



## Review article

# Hyaluronic acid association with bacterial, fungal and viral infections: Can hyaluronic acid be used as an antimicrobial polymer for biomedical and pharmaceutical applications?

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## ABSTRACT

The relationships between hyaluronic acid (HA) and pathological microorganisms incite new understandings on microbial infection, tissue penetration, disease progression and lastly, potential treatments. These understandings are important for the advancement of next generation antimicrobial therapeutical strategies for the control of healthcare-associated infections. Herein, this review will focus on the interplay between HA, bacteria, fungi, and viruses. This review will also comprehensively detail and discuss the antimicrobial activity displayed by various HA molecular weights for a variety of biomedical and pharmaceutical applications, including microbiology, pharmaceuticals, and tissue engineering.

## 1. Introduction

Polysaccharides are one of the major classes of biopolymers being exploited in the development of novel therapeutics in pharmaceutical and biomedical fields [1]. Biofilm-related infections and contamination of materials are major problems encountered specifically in the biomedical field [2]. Recent studies indicate that bacterial contamination in open wounds may adversely affect the formation of bone and newly formed connective tissue. Certainly, less bacterial burden in wound site improves clinical outcomes in regenerative therapy. A variety of polysaccharides, such as chitosan, and their derivatives have been explored for antimicrobial applications [3]. HA is a non-sulphated glycosaminoglycan (GAG) that is an essential component of the extracellular and pericellular matrixes of all tissues in the body. However, HA occurrence in various human tissues may differ in concentration and molecular size. For example, navel cords contain 4.10 mg/mL of HA, joint synovial fluid contains 1.50–3.60 mg/mL of HA, vitreous humour contains 0.14–0.34 mg/g of HA, and the dermis and epidermis contain 0.20–0.50 and 0.10 mg/g of HA, respectively. For instance, the synovial fluid contains HA with molecular weight around 6500 kDa [4]. HA is a natural biopolymer of increasing importance in the fields of biomedical engineering, pharmaceutical science and medicine [5]. Its

physicochemical properties are ideally suited for emerging bioengineering advances concerned with all aspects of bodily reconstruction and tissue regeneration [6,7]. The HA structure contains alternating repeat units of  $\beta$ -1,4-D-glucuronic acid- $\beta$ -1,3-N-acetyl-D-glucosamine. HA is the only GAG that is biosynthesized at the cell membrane and not linked to proteoglycans [8]. It is known to bind to its own synthases and to RHAMM and CD44 cell surface receptors [9], so it is critical to cell function responses [10]. The molecular size of HA is proportional to the activity of its synthesizing and degrading enzymes. Three isoforms of hyaluronan synthase (HAS) namely HAS-1, 2 and 3 exist in mammals and they produce HA of different sizes [11]. HA degradation *in vivo* begins on its linkage to HA receptors for endocytosis on the cell membrane [12]. It degrades quickly in the presence of hyaluronidases (HYAL) to shorter polymer chains that have size-dependent properties and functions [13]. Hyaluronidase-1 (HYAL-1), located in the lysosome, utilizes HA with any size as substrate to generate tetrasaccharides. While HYAL-2, located in the plasma membrane, breaks down HA to lower molecular weights of ~20 kDa [14].

The biological effects of HA depend heavily on molecular weight. HA with ultra-low molecular weights (0.4–4.0 kDa) acts as an inducer of heat shock proteins and has a non-apoptotic property. HA with super low molecular weights (below 60 kDa) and low molecular weights

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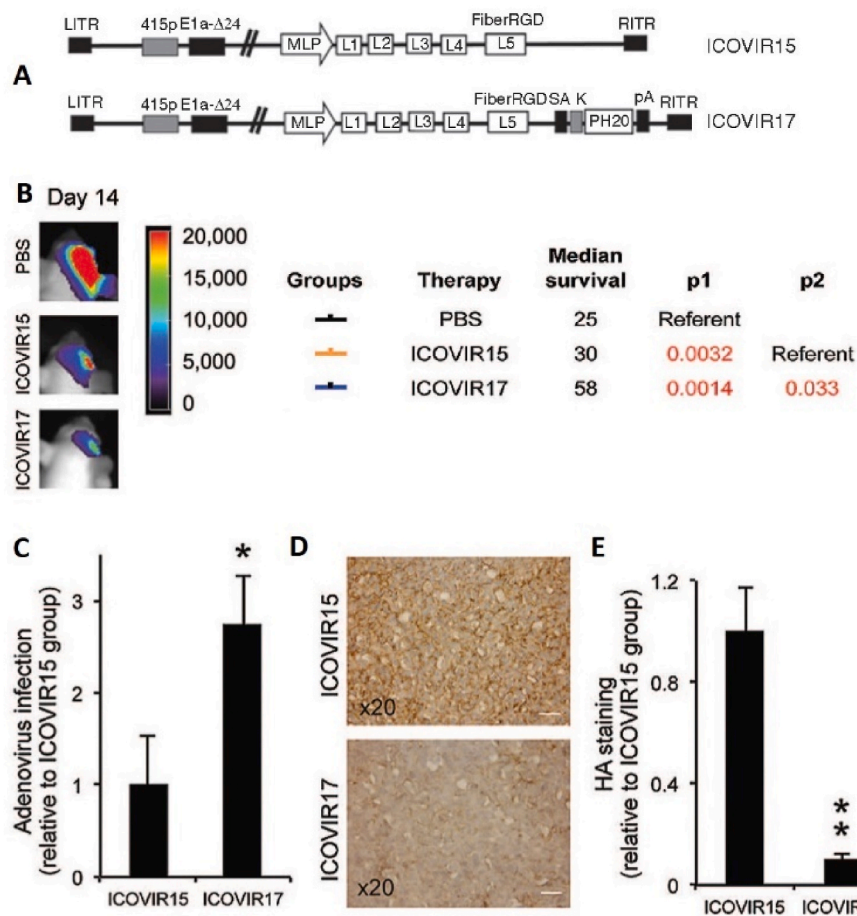
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**Fig. 1.** Oncolytic virotherapy. A) Oncolytic adenovirus (ICOVIR15) and oncolytic adenovirus expressing the promoter PH20 for hyaluronidase (ICOVIR17). Reproduced with Open Access permission from Ref. [52]. B) Kaplan–Meier survival curves of treated mice. P1 represents P value with PBS-treated group as reference and p2 represents P value with ICOVIR15 group as reference. C) Quantification of adenovirus staining. D) HA staining of brain with ICOVIR15 or ICOVIR17 treated groups. E) Quantification of HA levels. Bars, +SE (n = 5) and \*P < 0.05 ICOVIR15 and ICOVIR17 versus PBS control; P = 0.15 ICOVIR17 versus ICOVIR15 at day 24 (student t-test, two-sided). Bar = 50  $\mu$ m. Reproduced with permission from Attribution-Non Commercial-No Derivatives 4.0 International (CC BY-NC-ND 4.0) license from Ref. [51].

(60–200 kDa) possess immunostimulatory, angiogenic, and phlogotic activities. HA with a medium molecular weight of 200–500 kDa takes part in biological processes such as embryonic development, wound healing, and ovulation. By contrast, high molecular weight hyaluronic acid (>500 kDa) has anti-angiogenic activity, and can function as a space filler and a natural immunologic depressant [4]. HMW HA shows to inhibit neutrophil aggregation in a dose-dependent manner [15]. Which demonstrates that processes such as inflammation and wound healing could be modulated with the application of HA of different MW sizes for the development of biomedical engineering constructs [16,17]. However, many types of chemical modification are utilized to ensure HA integrity *in vivo* [18]. HA carboxylic acid groups are modified by for example by ester formation; while hydroxyl groups can be modified by for example utilizing glutaraldehyde [19]. HA derivatives often cross-linked using physical or covalent crosslinks [20]. Crosslinking strategies form 3 dimensional polymeric networks capable of storing water known as hydrogels. These gels have similar properties to biological tissues, and are inherently biocompatible [21]. HA has been reviewed recently with emphasis on chemistry [19,22], medicine [23], membranes for healing [24], reconstruction of tissues [21], delivery of pharmaceuticals [25], transplantation [26] and immune response modulation [27], but, to date, no review has been published on the emerging potential of HA as an antimicrobial biomaterial. Therefore, this review focuses on the relationship between HA and the three different classes of microbes (bacteria, fungi, and viruses), as well the intrinsic antimicrobial activity that exogenous HA may pose to combat microbial infections and improve wound healing for biomedical applications. This study acknowledges that the antimicrobial mechanisms presented on this review are only hypothetical, as there has never been any report in the literature that studied the precise antimicrobial mechanisms of action exerted by

