyaluronic acid prepared by a polyion complex method. *Scientific Reports*, 8(1), p.2336.

- [26]. de Belder, A.N. and Wik, K.O., 1975. Preparation and properties of fluorescein-labelled hyaluronate. *Carbohydrate Research*, 44(2), pp.251-257.
- [27]. Saravanakumar, G., Choi, K.Y., Yoon, H.Y., Kim, K., Park, J.H., Kwon, I.C. and Park, K., 2010. Hydrotropic hyaluronic acid conjugates: Synthesis, characterization, and implications as a carrier of paclitaxel. *International journal of pharmaceuticals*, 394(1-2), pp.154-161.
- [28]. Lee, S.C., Huh, K.M., Lee, J., Cho, Y.W., Galinsky, R.E. and Park, K., 2007. Hydrotropic polymeric micelles for enhanced paclitaxel solubility: in vitro and in vivo characterization. *Biomacromolecules*, 8(1), pp.202-208.
- [29]. Wilhelm, M., Zhao, C.L., Wang, Y., Xu, R., Winnik, M.A., Mura, J.L., Riess, G. and Croucher, M.D., 1991. Poly (styrene-ethylene oxide) block copolymer micelle formation in water: a fluorescence probe study. *Macromolecules*, 24(5), pp.1033-1040.
- [30]. Huh, K.M., Lee, S.C., Cho, Y.W., Lee, J., Jeong, J.H. and Park, K., 2005. Hydrotropic polymer micelle system for delivery of paclitaxel. *Journal of controlled release*, 101(1-3), pp.59-68.
- [31]. Kwon, S., Park, J.H., Chung, H., Kwon, I.C., Jeong, S.Y. and Kim, I.S., 2003. Physicochemical characteristics of self-assembled nanoparticles based on glycol chitosan bearing 5 β -cholanic acid. *Langmuir*, 19(24), pp.10188-10193.
- [32]. Saravanakumar, G., Min, K.H., Min, D.S., Kim, A.Y., Lee, C.M., Cho, Y.W., Lee, S.C., Kim, K., Jeong, S.Y., Park, K. and Park, J.H., 2009. Hydrotropic oligomer-conjugated glycol chitosan as a carrier of paclitaxel: synthesis, characterization, and in vivo biodistribution. *Journal of Controlled Release*, 140(3), pp.210-217.
- [33]. Lee, K.Y., Jo, W.H., Kwon, I.C., Kim, Y.H. and Jeong, S.Y., 1998. Structural determination and interior polarity of self-aggregates prepared from deoxycholic acid-modified chitosan in water. *Macromolecules*, 31(2), pp.378-383.
- [34]. Pedrosa, S.S., Goncalves, C., David, L. and Gama, M., 2014. A novel crosslinked hyaluronic acid nanogel for drug delivery. *Macromolecular Bioscience*, 14(11), pp.1556-1568.
- [35]. Ganesh, S., Iyer, A.K., Morrissey, D.V. and Amiji, M.M., 2013. Hyaluronic acid based self-assembling nanosystems for CD44 target mediated siRNA delivery to solid tumors. *Biomaterials*, 34(13), pp.3489-3502.
- [36]. Liu, Y., Sun, J., Cao, W., Yang, J., Lian, H., Li, X., Sun, Y., Wang, Y., Wang, S. and He, Z., 2011. Dual targeting folate-conjugated hyaluronic acid polymeric micelles for paclitaxel delivery. *International journal of pharmaceuticals*, 421(1), pp.160-169.
- [37]. Shen, Y., Li, Q., Tu, J. and Zhu, J., 2009. Synthesis and characterization of low molecular weight hyaluronic acid-based cationic micelles for efficient siRNA delivery. *Carbohydrate Polymers*, 77(1), pp.95-104.
- [38]. Bodnár, M., Daróczi, L., Batta, G., Bakó, J., Hartmann, J.F. and Borbély, J., 2009. Preparation and characterization of cross-linked hyaluronan nanoparticles. *Colloid and Polymer Science*, 287, pp.991-1000.
