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Review

Antiviral Properties of Alginate-Based Biomaterials: Promising Antiviral Agents against SARS-CoV-2

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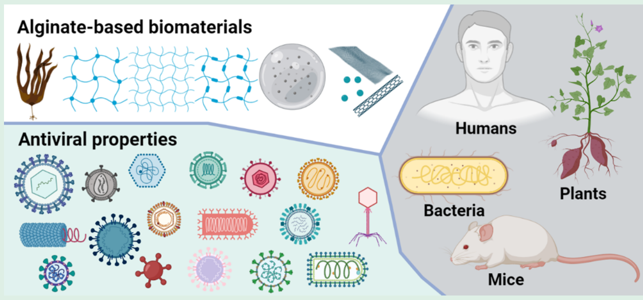
ABSTRACT: The COVID-19 pandemic has made it essential to explore alternative antiviral materials. Alginate is a biodegradable, renewable, biocompatible, water-soluble and antiviral biopolymer with many potential biomedical applications. In this regard, this review shows 17 types of viruses that have been tested in contact with alginate and its related biomaterials. Most of these studies show that alginate-based materials possess little or no toxicity and are able to inhibit a wide variety of viruses affecting different organisms: in humans by the human immunodeficiency virus type 1, the hepatitis A, B, and C viruses, Sindbis virus, herpes simplex virus type 1 and 2, poliovirus type 1, rabies virus, rubella virus, and the influenza virus; in mice by the murine norovirus; in bacteria by the T4 coliphage, and in plants by the tobacco mosaic virus and the potato virus X. Many of these are enveloped positive-sense single-stranded RNA viruses, like SARS-CoV-2, which render alginate-based materials highly promising in the COVID-19 pandemic.



KEYWORDS: *alginates, viruses, antiviral activities, biomaterials, SARS-CoV-2*

1. INTRODUCTION

Alginates, the salts of alginic acid, are natural anionic polymers that can be commonly extracted from brown seaweed of the class *Phaeophyceae*, mainly from the species *Laminaria hyperborea*, *Laminaria digitata*, *Macrocystis pyrifera*, and *Ascophyllum nodosum*.[1] *A. nodosum* has an alginate concentration of 22−30% of its dry weight, while *L. hyperborea*'s varies from 17−33% to 25−30% depending on the part of the algae from which the alginate is extracted.[2] Alginates can also be produced from bacteria such as *Pseudomonas aeruginosa*[3] and *Azotobacter vinelandii*.[4] Alginates are a linear polysaccharides composed of (1−4)-β-D-mannuronic acid (M) blocks and C-5 epimer α-L-glucuronic acid (G) blocks that can be distributed in several ways that directly affect the alginate's physical properties (Figure 1).[5]

Apart from the mannuronate-to-guluronate (M/G) ratio, other characteristics such as the molecular weight and the degree of acetylation also affect alginate's rheological properties.[6] Different sources produce alginate with different G and M contents and block lengths, thus creating many possible different structures with different properties. For example, the species *L. digitata* has a M-block content of 49%, while other available alginates range from 15% to 43%.[2] Commercial products of the most common salt form of alginic acid, sodium alginates (SA), usually present a molecular weight (M_W) that usually ranges from 32 000 to 400 000 g/mol.[5] The viscosity of alginate increases as the pH decreases (peaking around pH 3−

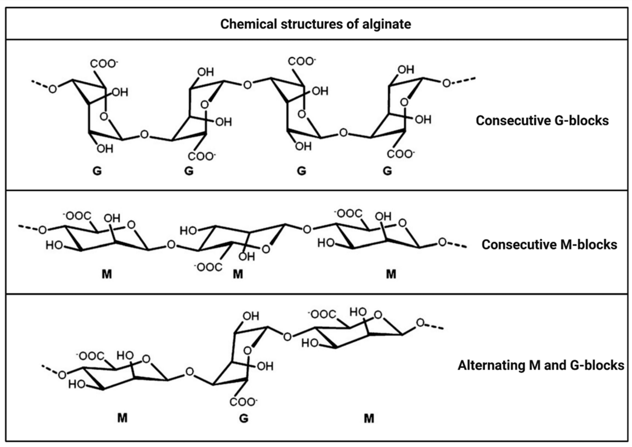


Figure 1. Chemical structures of alginate with a distribution of consecutive G-blocks (G), consecutive M-blocks (M), or alternating M and G subunits. Reproduced in part with permission from ref 5. Copyright 2012 Elsevier.