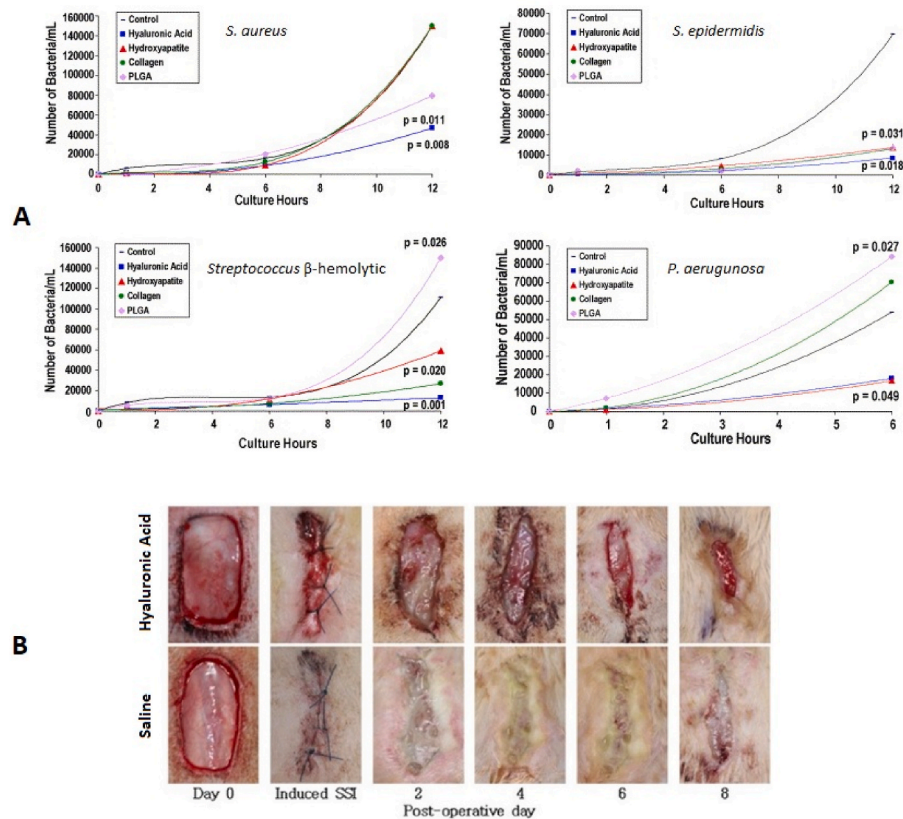
HA. With this review, we highlight and express the need to develop more research targeting to unveil the biomolecular mechanisms associated with the antimicrobial activity of HA.

## 2. The intrinsic relationship between microorganisms and HA metabolism

The growing demand for the commercial production of HA has shifted its production from animal to microbiological sources. HA was first isolated from streptococci A and C back in 1937 [28], and remains to this date the most cost sensitive and the best source for scaled production of HA. Furthermore, in the same matter, the commercial production of HA lyases has been shifting from animal sources such as from bovine testes to microbiological sources including *Bacillus* sp. and *Streptomyces roseofulvus* S10 [29–31].

The ability of many strains of bacteria (*Streptococcus pneumoniae*, *Bacillus anthracis*, and *Haemophilus influenzae*) and yeast (*Cryptococcus neoformans*) to produce HA is associated with an elevated pathogenic virulence factor [32]. Moreover, viruses such as *Paraneicum bursaria Chlorella virus* (PBCV-1) have also been discovered to present the HAS gene and to direct its host to produce HA in early infection [33,34].

The strains of bacteria that produce HA, incorporate the newly synthesized HA to the bacterial mucoid capsule, which confers to the bacteria resistance to opsonization, immune camouflage and protection against the host immune system [35,36]. Kass and Seastone have demonstrated that when group A and C streptococci have their mucoid capsule degraded by hyaluronidases, they decreased their infectiousness in mice, as their susceptibility to phagocytosis by leukocytes increase [37]. Other studies have confirmed the association between HA capsule production and virulence by deletion or mutation of the bacterial



**Fig. 2.** (A) Growth curves of different bacteria in the presence of various biomaterials. Reproduced with permission from Ref. [67]. Hyaluronic acid demonstrated statistically significant bacteriostatic effect against *S. aureus*, *S. epidermidis*, Streptococcus β-haemolytic apart from *P. aeruginosa*. (B) Evaluation of HMW HA effects on wound healing and wound contamination against saline treatment. Reproduced with permission from Ref. [70].

chromosome. Wessels et al. have shown that an acapsular mutant strain derived from mucoid bacteria was relatively increased sensitivity to phagocytosis and less virulent in mice than their wild strain [38]. Whereas the transfection of a mucoid plasmid into an acapsular strain was able to promote the production of HA and render the microorganism resistant to phagocytic killing [39].

It is also believed that the HA produced by mucoid bacteria can also serve as an adhesion contact point for host cells via linkages with HA-binding proteins [35]. Attachment of the bacteria to the host tissue shows a remarkable invasive infection capacity not observed with acapsular bacteria [40].

Microorganisms, such as bacteria, fungi and bacteriophages, are also able to produce HA degrading enzymes, generally called HA lyases to differ from hyaluronidases produced by vertebrates [41]. Some pathogenic Gram-positive bacteria produce membrane-bound HA lyases shown as a virulence factor in a variety of infection models, which is due to its role in facilitating local spread of infection [42]. For example, *Staphylococcus aureus* (*S. aureus*) produces HA lyases encoded in the *hysA* gene. When *hysA* positive and a mutant type *hysA* negative *S. aureus* were cultured in catheters prior to implantation in a murine model, the *hysA* positive bacteria were more invasive and increased lesion distribution and severity in comparison to *hysA* negative bacteria [43]. Group A streptococcus relies on the HA capsule to avoid phagocytosis by the innate immune system and to interact with epithelial cells, paradoxically, these bacteria also produce HA lyases. It is often assumed that HA lyases breakdown similar HA chains in human tissue in an effort to promote bacterial spread [44]. Van de Rijn et al. has demonstrated that streptococcal strains (A and C) at the stationary phase were able to degrade HA irrespective of their ability to produce HA [45].

The microbial ability to secrete HA lyases, highly associated with virulence, is also believed to be involved in the bacteriostatic

mechanism of action shown by exogenous HA. In session 3.4, a further discussion of this mechanism of action will be presented. Briefly, it is hypothesized that in HA-enriched substrates, the microbial HA lyases become saturated, leading to a decrease in microbial proliferation and tissue penetration.

Other microorganisms including *Paracoccidioides brasiliensis*, *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, *C. krusei* and *Malassezia pachydermatis* are also shown to produce HA lyases [46–48]. Bacteriophage viruses, such as virulent phage A25 and *Saphhexavirus* (vB\_EfaS\_TV16) are shown to present gene encoding HA lyase enzymes, which help with phage burst in mucoid bacteria [49,50]. This characteristic shown by bacteriophages is particularly interesting for phage therapy, a method to treat bacterial infections resistant to antibiotics [50].

Recently, oncolytic viruses have been employed in the aid to treat cancer (Fig. 1). For example, adenovirus expressing hyaluronidase (ICOVIR17) is known to mediate HA degradation in local glioblastoma, which enhanced viral spread throughout the tumour and resulted in significant tumour suppression and mice survival [51,52]. Also employed in the treatment of cancer, bacteria expressing hyaluronidase, such as *Salmonella typhimurium*, were capable of degrading HA deposited within pancreatic ductal adenocarcinoma tumours [53].

### 3. Antibiotic activity of HA

#### 3.1. Gram-positive bacteria

*S. aureus* are non-motile, Gram-positive, non-spore forming facultative anaerobic cocci bacteria. With a diameter circa 1 μm they grow via aerobic respiration from room temperature to 45 °C [54]. They are often located in mucous membranes and skin without symptoms [55]. They

are usually transmitted by contaminated surfaces, direct contact to predisposed humans and air borne. They are known to spread quickly in clinical settings, such as surgical wards [56].

*S. aureus* are often located on wound surfaces specifically chronic wounds [57]. Wound healing process during remodelling produces conditions which can often promote growth of *S. aureus* [58]. These opportunistic pathogens are involved in a number of life-threatening infectious diseases. For example, they commonly are the source of staphylococcal infections and initiate diseases such as osteomyelitis and food poisoning [59,60].

In surgical settings, *S. aureus* are a major reason for chronic biofilm formation which impacts cardiology and orthopaedics amongst other procedures. *S. aureus* infections are associated with implantable devices, such as in catheters, central blood lines, mechanical ventilation, prostheses, etc [61]. Treatment often consists of long antibiotics administration and in some cases additional surgeries, for the removal of infection causing device surfaces for example [43].

Amongst various polymers tested for antibacterial coatings, HA and its derivatives may offer a solution and long-term safety with a known ability to retard bacterial adhesion and the formation of biofilm [62]. HA has recently been investigated to assess its bacteriostatic properties and it shows dose-dependent effects on different microorganisms. The bacteriostatic activity of crosslinked HA films has shown to be higher in HMW than in LMW films [63]. HA coatings on titanium are decreased the density of *S. aureus* and adhesion, revealing the potential for application of HA-based coatings in osteosynthesis, orthopedics and dental surgery [64].

HA of varying MW and concentrations interacts with bacteria and trigger differing growth profiles. Three HA MW (low = 0.14 MDa, medium = 0.76 MDa and high = 1.3 MDa) were tested on *S. aureus* ATCC 9996, *Streptococcus mutans* ATCC 10449, and *Propionibacterium acnes*, with each showing reductions in proliferation in media containing HA. Moreover, the medium-sized MW showed the highest bacterial growth inhibition [65]. Regardless of HA MW and concentration, no bactericidal effects were detected.

Collagen, hydroxyapatite (HAp), PLGA and HYAFF-11™, a benzyl-ester LMW (0.2 MDa) HA derivative produced by Fidia Advanced Biopolymers, have been tested against *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228),  $\beta$ -haemolytic *Streptococcus* (ATCC 19615) cultures [66]. The results showed that HA significantly suppressed the growth rate of bacteria in comparison with the other biomaterials. (Fig. 2A). This was related to the saturation of the bacterial hyaluronidase by excessive HA, preventing the bacteria from maintaining tissue permeability [67].

Hyaloss is a benzyl alcohol esterification of hyaluronic acid. At higher percentages of esterification, HA becomes insoluble in water. These HA esters can be extruded to produce membranes and fibres, lyophilized to obtain sponges, or processed by spray-drying, extraction, and evaporation to produce microspheres. The degree of esterification influences the size of hydrophobic patches, which produces a polymer chain network that are rigid and less susceptible to enzymatic degradation. These materials show a bacteriostatic effect in the treatment of periodontal defects [68].