- [39]. Wang, D., Jiang, M., Wang, X., Wang, C., Ou, X. and Shang, L., 2023. Mechanical properties and immunotherapeutic effects of dissolving microneedles with different drug loadings based on hyaluronic acid. *Brazilian Journal of Pharmaceutical Sciences*, 59, p.e22690.
- [40]. Zhan, H., Ma, F., Huang, Y., Zhang, J., Jiang, X. and Qian, Y., 2018. Application of composite dissolving microneedles with high drug loading ratio for rapid local anesthesia. *European Journal of Pharmaceutical Sciences*, 121, pp.330-337.
- [41]. Zhuang, J., Rao, F., Wu, D., Huang, Y., Xu, H., Gao, W., Zhang, J. and Sun, J., 2020. Study on the fabrication and characterization of tip-loaded dissolving microneedles for transdermal drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, 157, pp.66-73.
- [42]. Feng, Y.H., Zhang, X.P., Li, W.X. and Guo, X.D., 2021. Stability and diffusion properties of insulin in dissolvable microneedles: a multiscale simulation study. *Langmuir*, 37(30), pp.9244-9252.
- [43]. Gao, Y., Vogus, D., Zhao, Z., He, W., Krishnan, V., Kim, J., Shi, Y., Sarode, A., Ukidve, A. and Mitragotri, S., 2022. Injectable hyaluronic acid hydrogels encapsulating drug nanocrystals for long-term treatment of inflammatory

- arthritis. *Bioengineering & Translational Medicine*, 7(1), p.e10245.
- [44]. Chi, Y., Huang, Y., Kang, Y., Dai, G., Liu, Z., Xu, K. and Zhong, W., 2022. The effects of molecular weight of hyaluronic acid on transdermal delivery efficiencies of dissolving microneedles. *European Journal of Pharmaceutical Sciences*, 168, p.106075.
- [45]. Chiu, Y.H., Chen, M.C. and Wan, S.W., 2018. Sodium hyaluronate/chitosan composite microneedles as a single-dose intradermal immunization system. *Biomacromolecules*, 19(6), pp.2278-2285.
- [46]. Wu, C., Liu, J., Zhai, Z., Yang, L., Tang, X., Zhao, L., Xu, K. and Zhong, W., 2020. Double-crosslinked nanocomposite hydrogels for temporal control of drug dosing in combination therapy. *Acta Biomaterialia*, 106, pp.278-288.
- [47]. Ning, X., Wiraja, C., Lio, D.C.S. and Xu, C., 2020. A double-layered microneedle platform fabricated through frozen spray-coating. *Advanced healthcare materials*, 9(10), p.2000147.
- [48]. Li, Y., Rodrigues, J. and Tomás, H., 2012. Injectable and biodegradable hydrogels: gelation, biodegradation and biomedical applications. *Chemical Society Reviews*, 41(6), pp.2193-2221.
- [49]. Li, J. and Mooney, D.J., 2016. Designing hydrogels for controlled drug delivery. *Nature Reviews Materials*, 1(12), pp.1-17.
- [50]. Yang, X., Wang, B., Peng, D., Nie, X., Wang, J., Yu, C.Y. and Wei, H., 2022. Hyaluronic acid-based injectable hydrogels for wound dressing and localized tumor therapy: a review. *Advanced NanoBiomed Research*, 2(12), p.2200124.
- [51]. Gao, Y., Li, Z., Huang, J., Zhao, M. and Wu, J., 2020. In situ formation of injectable hydrogels for chronic wound healing. *Journal of Materials Chemistry B*, 8(38), pp.8768-8780.
- [52]. Tu, Y., Chen, N., Li, C., Liu, H., Zhu, R., Chen, S., Xiao, Q., Liu, J., Ramakrishna, S. and He, L., 2019. Advances in injectable self-healing biomedical hydrogels. *Acta Biomaterialia*, 90, pp.1-20.