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3.5) due to hydrogen bonding of carboxylate groups that become protonated.[7] The increase of alginate's M_W can enhance the mechanical characteristics of the produced gels.[8] Controlling an alginate's molecular weight and its distribution can determine the alginate solution's viscosity pregelation and its rigidness afterward.[9] Because of its inherent biocompatibility, little or no toxicity, and affordability, alginate is a widely researched biomaterial for use as a tool in the field of biomedicine.[10] Although its *in vivo* and *in vitro* biocompatibility is well-known,[10] authors still disagree about how the biocompatibility is affected by the alginate's composition because of the different purity levels of the alginate studied in their reports.[11] This immunogenic reaction could be caused by remaining alginate impurities such as heavy metals or proteins.[12] In addition, highly pure alginate obtained from purification processes neither caused any reaction when implanted in animals[13] nor did alginate hydrogels produce any immunogenic reaction as an injectable system.[14] Alginates are mainly used in the form of hydrogels in tissue engineering and biomedicine, typically for wound dressings and drug delivery.[10] To create hydrogels, alginate polymer chains must be physically or chemically cross-linked.[15,16] The most frequently used method of producing hydrogels from an aqueous alginate solution is to immerse it in an ionic cross-linking aqueous solution with Ca^{2+}.[17,18] The structure of the G-blocks achieves a high degree of coordination of the divalent cations, which are considered to link exclusively to the guluronate blocks of the alginate chains to form junctions known as the "egg-box" model (Figure 2).[19,20]

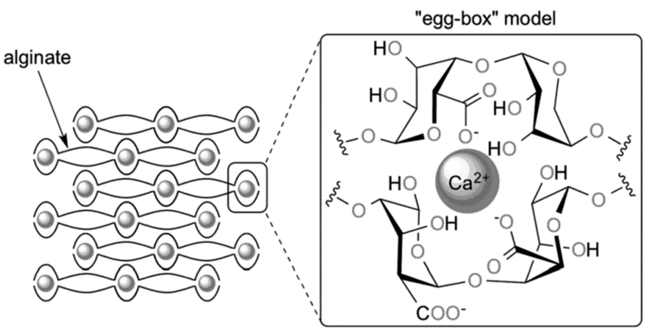

Figure 2. Egg-box model representation associated with the guluronate sequences of cross-linked alginate with calcium cations. Reproduced with permission under a Creative Commons Attribution 3.0 Unported License from ref 20. Copyright 2015 Royal Society of Chemistry.

Calcium chloride is one of the most frequently used agents to ionically cross-link alginate,[21,22] although zinc chloride is also used as cross-linker agent to provide antimicrobial activity and other desirable properties to this biopolymer.[23,24] However, both agents produce rapid uncontrolled gelation because of their excellent solubility in aqueous solutions.[5] Because of their lower solubility, calcium carbonate ($CaCO_3$) and calcium sulfate ($CaSO_4$) are able to increase the working time of alginate gels because they can reduce the gelation rate.[25] When using divalent cations, the rate of gelation needs to be as slow as possible to produce homogeneous materials with suitable mechanical performance.[26] The gelation temperature can also alter the gelation rate and the final gel physical properties.[27] An alginate with a high amount of G-blocks enhances the mechanical performance of the gel after being in contact with divalent cations, whereas those with a lower amount of G residues do not improve the mechanical properties.[28] A significant limitation of this type of cross-linking process is the short gel stability when exposed to long-term physiological conditions since exchange reactions with monovalent cations may dissolve the gels due to the release of divalent cations into the surrounding media.[29] Acid precipitation can also form alginate gels when the solution's pH is brought below the disassociation constant (pK_a) of the polymer.[30] Furthermore, alginate can be covalently cross-linked with agents such as glutaraldehyde, adipic acid dihydrazide, or poly(ethylene glycol)-diamine.[31,32] However, the covalent cross-linking agents can produce toxic side-effects and the nonreactive agents must be thoroughly eliminated from the resultant gels.[20] The physical properties of alginate hydrogels thus depend on the different types of cross-linking agents used in the reaction and on regulating the cross-linking densities.[33] Cross-linking agents with several functions allow a broad range of control over the degradation process as well as over the physical stiffness.[34] Covalent cross-linking may be approached with photo cross-linking and suitable chemical starters.[35] Alginates are nontoxic, biocompatible, and biodegradable materials.[36] Alginates cannot be degraded in mammals because they do not have the alginase enzyme to break the bonds of the biopolymer chains,[37] although alginates that are cross-linked with divalent cations in the form of hydrogels can release the cations into the surrounding media to degrade by ion exchange reaction such as exchange with Na^+ cations. Alginates can also be modified by partial oxidation or other methods to regulate their biodegradation properties.[38−40] Sulfated alginate is similar to the heparin structure and is famous for high blood compatibility in biomedical applications.[41−43] All these excellent alginate properties and the possibility of tailoring them via chemical modification or in combination with other materials for specific applications make it one of the most promising biopolymers in the biomedical field. In this review, a thorough search was made on the topic of alginate-based materials used as antiviral agents. In fact, antiviral polysaccharides have been proposed as ideal candidates to combat the SARS-CoV-2 coronavirus, which causes COVID-19, via pharmacotherapeutic applications.[44] A layer-by-layer nanocoating strategy has also been proposed to coat surfaces of masks, clothing, and work surfaces in places such as wet markets to prevent the spread of viruses in the present and future pandemics. We here analyze all of the alginate-based materials that have shown antiviral capacity against a broad range of viruses in the literature and compare them with SARS-CoV-2 to study the possibility of antiviral success against this new virus.