Study *in vivo* with Hyruan Plus®, a linear HMW HA (3 MDa) produced by LG life sciences (Iksan/South Korea) [65], was placed in surgical wounds inoculated with *S. aureus* (SC 2406). As observed, the HA increased wound healing through its bacteriostatic properties, as shown in Fig. 2B [69,70]. In this study, the animal treated with HA had their wounds healed faster (wound area  $26.54\% \pm 6.12\%$ ) and showed less purulence (bacterial count  $4.69 \pm 0.45 \log\text{CFU/mL}$ ) in comparison with the control group (wound area  $50.59\% \pm 5.50\%$  and bacterial count  $5.31 \pm 0.27 \log\text{CFU/mL}$ ).

Sepafilum® (Genzyme Corporation, Japan) is a gel made of HMW HA and carboxymethylcellulose (CMC) at a 2:1 ratio by weight [71]. For these materials, as the concentration of HA in *S. aureus* ATCC 27217 cultures increased, the optical density (OD) of the bacteria medium

decreased. Thereby demonstrating the bacteriostatic properties of HA against this bacterial strain [72]. Other antimicrobial studies using Gram-positive bacteria from the streptococcus and enterococcus species showed that HMW HA (1.8 MDa) against *Streptococcus mutans* ATCC 25175, *Enterococcus faecalis* ATCC 29212 and *Enterococcus hirae* ATCC 10541 also possesses dose-dependent bacteriostatic effects [73]. A porous tissue engineering scaffold produced from HA (125 kDa) decreased the colony formation units (CFU) from  $1 \times 10^7$  (control) to  $1.1 \times 10^3$  (HA) of *S. aureus* ATCC 6538 [74].

In orthodontics, bacteria proliferation and biofilm formation are the main cause of gingivitis, plaque formation, and periodontitis, which can lead to tooth loss [75]. This can be interrupted with tooth brushing and mouthwash application. For example, Gengigel® is a mouthwash containing 0.2% of HA with reported antimicrobial activity against *S. aureus* and *S. mitis* [76] and *Streptococcus constellatus* [77].

Colonization of *S. aureus* and *Streptococcus pneumoniae* in the airways is responsible for respiratory tract infections (RTI). A pharmaceutical solution of HMW HA (1 MDa) Yabro® (IBSA, Lugano, Switzerland) has been developed to treat the main symptoms of emphysema and asthma [78]. Two different concentrations (0.15 and 0.3% v/v) of Yabro® have been tested for antimicrobial activity against *S. aureus* and *S. pneumoniae*. HA was found to reduce 56% of biofilm formation [79].

In ophthalmological applications, HA is used in eye drop solutions to increase the solution viscosity for lubrication purposes [80]. Moreover, HA is also used in the fabrication of contact lenses as a wetting or comfort agent [81]. Both ophthalmological products are known to suffer from microbial contamination [82]. The contamination of eye drops and contact lenses pose risks to the patients using them, as they can transfer the bacteria to the eye and leading to the development of corneal infections, such as bacterial keratitis [83]. In bacterial keratitis the most common infections are associated with Gram positive bacteria, such as Staphylococci [84]. In contact lenses, specifically, bacterial populations develop into biofilms, which provide protection against higher dosages of antibacterial drugs in contrast to their free-floating planktonic forms [82].

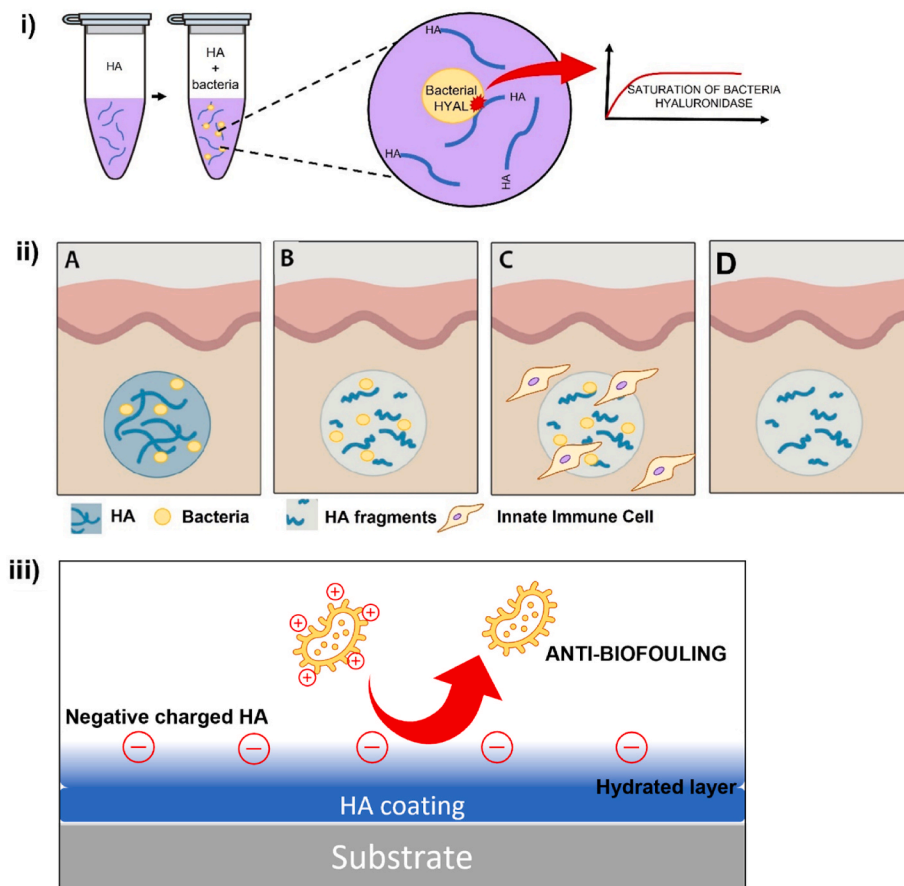
### 3.2. Gram-negative bacteria

*Escherichia coli* (*E. coli*) is a popular organism for the study of the basic mechanisms of molecular genetics. *E. coli* is Gram-negative bacillus, with 1  $\mu\text{m}$  in length and 0.35  $\mu\text{m}$  in width, but this depends on strain type and growth conditions. It may contain whip-like flagella for movement, or hair-like pili for the attachment to surfaces [85].

Sepafilum® shows bacteriostatic effect against *E. coli* ATCC 25922 in a concentration-dependent manner [72]. A porous tissue engineering scaffold produced from HA (125 kDa) has bacteriostatic effects by decreasing the colony formation units (CFU) of *E. coli* (ATCC 11229) from  $3.9 \times 10^7$  (control) to 50 (with HA) [74]. This result was confirmed elsewhere for HMW HA (1 MDa) [86,87].

*Porphyromonas gingivalis* (ATCC 33277), *Prevotella oris* (ATCC 33573), and *Actinobacillus actinomycetemcomitans* Y4 have been tested against three HA MWs (0.14, 0.76, and 1.3 MDa). Each bacteria displayed distinct growth inhibition indexes, where *A. actino* showed higher growth inhibition in comparison to *P. oris* and *P. gingivalis*, respectively [65]. HA (HYAFF-11™) significantly suppresses the growth rate of *Pseudomonas aeruginosa* (ATCC 27853), when compared to collagen, HAp, and PLGA [67]. Hyabest® (S) LF-P shows antibacterial activity against *Proteus mirabilis* (ATCC 35508) [88]. While Gengigel®, displayed bacteriostatic effects when evaluated against *E. coli*, and other microorganisms found in dental plaque, such as *Fusobacterium nucleatum* and *Eikenella corrodens* [77].

Urinary tract infections (UTI) are associated with uropathogenic *E. coli*. Antibiotics can provide symptomatic relief; however, they do not prevent recurrence. In this situation, clinical evidence suggests intravesical HA therapy helps reduce UTI [89]. HMW HA (1.5–1.8 MDa) reduces bacterial adherence and urothelial disruption by uropathogenic



**Fig. 3.** Proposed antimicrobial mechanisms of HA shown in the literature. i) The direct bacteriostatic mechanism of action of HA shown in *in vitro* studies is believed to be due to bacteria hyaluronidase saturation by excess HA, making the bacteria unable to efficiently breakdown HA and decreasing bacteria proliferation rate. ii) In studies *in vivo*, the antimicrobial mechanism of HA is hypothesized to be indirect: A) bacterial lyase breaks down HA, making bacteria unable to attach to the substrate (anti-adherent/antiadhesive substrate), which inhibit bacteria colonization. B) In turn, the LMW fragments of HA are released. C) These LMW fragments recruit innate immune cells, such as neutrophils, to the infection site. D) Neutrophils are able to obliterate bacteria. iii) Anti-biofouling properties of HA based on hydrophilicity and net negative charge that repels positive charged cell wall from bacteria.

*E. coli* (strain TPA493J) [90].

In respiratory tract infections (RTI), administration of HA into the airways reduces occurrence. Two different concentrations (0.15 and 0.3% v/v) of HMW Yabro® have been tested for antimicrobial activity against *Moraxella catarrhalis* and *Haemophilus influenzae*. HA was found to reduce up to 30% of biofilm formation from these two bacteria species [79].

### 3.3. Mycobacteria

Mycobacteria invade the lungs through the interaction with GAG, such as HA. Three strains of mycobacteria (*M. tuberculosis* H37Rv, *M. smegmatis* mc<sup>2</sup>155, and *M. avium* type 4) were used to determine the influence of HA on infection and disease. Interestingly, HA promotes mycobacteria invasion and proliferation [91].