- [53]. Lavanya, K., Chandran, S.V., Balagangadharan, K. and Selvamurugan, N.J.M.S., 2020. Temperature-and pH-responsive chitosan-based injectable hydrogels for bone tissue engineering. *Materials Science and Engineering: C*, 111, p.110862.
- [54]. Bowers, S. and Franco, E., 2020. Chronic wounds: evaluation and management. *American family physician*, 101(3), pp.159-166.
- [55]. Xu, Z., Han, S., Gu, Z. and Wu, J., 2020. Advances and impact of antioxidant hydrogel in chronic wound healing. *Advanced healthcare materials*, 9(5), p.1901502.
- [56]. Gao, Y., Li, Z., Huang, J., Zhao, M. and Wu, J., 2020. In situ formation of injectable hydrogels for chronic wound healing. *Journal of Materials Chemistry B*, 8(38), pp.8768-8780.
- [57]. Zhang, X., Guo, X., Wu, Y. and Gao, J., 2021. Locally injectable hydrogels for tumor immunotherapy. *Gels*, 7(4), p.224.
- [58]. Norouzi, M., Nazari, B. and Miller, D.W., 2016. Injectable hydrogel-based drug delivery systems for local cancer therapy. *Drug discovery today*, 21(11), pp.1835-1849.
- [59]. Chen, F., Wang, Y., Gao, J., Saeed, M., Li, T., Wang, W. and Yu, H., 2021. Nanobiomaterial-based vaccination immunotherapy of cancer. *Biomaterials*, 270, p.120709.
- [60]. Sahin, U. and Türeci, Ö., 2018. Personalized vaccines for cancer immunotherapy. *Science*, 359(6382), pp.1355-1360.
- [61]. Schumacher, T.N. and Schreiber, R.D., 2015. Neoantigens in cancer immunotherapy. *Science*, 348(6230), pp.69-74.
- [62]. Nikjoo, D., van der Zwaan, I., Brülls, M., Tehler, U. and Frenning, G., 2021. Hyaluronic acid hydrogels for controlled pulmonary drug delivery—a particle engineering approach. *Pharmaceutics*, 13(11), p.1878.
- [63]. Tomihata, K. and Ikada, Y., 1997. Crosslinking of hyaluronic acid with glutaraldehyde. *Journal of Polymer Science Part A: Polymer Chemistry*, 35(16), pp.3553-3559.
- [64]. Citernesi, U.R., Beretta, L. and Citernesi, L., IRA Istituto Ricerche Applicate Srl, 2018. *Cross-linked hyaluronic acid, process for the preparation thereof and use thereof in the aesthetic field*. U.S. Patent 10,117,822.
- [65]. Reddy, N., Reddy, R. and Jiang, Q., 2015. Crosslinking biopolymers for biomedical

- applications. *Trends in biotechnology*, 33(6), pp.362-369.
- [66]. Umashankar, P.R., Mohanan, P.V. and Kumari, T.V., 2012. Glutaraldehyde treatment elicits toxic response compared to decellularization in bovine pericardium. *Toxicology international*, 19(1), p.51.
- [67]. Li, Y., Han, M., Liu, T., Cun, D., Fang, L. and Yang, M., 2017. Inhaled hyaluronic acid microparticles extended pulmonary retention and suppressed systemic exposure of a short-acting bronchodilator. *Carbohydrate polymers*, 172, pp.197-204.
- [68]. Killeen, P.R., 2005. An alternative to null-hypothesis significance tests. *Psychological science*, 16(5), pp.345-353.
- [69]. Nikjoo, D., van der Zwaan, I., Brülls, M., Tehler, U. and Frenning, G., 2021. Hyaluronic acid hydrogels for controlled pulmonary drug delivery—a particle engineering approach. *Pharmaceutics*, 13(11), p.1878.