2. ALGINATE-BASED MATERIALS WITH ANTIVIRAL PROPERTIES

In view of the alarming global spread of the COVID-19 and possible future pandemics, the development of new antiviral agents is gaining much importance.[45] The objective of this review was to examine the possibilities of alginate in pure form, modified, and in combination with other materials to be used as an antiviral agent and its promising potential as antiviral action against SARS-CoV-2.

2.1. Alginic Acid/Sodium Alginate and Their Derivatives.

Alginic acid has been tested against the rabies virus (RAV) in chicken-embryo-related (CER) cells, in which the initial step was affected by alginate's antiviral activity.[46] Alginate's inhibitory effect on the RAV was shown to be dose-dependent at concentrations that ranged from 1 to 100 μg/mL. Alginic acid has also shown antiviral activity against

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the enveloped Baltimore group IV rubella virus (RV) infection on Vero cells.[47] SA has also exhibited antiviral capacity against some plant viruses. For example, an alginate inhibited potato virus X (PVX) infectivity by 95% when *Chenopodium quinoa* was used as host,[48] as in previous studies of alginate's antiviral activity. Thus, a strong antiviral effect of SA at a concentration of 1 $\mu g/\mu L^{-1}$ against PVX was demonstrated. However, a few studies of SA have reported no antiviral activity, as sodium alginate and mannuronic-acid-rich alginate did not show any inhibition effect against vesicular stomatitis virus (VSV)[49] or against herpes simplex virus type 2 (HSV-2),[50] respectively. Alginate hydrogels have also been shown to possess antiviral capacity against herpes simplex virus type 1 (HSV-1) when used as a sulfated compound was dependent on the sulfate contents of the polysaccharides and the chemical properties of the sulfated alginate.[51] The *in vitro* characteristics reported in this study suggested that sulfated alginate is an interesting candidate for further antiviral research. However, SA was less effective against HSV-1 than other sulfated polysaccharides, as shown by its half-maximal inhibitory compound concentration (IC_{50}), which ranged from 10 to 15 mg/mL, which is 10-fold greater than those of fucoidans, and sulfated polysaccharides.[51,52]

A guluronic acid-rich SA derived from *Sargassum tenerrimum* was found to have an anti-HSV-1 effect.[52] Its antiviral activity increased with increasing sulfate ester content. However, another study showed that mannuronic-acid-rich alginate (M/G ratio = 1.88) extracted from *Sargassum trichophyllum* brown algae had no effect against HSV-2.[50] SA and its sulfated derivatives exerted a strong antiviral inhibitory effect against HSV-1 due to direct interferation with virions and inhibition of viral adsorption/attachment to cells.[53]