HA has been widely utilized to encapsulate drugs, including antibiotics. Aminoglycoside antibiotics (such as streptomycin) are highly hydrophilic. The hydrophilicity of this drug poses pharmacological challenges, particularly in the treatment of intracellular bacterial infections, such as those from mycobacteria. Due to its high hydrophilicity, it has poor penetration within the eukaryotic cell membranes, often high doses of antibiotic still display subtherapeutic concentration inside the cell [92]. It was speculated that HA could be an antibiotic carrier for the treatment of intracellular bacterial infections. Antibiotics conjugated to HA can be phagocytised by infected eukaryotic cells through a CD44-mediated pathway [93].

### 3.4. Bacteriostatic mechanism of action from HA

#### 3.4.1. Bacteria expressing HA lyases

Although bacteria show different susceptibility to HA, it is of interest

to understand the exact mechanism involved in the antimicrobial activity of HA. The expression of bacterial exoenzymes has also been correlated to their virulence. HA lyase is shown to be a pathogenic bacterial spreading factor that catalyses the degradation of HA through an enzymatic  $\beta$ -elimination process [94]. Because HA is an important constituent of the extracellular matrix (ECM), its bacterial degradation may contribute to bacterial spread via increased tissue permeability, causing wound infection, respiratory disease, and sepsis [67].

However, some studies have shown that the bacteriostatic effect of soluble HA *in vitro* may be attributed to the saturation of the bacterial hyaluronidase by excess of HA in the medium [67]. Thus, slowing their proliferation profiles (Fig. 3i). Some studies in the treatment of wounds speculate that the lower proliferation rate of bacteria *in vivo* in the presence of excess HA is due to the bacterial lyase being unable to break down HA efficiently (Fig. 3ii), which prevents the bacteria from maintaining the elevated levels of permeability in tissue [70]. This leads to the inability of bacteria to attach and form biofilms in the wound area (antiadherent/antiadhesive substrate), inhibiting bacteria colonization [79,95]. In turn, the LMW fragments of HA generated by bacterial lyases, further instigate the ability of the host's immune system to remove pathogens [96]. For instance, it is well known that LMW HA fragments activate toll-like receptors for the stimulation of leukocyte recruitment and adhesion to the injured site [97,98]. These LMW HA fragments enhance neutrophil aggregation and macrophage activation [15], which are two of the most important immune cells associated with the combat of bacterial infections [99,100].

#### 3.4.2. Bacteria unable to express HA lyases

The above-mentioned antibacterial mechanisms of action of HA are related to bacteria that can synthesize HA lyases. However, as shown previously, bacteria which cannot synthesize HA lyases are also unable

**Table 1**  
Summary of HA loaded or conjugated to antibiotic agents.

HA MW	Processing	Antibacterial agent	Bacteria type	Outcome
0.2 MDa	HA-cholesterol nanohydrogels (NHs)	Levofloxacin (LVF)	<i>P. aeruginosa</i> (PAO1), methicillin-susceptible <i>S. aureus</i> (MSSA - ATCC 6538P) and methicillin-resistant <i>S. aureus</i> (MRSA - USA300-0114)	LVF-NHs removes bacterial infections [109]
0.25 MDa	Deacetylation	Streptomycin	<i>S. aureus</i> and <i>Listeria monocytogenes</i>	LVF-NHs eradicated bacteria in intracellular infections Conjugation of streptomycin to HA decreased bacterial burden <i>in vivo</i> with no nephrotoxicity [93]
0.12–0.15 MDa	Collagen (COL)-conjugated	Tobramycin or ciprofloxacin	<i>P. aeruginosa</i> (ATCC 9027)	Antibiotic-loaded collagen-HA matrix for a skin substitute was found to inhibit bacteria growth [110]
Not shown	Chemical conjugation and physical absorption	Ciprofloxacin or vancomycin	<i>P. aeruginosa</i> , <i>S. aureus</i> , and <i>B. subtilis</i>	HA microgel loaded with antibiotics showed long lasting antibiotic release, while preventing bacterial infections with no toxicity to corneal endothelium [111]
0.35 MDa	HA and polyvinylpyrrolidone (PVP) blend	Ciprofloxacin	<i>S. aureus</i> , <i>E. coli</i> and <i>P. aeruginosa</i>	Multi-layered films showed biocompatibility, antibacterial activity, and resorbed <i>in vivo</i> [112]
0.17 MDa	Polyelectrolyte complexes (PECs)	Gentamicin	Not shown	HA derivative PECs can modulate the availability of the gentamicin by increasing the half-life and extending the release time of the antibiotic [113]
$1.8 \times 10^6$ g/mol	HA/COL/chitosan (CHI) blend	Gentamicin	<i>S. aureus</i> (ATCC6538), <i>E. coli</i> (ATCC8739) and <i>P. aeruginosa</i> (ATCC15442)	Films based on natural polymers enriched in gentamicin sulphate inhibit the growth of Gram negative and positive bacteria [114]
Not shown	COL/HA polyelectrolyte multilayers (PEMs)	LL-37 peptide	<i>E. coli</i> (DH10B strain)	The LL-37-modified PEMs prevented bacterial adhesion, killed bacteria in broth and neutralized an <i>E. coli</i> culture [115]
Not shown	Poly( <i>N</i> -isopropylacrylamide)-grafted HA	Chlorhexidine, gentamicin, rifampicin, and vancomycin	<i>S. aureus</i> (ATCC 25923), MRSA (ATCC 43300), <i>S. epidermidis</i> (ATCC 35984), and <i>E. coli</i> (ATCC 25922)	HApN can be loaded with antiseptic or antibiotics, which prevents the growth of multiple bacteria (prophylactic effect) [116]
0.0095 MDa	Oleylamine (OLA)-HA conjugates	Vancomycin	<i>S. aureus</i> (ATCC 25923) and MRSA (ATCC 700699)	HA-OLA conjugates self-assembled into polymersomes entrapping Vancomycin and showed enhanced <i>anti</i> -MRSA activity compared to free drug [117]
Not shown	HA-Graphene oxide composite	Silver nanoparticles (AgNPs)	<i>S. aureus</i>	HA-Graphene Oxide Composites loaded with AgNPs showed excellent antibacterial activity against <i>S. aureus</i> [118]
Not shown	HA-CHI blend	AgNPs	<i>E. coli</i> (ATCC 25922), <i>S. aureus</i> (ATCC 35556), MRSA (ATCC, p1030, p1077), <i>P. Aeruginosa</i> (strain 01847) and <i>Klebsiella pneumoniae</i>	Chitosan-HA/AgNPs composite sponges showed potent antimicrobial property against the tested microorganisms [119]
1.3 MDa	HA/polycaprolactone (PCL) nanofibrous membranes (NFMs)	AgNPs	<i>S. aureus</i> (BCRC 10451) and <i>E. coli</i> (BCRC 11634)	The release of Ag from HA/PCL + Ag NFMs plateaued after 4 days, which confirmed the short-term anti-bacterial effect [120]
0.2 MDa	HA/CHI	Vancomycin	MRSA	vancomycin-loaded CC/AHA injectable hydrogels showed pH-dependent vancomycin delivery [121]
0.6–1.1 MDa	Electrosprayed films	Cefoxitin (Cef)	<i>Klebsiella pneumoniae</i> (Xen39), <i>S. aureus</i> (Xen 36), and <i>Listeria monocytogenes</i> (EDGE)	Nanofiber scaffolds of HA containing Cef may be used in dressings to control postoperative infections [122]
0.2 MDa	HA/COL/Alginate matrix	AMP tet213 peptide	<i>E. coli</i> (ATCC25922), MRSA (ATCC33592), and <i>S. aureus</i> (ATCC6538)	AMP-loaded wound dressing released AMP in a sustainable manner, exhibiting antimicrobial activity against different bacterial strains [123]
Not shown	HA/Aloe vera NP	Doxycycline	<i>E. coli</i> and <i>S. aureus</i>	Nanocarriers affected both bacteria [124]
0.05 MDa	Octenyl succinic anhydride (OSA)-modified HA	DJK-5 peptide	<i>P. aeruginosa</i> (LESB58)	Upon subcutaneous administration, the toxicity of the DJK-5 in nanogels was decreased four-fold compared to non-formulated peptide [125]
0.7–1.0 MDa	HA/CHI PEMs	Triclosan (TRI) and rifampicin (RIF)	<i>E. coli</i> (ATCC 11229)	PEMs-loaded TRI and RIF showed good antimicrobial coating for PET devices [87]
Not shown	11-amino-1-undecanethiol (AT)-conjugated HA nanogel	LLKK18 peptide	<i>Mycobacterium avium</i> (ATCC 2447 and 25291) and <i>M. tuberculosis</i> H37Rv	Intra-tracheal administration of peptide-loaded nanogels significantly reduced infection levels in mice [126]
0.6–1.1 MDa	Polyethylene oxide (PEO)-HA nanofibers	Kanamycin	<i>Listeria monocytogenes</i> (EDGE) and <i>P. aeruginosa</i> (PA01)	The kanamycin-PEO-HA nanofibers inhibited bacterial growth, suggesting its use to coat prosthetic implants to prevent secondary infections [127]
2 MDa	Eggshell membrane composite	KR-12 peptide	<i>S. aureus</i> (ATCC 25923), MRSA (ATCC 43300) and <i>E. coli</i> (ATCC 25922)	<i>In vitro</i> results revealed that the composite membrane had excellent antibacterial activity against all bacteria tested and it could prevent MRSA biofilm formation on its surface [128]

(continued on next page)

Table 1 (continued)