- [70]. El-Sherbiny, I.M., McGill, S. and Smyth, H.D., 2010. Swellable microparticles as carriers for sustained pulmonary drug delivery. *Journal of pharmaceutical sciences*, 99(5), pp.2343-2356.
- [71]. Nsereko, S. and Amiji, M., 2002. Localized delivery of paclitaxel in solid tumors from biodegradable chitin microparticle formulations. *Biomaterials*, 23(13), pp.2723-2731.
- [72]. Mangal, S., Nie, H., Xu, R., Guo, R., Cavallaro, A., Zemlyanov, D. and Zhou, Q., 2018. Physico-chemical properties, aerosolization and dissolution of co-spray dried azithromycin particles with l-leucine for inhalation. *Pharmaceutical research*, 35, pp.1-15.
- [73]. Rouquerol, J., Rouquerol, F., Llewellyn, P., Maurin, G. and Sing, K., 2013. *Adsorption by powders and porous solids: principles, methodology and applications*. Academic press.
- [74]. Thommes, M., Köhn, R. and Fröba, M., 2002. Sorption and pore condensation behavior of pure fluids in mesoporous MCM-48 silica, MCM-41 silica, SBA-15 silica and controlled-pore glass at temperatures above and below the bulk triple point. *Applied surface science*, 196(1-4), pp.239-249.
- [75]. Huang, G. and Huang, H., 2018. Application of hyaluronic acid as carriers in drug delivery. *Drug delivery*, 25(1), pp.766-772.
- [76]. Yao, J., Fan, Y., Du, R., Zhou, J., Lu, Y., Wang, W., Ren, J. and Sun, X., 2010. Amphoteric hyaluronic acid derivative for targeting gene delivery. *Biomaterials*, 31(35), pp.9357-9365.
- [77]. Jang, Y.L., Ku, S.H., Lee, S.J., Park, J.H., Kim, W.J., Kwon, I.C., Kim, S.H. and Jeong, J.H., 2014. Hyaluronic acid-siRNA conjugate/reducible polyethylenimine complexes for targeted siRNA delivery. *Journal of Nanoscience and Nanotechnology*, 14(10), pp.7388-7394.
- [78]. Motokawa, K., Hahn, S.K., Nakamura, T., Miyamoto, H. and Shimoboji, T., 2006. Selectively crosslinked hyaluronic acid hydrogels for sustained release formulation of erythropoietin. *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*, 78(3), pp.459-465.
- [79]. Galer, C.E., Sano, D., Ghosh, S.C., Hah, J.H., Auzenne, E., Hamir, A.N., Myers, J.N. and Klostergaard, J., 2011. Hyaluronic acid-paclitaxel conjugate inhibits growth of human squamous cell carcinomas of the head and neck via a hyaluronic acid-mediated mechanism. *Oral oncology*, 47(11), pp.1039-1047.
- [80]. Liu, E., Zhou, Y., Liu, Z., Li, J., Zhang, D., Chen, J. and Cai, Z., 2015. Cisplatin loaded hyaluronic acid modified TiO₂ nanoparticles for neoadjuvant chemotherapy of ovarian cancer. *Journal of Nanomaterials*, 2015(1), p.390358.
- [81]. El Kechai, N., Bochot, A., Huang, N., Nguyen, Y., Ferrary, E. and Agnely, F., 2015. Effect of liposomes on rheological and syringeability properties of hyaluronic acid hydrogels intended for local injection of drugs. *International journal of pharmaceutics*, 487(1-2), pp.187-196.
- [82]. Kotcharat, N., Charoenkanburkang, P. and Luckanagul, J.A., 2020. Grafted hyaluronic acid nanogel for the incorporation of poly (I: C) as an immunostimulatory adjuvant. *SRP*, 11, pp.247-253.
- [83]. D'Este, M., Alini, M. and Eglin, D., 2012. Single step synthesis and characterization of

- thermoresponsive hyaluronan hydrogels. *Carbohydrate polymers*, 90(3), pp.1378-1385.