HA MW	Processing	Antibacterial agent	Bacteria type	Outcome
420 000 g/mol		Cateslytin	<i>Micrococcus luteus</i> (A270) and <i>S. aureus</i> (ATCC25923)	HA-CTL-C/CHI films fully inhibit the development of <i>S. aureus</i> which is a common and virulent pathogen encountered in care-associated diseases [129]
Not shown		Green tea (GT)	<i>E. coli</i> (KCTC1041), <i>Salmonella typhimurium</i> (KCTC2054), <i>Pseudomonas putida</i> (KCTC1134), <i>B. subtilis</i> (KCTC2217) and <i>S. aureus</i> (KCTC1916)	Microneedles composed of GT extract and HA exhibit ~95% growth reduction of Gram-negative and positive bacteria [130]
0.35 MDa		Carvacrol prodrugs	Gram-negative <i>Klebsiella pneumoniae</i> (ATCC 700603), <i>E. coli</i> (ATCC 25922), <i>Acinetobacter baumannii</i> (ATCC 19606), and <i>P. aeruginosa</i> (ATCC 27853) and Gram-positive MRSA (ATCC 43300), <i>S. aureus</i> (ATCC 29213), <i>S. epidermidis</i> (RP62A), <i>E. faecalis</i> (ATCC 29212), <i>E. faecium</i> 64/3, <i>Streptococcus agalactiae</i> (ATCC 2603), <i>Streptococcus pneumoniae</i> (ATCC 49916), and <i>Streptococcus pyogenes</i> (12RF)	HA-loaded carvacrol prodrugs shows better minimum inhibitory concentration (MIC) values against <i>E. faecium</i> and <i>E. faecalis</i> compared to those of carvacrol [131]
Not shown	HA/adipic acid dihydrazide hydrogel	Vancomycin	MRSA (ATCC 29213)	Vancomycin-loaded gels exhibited excellent drug release and in vitro antimicrobial activity with minimal cell toxicity [132]
8–10 MDa	Genipin-crosslinked gelatin/hyaluronic acid gel	Hinokitiol	<i>S. aureus</i> (ATCC 25923) and <i>E. coli</i> (ATCC 10798)	Gels loaded with hinokitiol showed significant antibacterial activity [133]
Not shown	HA/palm oil-based organogel	Maraviroc	<i>L. crispatus</i> (ATCC 33197)	HA/palm oil-based organogel could be exploited for vaginal delivery of maraviroc microbicide [134]
Not shown	Polyurethane-HA microfibers	Ethanol extract of propolis (EEP)	<i>S. aureus</i> and <i>E. coli</i>	EEP-incorporated samples caused formation of considerable inhibition zones against these bacteria [135]
140 000 g/mol	HA nanocapsules (NCs)	Polyhexanide (PH)	<i>S. aureus</i> (ATCC 29213 and ATCC 43300) and <i>E. coli</i> (ATCC 25922)	HA-loaded PH exhibited antibacterial action [136]
0.035 MDa	OSA-modified HA	Novicidin peptide	<i>S. aureus</i> (ATCC 25923) and <i>E. coli</i> (ATCC 25922)	Self-assembly of novicidin with HA into nanogels significantly improved the safety profile when tested in HUVECs and NIH 3T3 cells, whilst showing no loss of antimicrobial [137]
Not shown		Ag NPs	<i>S. aureus</i>	HA-loaded Ag NPs showed some antibacterial activity, however the highest antimicrobial properties was shown to chitosan-loaded Ag NPs [138]
401.3 g mol <sup>-1</sup>	Polyelectrolyte-assembly over fabric viscose (CV)	Surfactant MKM	<i>E. coli</i> , <i>S. aureus</i> and <i>S. agalactiae</i>	Exceptional antimicrobial activity has been shown to CV-functionalized MKM, making it highly interesting for potential use in medicine [139]
HA-EP3 (1600 KDa–2500 K Da)	Mesoporous microparticle crosslinked using divinyl sulfone (DVS)	Vancomycin		Drug release profile of these mesoporous microparticles showed that the crosslinking ratio increased the drug releasing amount decreased [140]
1.5 to 2.2 million Da	HA and HA/Sucrose particles were synthesized using two different crosslinkers: DVS and glycerol diglycidyl ether (GDE)	Ciprofloxacin	<i>E. coli</i> (ATCC 8739), <i>P. aeruginosa</i> (ATCC 10145), <i>S. aureus</i> (ATCC 6538), and <i>B. subtilis</i> (ATCC 6633)	No bacterial effect was observed for bare HA and HA/sucrose particles. However, ciprofloxacin-loaded particles showed MIC and MBC values of 0.25–2 mg ml <sup>-1</sup> and 0.25–4 mg ml <sup>-1</sup> against all bacteria species, respectively [141]

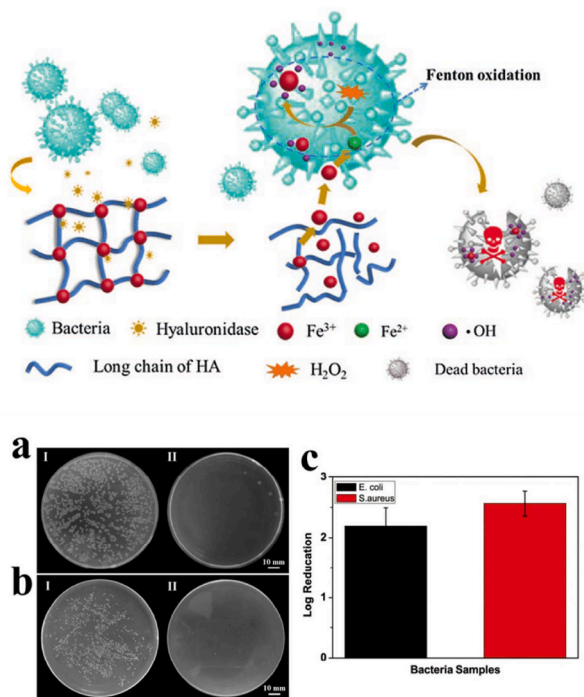
to proliferate in HA coatings. To explain this phenomenon, The anti-biofouling property of HA which is attributed to the chemical and physical characteristics of HA molecule may contribute to the lower rates of contamination, colonization, and biofilm formation in HA substrates (Fig. 3iii).

Many polymers have shown to possess anti-biofouling properties, such as low-attachment hydrophobic materials, hydrophilic polymers, and zwitterionic materials [101]. HA is composed of alternating units of glucuronic acid  $\beta(1-3)$  and *N*-acetylglucosamine  $\beta(1-4)$ , which possess abundant amide (CO–NH) and carboxyl (COOH) groups. These groups provide a net negative charge and hydrogen-bond donors/acceptors improving surface hydration [102]. HA's negative net charge can induce steric repulsion of the bacteria cell wall (also negatively charged), thus improving its antifouling performance against bio contaminants in comparison to other hydrophilic biopolymers, such as chitosan (a cationic hydrophilic polymer) [103]. For example, HMW HA/dopamine conjugates exhibited non-fouling properties in various biomaterials

commonly used as implantable substrates (i.e., polyimide, gold, poly(methyl methacrylate) (PMMA), polytetrafluoroethylene, and polyurethane [104,105]. In another study, LMW HA was modified using bisphosphonic acid derivatives. These derivatives were developed as coatings for titanium Grade 4 used in implants. Non-modified HA was able to reduce the colonization of *S. aureus* P 209 and *E. faecium* Ef79OSA, and modified HA significantly decreased bacterial colonization of *S. aureus* and *E. faecium* and *P. aeruginosa* ATCC 27,853 [106]. Moreover, the development of coatings based on polyelectrolyte multilayers consisting of HA and chitosan have been extensively studied for biomedical applications and suppression of bacterial and protein attachment on implantable devices such as intraocular lenses, stents and catheters) to reduce implant-related infections [107].

### 3.5. HA derivatives for antibiotic delivery applications

HA has been widely used to encapsulate a wide range of drugs,



**Fig. 4.** Illustration of bacteria-responsive, iron-releasing ( $\text{Fe}^{3+}$ ) HA hydrogels. Bacteria (turquoise) secrete HA lyases, degrade the HA releasing  $\text{Fe}^{3+}$  ions.  $\text{Fe}^{3+}$  is absorbed by the bacteria and reduced intracellularly to  $\text{Fe}^{2+}$ .  $\text{Fe}^{2+}$  reacts with  $\text{H}_2\text{O}_2$  to form a hydroxyl radical, killing the bacterial cell (grey). Photographs of actual CFUs of *E. coli* (a) and *S. aureus* (b) on agar plates from diluted bacterial suspension without (i) and with (ii) HA-Fe-EDTA hydrogel treatment. (c) Log reduction of the HA-Fe-EDTA hydrogels against *E. coli* and *S. aureus* calculated from the photographs (pristine Luria–Bertani broth was used as control group) (mean  $\pm$  SD,  $n = 3$ ). Reproduced with permission from Standard ACS Editors' Choice usage agreement [108].

including antibiotics. In this section, the latest studies utilizing HA derivatives as vehicles for antibiotic drugs are reviewed, as shown in Table 1.

Encapsulating antibiotics in HA matrixes combines a synergistic strategy for the targeted treatment of bacterial infections. For example, Tian et al. designed an antimicrobial hydrogel based on HA [108]. Bacterial HA lyases target the degradation of the HA hydrogel, which releases  $\text{Fe}^{3+}$ . Bacteria take up  $\text{Fe}^{3+}$  and reduce it into  $\text{Fe}^{2+}$ . Subsequently,  $\text{Fe}^{2+}$  reacts with  $\text{H}_2\text{O}_2$  to form hydroxyl radicals leading to bacterial cell death (Fig. 4).

Furthermore, aminoglycoside antibiotics (such as streptomycin) are hydrophilic. This poses pharmacological challenges, particularly in the treatment of intracellular bacterial infections. Due to its hydrophilicity, it has poor penetration within eukaryotic cell membranes, often high doses of antibiotic may still display subtherapeutic concentration inside the cell [92]. It has been proposed that HA could be an antibiotic carrier for the treatment of intracellular bacterial infections. Antibiotics conjugated to HA were able to be phagocytised by infected eukaryotic cells through a CD44-mediated pathway and were able to eradicate bacteria in intracellular infections [93].

#### 4. Antifungal activity of HA

Fungi are a type of eukaryote. They contain membrane-bound sub-compartments that separate specific functions, particularly the nucleus. Fungi can be single-celled, like yeast, or multicellular, like mould. Similar to bacteria, fungi can proliferate via asexual and sexual replication. Fungi are important to society, and are used in food preparations, such as *Saccharomyces cerevisiae*, a yeast used in the fermentation of

sugars to produce alcohol and to raise bread. Fungi are also important in pharmaceuticals, for example *Penicillium chrysogenum* produces penicillin [142]. Alterations in the intestinal microbiome are associated with many diseases, and alterations (dysbiosis) of fungal communities may contribute to disease. Many factors are likely able to promote fungal dysbiosis including exposure to antibiotic, for example, exposure to antibacterial antibiotics has long been known to promote the overgrowth of *Candida* in the gut. Likewise, treatment with antifungal antibiotics reduces the prevalence of some fungi, while it increases the prevalence of others [143]. HMW HA (1.8 MDa) displays intrinsic antifungal properties against *Candida glabrata* ATCC 90030 and *Candida parapsilosis* ATCC 22019 (Fig. 5), with fungistatic activity reported to be dose-dependent [73]. Other study, evaluated the fungistatic activity of LMW HA (1630 kDa) against *Candida albicans* (ATCC 10231, 18804, and 11006) [144]. This study reported that HA solutions displayed concentration-dependent activity (0.5, 1.0, and 2.0  $\text{mg mL}^{-1}$ ) against candida strains, where greatest inhibition was observed against *C. albicans* ATCC 11006 strain and weakest inhibition was observed against ATCC 18804. No candidacidal activity was reported in the study [144].

#### 4.1. HA derivatives for antimycotic delivery applications

The three classes of antifungals currently in clinical use are polyenes (i.e., Amphotericin B), triazoles (i.e., Ketoconazole), and echinocandins (i.e., Caspofungin) [107]. Polyenes and triazoles are exhibiting declining efficacy due to the development of drug-resistant fungal strains [145]. In order to overcome the antifungal resistance, novel formulations availing of various drug delivery matrixes and adjuvants have been studied. In this landscape, HA has been employed as to enhance hydrophobic molecules to improve their bioavailability (shown in Table 2). Poor water soluble clotrimazole has been encapsulated in ionic polymeric micelles based on HA to improve clotrimazole's bioavailability [146]. Another study has used HA hydrogels to load selenium and ketoconazole nanoparticles for the topical treatment of seborrheic dermatitis [147]. Amphotericin B has also been incorporated into HA microneedle patches for a topical ocular drug delivery treatment in corneal fungal infections [148].

#### 5. Antiviral activity of HA

The influence of HA on viruses is an emerging topic and to date there has been relatively few studies. However, evidence of antiviral activity of HA has been shown *in vitro* with respect to the herpes simplex virus (HSV-2) [156], while interestingly other studies on type 1 HSV-1, show that HA could be involved in HSV-1 infection in brain and skin tissues [157]. In the case of HIV infection, exogenous HA reduced HIV infection of unstimulated  $\text{CD4}^+$  T helper (Th) cells in a CD44-dependent manner, while, hyaluronidase-mediated degradation of endogenous HA on the cell surface aid HIV binding and infection of unstimulated  $\text{CD4}^+$  Th cells [158].

The levels of HA produced by rheumatoid arthritis (RA) synovial cell lines have been examined, they were shown to be resistant to infection with Newcastle disease virus (NDV), vesicular stomatitis virus (VSV), and rubella virus (RV). While normal foetal synovial cells lines were susceptible to NDV, VSV, and RV. Interestingly, RA cells became infected when HA was degraded, HA prevented infection of normal synovial cells with VSV [159].

Another study has shown that different cell lines pre-treated with HMW HA (1800 kDa) demonstrated strong antiviral activity against Coxsackievirus B5 (COXB5), mumps virus (MV) and influenza virus A/H1N1, with mild antiviral activity against HSV-1 and porcine parvovirus (PPV), and no activity against Adenovirus 5 (ADV-5), human Herpesvirus-6 (HHV-6), porcine reproductive and respiratory syndrome virus (PRRSV) as shown in Fig. 6. In all cases, no virucidal activity of HA was observed [160].



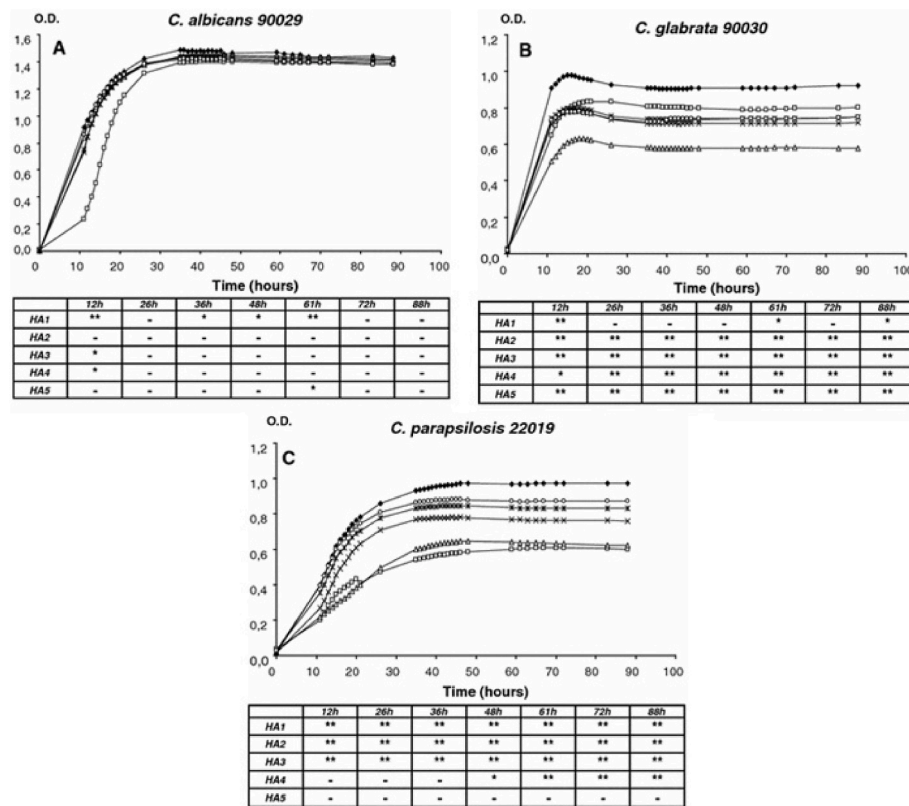


Fig. 5. Growth profile of *C. albicans* ATCC 90029 (a), *glabrata* ATCC 90030 (b), and *C. parapsilosis* ATCC 22019 (c) at  $5 \times 10^6$  CFU/mL exposed to HA 1837 kDa. Five different concentrations of HA were used:  $4 \text{ mg mL}^{-1}$  (square),  $2 \text{ mg mL}^{-1}$  (triangle),  $1 \text{ mg mL}^{-1}$  (times),  $0.5 \text{ mg mL}^{-1}$  (snowflake) and  $0.25 \text{ mg mL}^{-1}$  (circle), and no HA (filled diamonds). \*\*Highly significant ( $P < 0.01$ ); \*significant ( $P < 0.05$ ); - not significant ( $P > 0.05$ ). Reproduced with permission [73].

As alluded to above, HA degradation is a virulence factor in a variety of infection models and facilitates local spread of the pathogens. The Zika flavivirus infection, a concern during pregnancy, infects human placentas, inducing defects in the developing foetus. The Flavivirus non-structural protein 1 (NS1) alters the GAG on the endothelium of placenta, causing hyperpermeability *in vitro* and vascular leakage *in vivo* in a tissue-dependent manner. NS1 induced shedding of HA and heparan sulphate (HS) as well as altering the expression of CD44 and LYVE-1 HA receptors on stromal fibroblasts and Hofbauer macrophages in villous cores. The mechanism behind this is postulated to be the stimulation of hyaluronidase in NS1-treated trophoblasts which leads to HA degradation [161].

### 5.1. Antiviral mechanism of action from HA and other polysaccharides

Researchers have proven that the antiviral activity of polysaccharides is associated with their anionic groups and chemical modifications with the inclusion of sulphate groups. According to their structural features, polysaccharides can inhibit the virus cycle at different stages, such as at the internalization, uncoating, and transcription phases, or even by directly killing the virus (Fig. 7) [162]. The antiviral mechanisms of HA polysaccharides involve two major criteria: (1) inhibit the virus activity by binding to envelope ligand sites thus inactivating the virus itself; (2) which inhibits the viral docking, internalization and uncoating in host cells; and (3) improve the immune response of the host against the virus, thus, indirectly inhibiting the virus replication process and fastening the viral clearance [163]. The latter mechanism of action can also be also applied as a strategy for the

delivery of novel vaccines. Where HA can boost the immune response of the host cells by activating the production of antiviral immune factors [164]. This will be further discussed in the next section 5.2.