- [84]. D'Este, M., Eglin, D. and Alini, M., 2014. A systematic analysis of DMTMM vs EDC/NHS for ligation of amines to hyaluronan in water. *Carbohydrate polymers*, 108, pp.239-246.
- [85]. Li, Y., Wang, X., Gao, Y., Zhang, Z., Liu, T., Zhang, Z., Wang, Y., Chang, F. and Yang, M., 2024. Hyaluronic acid-coated polypeptide nanogel enhances specific distribution and therapy of tacrolimus in rheumatoid arthritis. *Journal of Nanobiotechnology*, 22(1), p.547.
- [86]. Brand, D.D., Latham, K.A. and Rosloniec, E.F., 2007. Collagen-induced arthritis. *Nature protocols*, 2(5), pp.1269-1275.
- [87]. Shebl, R.I., Amer, M.E., Abuamara, T.M., Matar, E.R., Ahmed, H.F., Gomah, T.A., El Moselhy, L.E., Abu-Elghait, M. and Mohamed, A.F., 2021. Staphylococcus aureus derived hyaluronic acid and bacillus Calmette-Guérin purified proteins as immune enhancers to rabies vaccine and related immuno-histopathological alterations. *Clinical and Experimental Vaccine Research*, 10(3), p.229.
- [88]. Dedloff, M.R., Effler, C.S., Holban, A.M. and Gestal, M.C., 2019. Use of biopolymers in mucosally-administered vaccinations for respiratory disease. *Materials*, 12(15), p.2445.
- [89]. Rooijackers, E., Groen, J., Uittenbogarrd, J., Van Herwijnen, J. and Osterhaus, A.D.M.E., 1996. Development and evaluation of alternative testing methods for the in vivo NIH potency test used for the quality control of inactivated rabies vaccines. *Developments in Biological Standardization*, 86, pp.137-145.
- [90]. Eid, R.A., Ahmed Zaki, M.S., Alghamd, M.A., Wares, A., Eldeen, M.A., Sayed Massoud, E.E. and Haidara, M.A., 2020. Ameliorative Effect of Vitamin E on Biochemical and Ultrastructural Changes in Artemether-induced Renal Toxicity in Rats. *International Journal of Morphology*, 38(2).
- [91]. Bussio, J.I., Molina-Perea, C. and González-Aramundiz, J.V., 2019. Hyaluronic acid nanocapsules as a platform for needle-free vaccination. *Pharmaceutics*, 11(5), p.246.
- [92]. Vicente, S., Peleteiro, M., Gonzalez-Aramundiz, J.V., Díaz-Freitas, B., Martínez-Pulgarín, S., Neissa, J.I., Escribano, J.M., Sanchez, A., González-Fernández, Á. and Alonso, M.J., 2014. Highly versatile immunostimulating nanocapsules for specific immune potentiation. *Nanomedicine*, 9(15), pp.2273-2289.
- [93]. Bussio, J.I., Molina-Perea, C. and González-Aramundiz, J.V., 2018. Lower-sized chitosan nanocapsules for transcutaneous antigen delivery. *Nanomaterials*, 8(9), p.659.
- [94]. Neun, B.W. and Dobrovolskaia, M.A., 2010. Qualitative analysis of total complement activation by nanoparticles. In *Characterization of Nanoparticles Intended for Drug Delivery* (pp. 237-245). Totowa, NJ: Humana Press.
- [95]. Dickinson, J. and Fowler, S.J., 2002. Quantification of proteins on Western blots using ECL. *The protein protocols handbook*, pp.429-437.
- [96]. González-Aramundiz, J.V., Peleteiro, M., González-Fernández, Á., Alonso, M.J. and Csaba, N.S., 2018. Protamine nanocapsules for the development of thermostable adjuvanted nanovaccines. *Molecular pharmaceutics*, 15(12), pp.5653-5664.