### 5.2. HA derivatives for antiviral delivery applications

HA has been used to encapsulate a wide range of antiviral drugs (summarized on Table 3). It is worth to mention that the use of HA as an encapsulant for the delivery of inactivated viruses and antigens can boost immunization efficiency in the development of vaccines [165], even in the current context of SARS-CoV-2 [166]. In this regard, mice immunized with a HA complex carrying the hepatitis B surface antigen (HBsAg) exhibited a significant increase (6-fold) in cellular immune response and (120-fold) in humoral immune response relative to mice vaccinated with HBsAg alone. This shows that HA employed for the production of these novel vaccine carriers was pivotal for enhancing immune activation at the delivery site against hepatitis B [164]. Other HA-based vaccines showed remarkable protection against rabies [167] and Ebola viruses [168].

Intranasal administration of vaccines has been developed as an innovative form of immunization. In this context, the pharmacokinetics for novel HA vaccine delivery systems appeared to be optimum when using HA with lower MWs (<67 kDa), as they showed more rapid systemic distribution than higher MWs (>215 kDa) [169]. New cationic liposome-HA nanoparticles were developed as a carrier for F1-V, a recombinant antigen for plague virus. The study showed that these novel nanoparticles induced potent humoral immune responses as an intranasal vaccine platform against *Yersinia pestis* [170]. Another study used

**Table 2**  
Summary of HA loaded or conjugated to antimycotic agents.

HA MW	Processing	Antimycotic agent	Fungi type	Outcome	Reference
420,000 g/mol		Cateslytin	<i>Candida albicans</i>	HA-CTL-C films fully inhibit the development of <i>C. albicans</i> , which are common pathogens encountered in care-associated diseases	[129]
Not shown		Glycyrrhetic acid (1818 $\beta$ -GA)	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. krusei</i> , <i>C. guillier-Mondii</i> , <i>C. lusitanae</i> , <i>Saccharomyces cerevisiae</i> and <i>Zygosaccharomyces</i> spp.	18 $\beta$ -GA and HA, alone and in combination show antimycotic activity, being considered as possible alternative to azole antifungal agents for the topical treatment of vulvovaginal candidiasis	[149]
0.001 MDa	poly( <i>N</i> -isopropylacrylamide)/HA gels	Ketoconazole (KCL)	<i>C. albicans</i>	<i>In vivo</i> antimicrobial study shows growth inhibition of <i>C. albicans</i> inoculated in rabbit eyes	[150]
0.8–1.17 MDa	HA gel -integrated liposomes	Fluconazole		Potential use for the treatment of fungal keratitis	[151]
1.01 MDa	HA-H5 PEMs	Polypeptide histatin-5 (H5)	<i>C. albicans</i> (ATCC 10231)	The PEM inhibited fungal attachment/adhesion, significantly reduced fungal biofilm formation, and showed synergistic effects with the antifungal drug miconazole. This novel coating is expected to treat Candida-associated denture stomatitis	[152]
15,000–30,000 g/mol	Eudragit RL100/amphotericin B NP coated with HA	Amphotericin B	<i>C. albicans</i> (ATCC 14053)	Nanoparticles showed rapid <i>C. albicans</i> elimination and efficacy against <i>in vivo</i> vulvovaginal candidiasis	[153]
Not shown	HA-loaded MZ based gel	Miconazole (MZ)	<i>C. albicans</i>	HA-loaded MZ based gel demonstrated better antifungal activity, indicating its potential in oral thrush pharmacotherapy	[154]
1.3 MDa		Clotrimazole	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. tropicalis</i> and <i>S. cerevisiae</i>	CTZ-loaded HA gel showed fungistatic activity for more than 24h. This formulation shows to be a potential drug delivery system for local therapy of vaginal candidiasis and other similar infections	[155]
401.3 g mol <sup>-1</sup>	Polyelectrolyte-assembly over fabric viscose (CV)	Surfactant MKM	<i>C. albicans</i> and <i>C. glabrata</i>	Exceptional antimicrobial activity was shown for the functionalized CV, making it highly interesting for potential use in medicine	[139]
1000 Da	HA polynucleotide (HA-PN)	Ketoconazole	<i>C. albicans</i>	New Zealand White rabbits presenting fungal keratitis were used for <i>in vivo</i> antimicrobial studies, showing that this formulation cured 91.7% of rabbits in comparison to commercial ketoconazole eye drop (66.7%).	[150]

HA to encapsulate inactivated influenza virus for a flu vaccine using HA (HYAFF) microspheres. The microspheres showed significant higher immunization response following intranasal administration than those induced by traditional intramuscular immunization at the same vaccine dose [171]. A quadrivalent influenza virus vaccines based on HA with a MW of 27 kDa was developed. The quadrivalent influenza virus HA vaccine induced serum IgG and intranasal-secreted IgA with antigen-specificity [172].

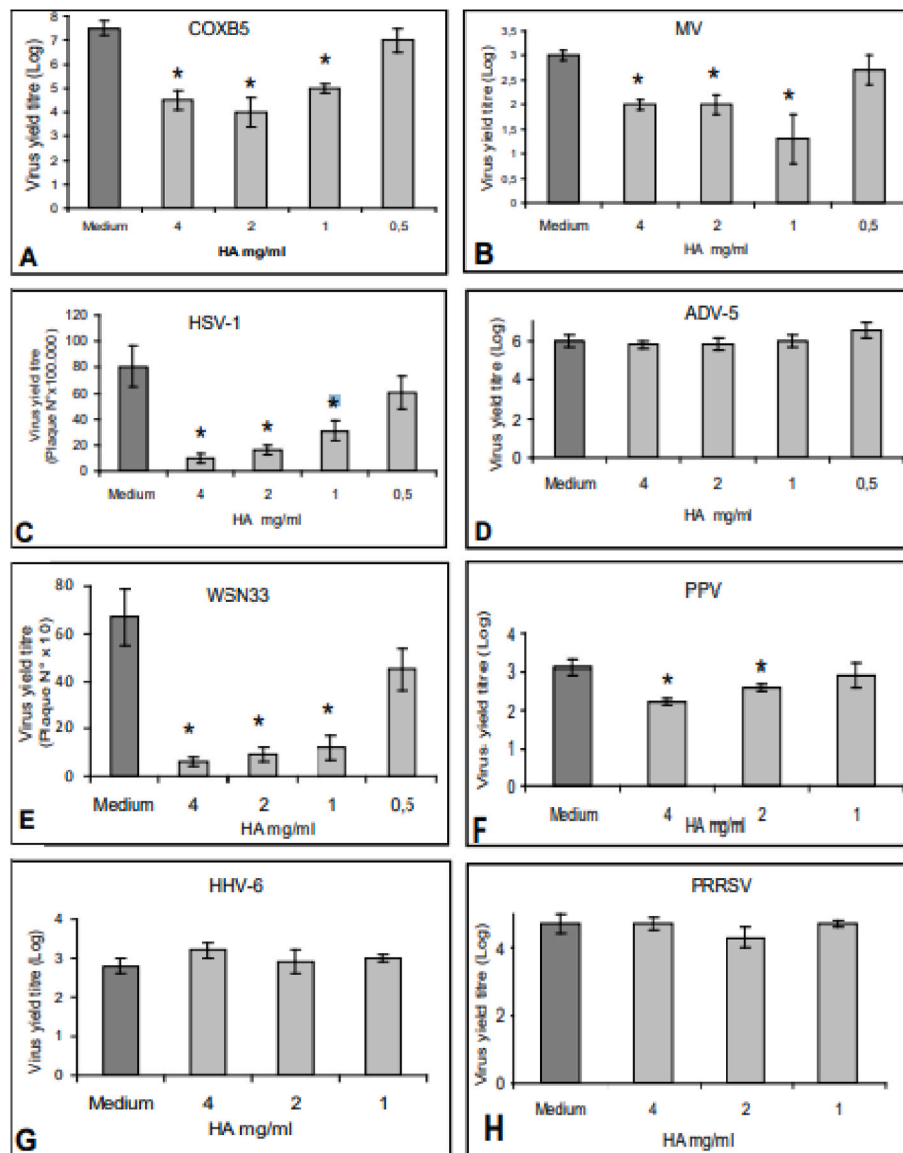
The transcutaneous route of administration of vaccines through the use of microneedle patches has gained great interest in the last couple of years. It has been shown that dissolvable HA microneedle tips were able to deliver antigens derived from influenza virus, including A/H1N1, A/H3N2 strains and A/canine/VC378/2012, and promote a humoral response two times higher than those induced by intramuscular injections [173,174].

## 6. Antimicrobial properties of HA in comparison to other biopolymers

When comparing the antimicrobial effects of HA against other polymers sourced in nature, it can be noticed that chitosan and alginates also show to possess antimicrobial properties similar to those presented by HA. All polymers and their derivatives show an antimicrobial effect which is dependent to dose/concentration and MW. However, the antimicrobial mechanism of action is distinct for each polymer. For example, positive charged chitosan molecules (either LMW or HMW), through electrostatic interaction with the membrane of bacteria, change

the bacterial membrane permeability which inhibits their proliferation. Moreover, it is expected that LMW chitosan can penetrate through the microbial membrane and interact with its DNA. This will lead to the inhibition of the microbial mRNA and protein synthesis [178,179]. It is also postulated that the antifungal mechanism of action of LMW chitosan is associated with its ability to be taken up by the cells and to chelate intracellular metals, to suppress spore elements and to bind to essential nutrients to fungal growth [180]. However, the use of chitosan for antimicrobial applications is hampered due to belonging to a weak class of polyamine (with a pKa of around 6.5) which imparts pH-responsiveness. Chitosan shows antifungal activity only under acidic conditions [181].

For chitosan, its degree of acetylation also influences the antimicrobial effectiveness of the polymer. It is observed that the antimicrobial effect of chitosan improves as the degree of acetylation decreases [180]. In Gram-negative *E. coli*, its growth rate is highly inhibited with LMW (5 kDa) chitosan. While, Gram-positive *S. aureus* is more susceptible to inhibition in HMW (~305 kDa) chitosan formulations [182]. Chitosan nanoparticles [183] and derivatives also present antimicrobial effects [184]. Where carboxy methylation of chitosan has significantly improved antibacterial [185,186] and antifungal effects [181]. Other chitosan derivatives such as quaternary ammonium chitosan nanoparticles also decreases the contamination of poly(methyl methacrylate) (PMMA) bone cement [187]. The antiviral activity of chitosan has also been observed in plants [188] and mammals [189,190], where its antiviral activity has been shown to increase as its molecular weight decreases [162]. It is postulated that chitosan can inhibit SARS-CoV-2



**Fig. 6.** Virus yield of various infected cell lines after the exposure to HA in different concentrations. A) VERO cells infected with COXB5, B) VERO cells infected with MV, C) VERO cells infected with HSV-1, D) VERO cells infected with ADV-5, E) WSN33, F) PK15 cell line infected with PPV, G) JJHAN cell line infected with HHV-6, and H) MARC145 cells infected with PRRSV. Open access reprinting from Ref. [160].

infection, by preventing the viral spike protein from docking in angiotensin converting enzyme 2 (ACE-2) on the surface of host cells [166].

In case of alginate and its derivatives, the study by Salem et al., shows that algi-1,2-phenelinide has bigger bacterial inhibition to both Gram positive and Gram negative bacteria, however, algi-4-chloro-1,2-phenelinide has a more prominent inhibition effect on fungi [191]. In another study, utilizing polyurethane and alginate blend scaffolds, the incremental addition of alginate content (up to 1%) increased the hydrophilicity of the scaffold, thus decreasing bacterial adhesion and proliferation [192]. Other studies have demonstrated that alginates also possess antiviral properties [193,194]. Although the antimicrobial properties of chitosan and alginates may compare to those presented by HA, the latter still possess other beneficial properties (biocompatibility and immune regulator) that contribute to outperform the two previous biomaterials. For example, alginates with high mannuronic acid or impurity content are potentially immunogenic, which decreases its desirability for applications such as antimicrobial wound dressings or

catheter coatings [195]. Regarding chitosan, due to its cationic nature, the adsorption of proteins in chitosan coatings may prove difficult its applications as an antimicrobial coating for catheters [103].

## 7. Conclusion and perspectives

Bacteria, fungi, and viruses are in close association with HA and its metabolism to surpass host defences and thrive. Specifically, in the case of bacteria, there are two main contributing factors to increase virulence. One is associated with their ability to produce a mucoid capsule containing HA, which decreases their recognition by surveillant host immune cells. The other contributing factor is associated with their ability to produce HA lyases to degrade endogenous HA, which enhances bacterial penetration and spread within tissues. However, treatment of wounds with exogenous HA are shown to decrease bacterial infection. Their ability to produce HA lyases can be exploited to counteract their virulence. Some researchers postulated that bombarding wounded sites

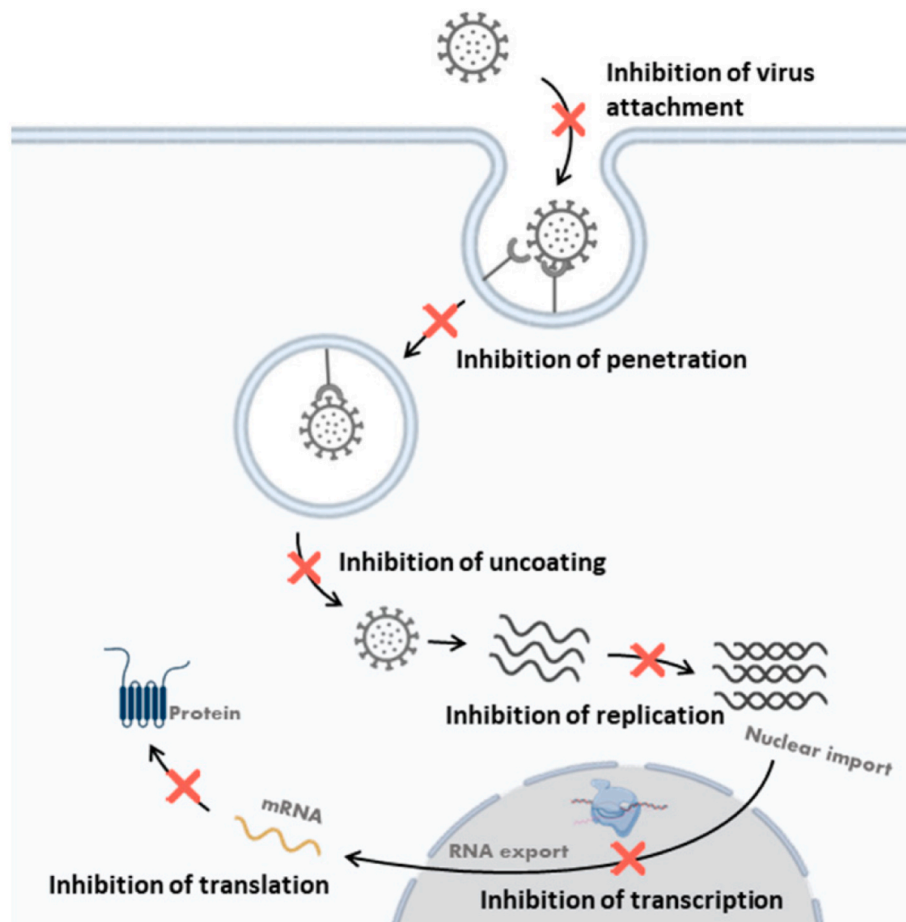


Fig. 7. Steps of viral replication inhibition by antiviral polysaccharides. Reprinted with permission from Ref. [162].

with HA may overwhelm bacteria capable of producing HA lyases, thereby suppressing bacterial growth. In the studies presented in this review, all molecular weights of HA exhibit antimicrobial activity at some level, but it is worth mentioning that HMW HA shows better antimicrobial properties in comparison to LMW HA. It does not come as a surprise, as HMW HA contains longer polymeric chains which HA lyases will take longer to break down in comparison to LMW HA. Moreover, HA also possess physical properties that enable HA matrixes to repel bacteria adhesiveness (anti-biofouling effect) through its super-hydrophilicity and negative net charge.

The exact mechanism of action by which HA can down-regulate the proliferation of bacteria and fungi, and the infection of healthy cells with some viral strains, needs to be studied in more detail. While there has been relatively little attention to date on the antiviral activity of HA, it offers intriguing possibilities for the treatment of a variety of ailments such as Herpes, HIV, rheumatoid arthritis (RA), mumps and others. Research findings associated with HA encoding viruses may be of particular interest to treat SARS-CoV-2 infections. It is known that during SARS-CoV-2 infection, the lungs of patients produce a gel like substance that lines the alveoli and decreases oxygen exchange in the lungs [196]. This overproduction of HA may be associated with the virus itself as it can induce the production of HA by using the host translational machinery. The treatment of these patients with HA synthase inhibitors may overt COVID-19 symptoms [197]. Moreover, viruses

encoding HYAL genes maybe be used for the targeted treatment of multiple drug-resistant bacterial infections (bacteriophages), or these viruses can be used for the treatment of cancers (oncolytic viruses).

Researchers in the field of microbiology have an exciting new area for exploration in order to unveil the biochemical cascade mechanisms that are associated with the intrinsic antimicrobial activity of HA. The encapsulation of many antimicrobial agents in HA substrates results in novel therapeutics that can target and enhance the delivery of drugs to the local infection site. Moreover, as HA also possess bacteriostatic properties, the encapsulation of antibiotics in HA matrixes can have a synergistic effect to hamper bacterial proliferation and treat multidrug-resistant bacterial strains. When HA is utilized for prophylactic purposes against microbial infections, the use of LMW HA would be recommended as they can boost local immune surveillance, being commonly employed as adjuvants in vaccine development.

HA hydrogels, coatings and films show excellent biocompatibility, biodegradability, immunomodulatory, and antimicrobial properties, all of which are suitable for the development of tissue engineering constructs and biomedical-associated devices that may impact positively healthcare-associated infections. In conclusion, HA may prove to be a complete biopolymer that possesses all desirable properties that are important for the development of contact lenses, wound dressings, scaffolds for tissue reconstruction, implants coatings, and drug delivery systems.

**Table 3**  
Summary of HA loaded or conjugated to anti-viral agents.

HA MW	Modification	Drug	Virus	Outcome	Reference
27 kDa	Tetraglycine-l-octaarginine	Inactivated H1N1 A/New Caledonia/20/99 IVRI16 (NCL) viruses	PR8 viruses	Infection with PR8 viruses were completely circumvented through nasal immunization with a mixture of inactivated NCL viruses and tetraglycine-l-octaarginine-linked HA	[175]
650 kDa	Cyclodextrin (CD)	Acyclovir (Acy)	Herpes viruses (HSV-1, HSV-2 and VZV)	HA-CD/Acy complex showed good antiviral activity together with a delayed release of Acy from HA-CD/Acy	[176]
Not shown	HA/palm oil-based organogel	Maraviroc	TZM-b1 cells inoculated with HIV-1 NL4-3 virus	HA/palm oil-based organogel could be exploited for vaginal delivery of maraviroc as anti-HIV microbicide	[134]
Not shown		Zidovudine and Lamivudine	TZM-b1 cells inoculated with HIV-1 virus	HIV reverse transcriptase inhibitors Zidovudine and Lamivudine were successfully encapsulated into the HA polymer assembly in a noncovalent manner. The supramolecular assembly exert potent antiviral activity and allow sustained drug release	[177]

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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