- [97]. Danaei, M.R.M.M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S. and Mozafari, M.R., 2018. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*, 10(2), p.57.
- [98]. Ganguly, P., Breen, A. and Pillai, S.C., 2018. Toxicity of nanomaterials: Exposure, pathways, assessment, and recent advances. *ACS Biomaterials Science & Engineering*, 4(7), pp.2237-2275.
- [99]. Ghebrehwet, B., 2016. The complement system: an evolution in progress. *F1000Research*, 5, p.2840.
- [100]. HogenEsch, H., O'Hagan, D.T. and Fox, C.B., 2018. Optimizing the utilization of aluminum adjuvants in vaccines: you might just get what you want. *npj Vaccines*, 3(1), p.51.
- [101]. Graciela, C.Q., José Juan, E.C., Gieraldin, C.L., Xóchitl Alejandra, P.M. and Gabriel, A.Á., 2023. Hyaluronic Acid—extraction methods, sources and applications. *Polymers*, 15(16), p.3473.

- [102]. León-López, A., Morales-Peñaloza, A., Martínez-Juárez, V.M., Vargas-Torres, A., Zeugolis, D.I. and Aguirre-Álvarez, G., 2019. Hydrolyzed collagen—sources and applications. *Molecules*, 24(22), p.4031.
- [103]. Salwowska, N.M., Bebenek, K.A., Żądło, D.A. and Wcisło-Dziadecka, D.L., 2016. Physicochemical properties and application of hyaluronic acid: a systematic review. *Journal of cosmetic dermatology*, 15(4), pp.520-526.
- [104]. Chauhan, N., Vasava, P., Khan, S.L., Siddiqui, F.A., Islam, F., Chopra, H. and Emran, T.B., 2022. Ethosomes: A novel drug carrier. *Annals of Medicine and Surgery*, 82.
- [105]. Tezel, A. and Fredrickson, G.H., 2008. The science of hyaluronic acid dermal fillers. *Journal of Cosmetic and Laser Therapy*, 10(1), pp.35-42.
- [106]. Zamboni, F., Keays, M., Hayes, S., Albadarin, A.B., Walker, G.M., Kiely, P.A. and Collins, M.N., 2017. Enhanced cell viability in hyaluronic acid coated poly (lactic-co-glycolic acid) porous scaffolds within microfluidic channels. *International journal of pharmaceuticals*, 532(1), pp.595-602.
- [107]. Saranraj, P. and Naidu, M.A., 2013. Hyaluronic acid production and its applications—a review. *Int J Pharm Biol Arch*, 4(5), pp.853-59.
- [108]. Harding, S.E., Tombs, M.P., Adams, G.G., Paulsen, B.S., Inngjerdingen, K.T. and Barsett, H., 2017. *An introduction to polysaccharide biotechnology*. CRC Press.
- [109]. Chen, J., 1999. W and Abatangeio, Y. 1999. Hyaluronic acid in wound repair. *Wound Repair and Regeneration*, pp.79-89.
- [110]. Willoughby, D.A. ed., 1994. *First International Workshop on Hyaluronan in Drug Delivery: Proceedings of an Extended Panel Discussion Held in Windsor, UK on 29 September, 1992*. Royal Society of Medicine Services with financial support from Hyal Pharmaceutical Corporation.
- [111]. Huang, G. and Huang, H., 2018. Application of hyaluronic acid as carriers in drug delivery. *Drug delivery*, 25(1), pp.766-772.
- [112]. Pashkina, E., Bykova, M., Berishvili, M., Lazarev, Y. and Kozlov, V., 2025. Hyaluronic Acid-Based Drug Delivery Systems for Cancer Therapy. *Cells*, 14(2), p.61.
- [113]. Matalqah, S., Lafi, Z. and Asha, S.Y., 2024. Hyaluronic acid in nanopharmaceuticals: an overview. *Current Issues in Molecular Biology*, 46(9), pp.10444-10461.