SA hydrogel films combined with lipids and two natural extracts with a high content of phenolic compounds, such as those obtained from green tea (GTE) and grape seed (GSE), demonstrated viral inhibition against murine norovirus (MNV) and hepatitis A virus (HAV).[54] GTE and GSE had previously been found to present antiviral activity against human norovirus surrogates and HAV,[55−58] indicating that these natural extracts could be employed in active food packaging to inhibit enteric viruses. In this assay, alginate hydrogels combined with the extracts showed a reduction of MNV titers by 2.00 and 1.92 logarithm median tissue culture infectious dose (log $TCID_{50}$/mL), for 0.5 and 0.75 g GTE extract/g alginate, respectively, and by and 0.96 and 1.67 log TCID50/mL, for 0.5 and 0.75 g GSE extract/g alginate, respectively (see Figure 3A).

The HAV titers were also reduced in the GTE and GSE alginate films (see Figure 3B). Although alginate biofilms combined with GTE or GSE demonstrated viral inhibition in MNV and HAV, they had a lower antiviral capacity than the pure natural extracts,[56,58] indicating that the extracts could interfere with the release of the alginate film's active compounds. This assay reported that alginate hydrogels combined with GTE were marginally more successful in inhibiting MNV and HAV than those combined with GSE. Another study[59] investigated further GTE with anti-MNV and anti-HAV capacity by developing edible alginate films with incorporated oleic acid and GTE (A-OA-GTE) to coat strawberries and raspberries. In this study, the effect of the film-forming dispersion pH on viral inhibition was analyzed at different temperatures (10 and 25 °C). A-OA-GTE films prepared in acidic media (pH = 5.5) showed superior antiviral activity than those prepared under neutral conditions (pH = 7.0) at 37 °C. Significant reductions were also observed for MNV at 25 °C, whereas there was no activity at 10 °C because viral particles generally thrive at lower temperatures. In the case of HAV, relevant variations were observed for alginate-GTE films prepared at pH 5.5 after overnight incubation at 25 and 37 °C. However, alginate-GTE films prepared at pH 7 did not have a significant effect on HAV after overnight incubation at either 10, 25, or 37 °C, which is inconsistent with what was reported in ref 58 on pure GTE. Pure GTE was thus highly successful in inhibiting HAV and MNV at neutral pH (pH = 7.0) but showed no activity at the acidic pH (pH = 5.5) due to the variation in the GTE content. The variations in the viral inhibition between pure extract and alginate-GTE hydrogels may therefore be ascribed to the processes followed in characterizing the antiviral activity.

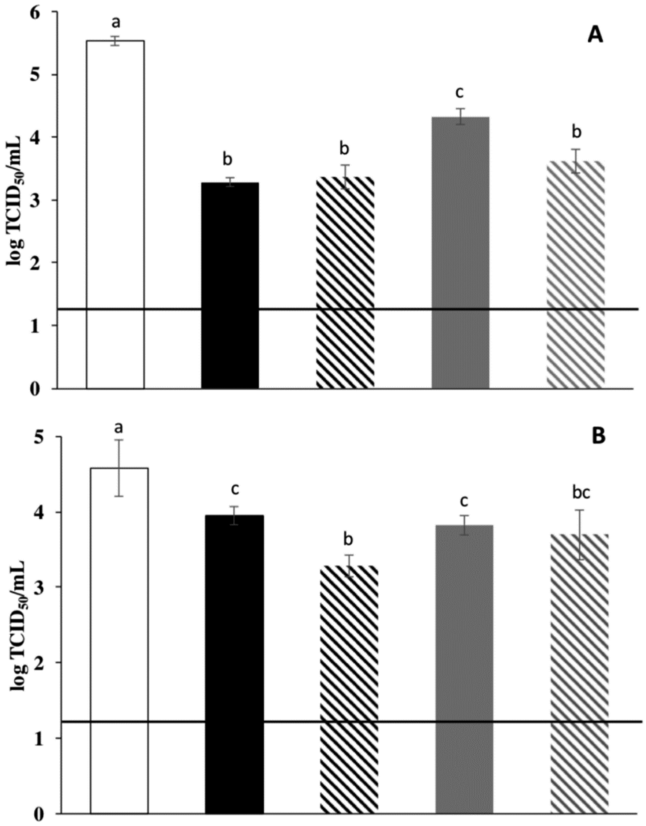

Figure 3. Represented $TCID_{50}$ per mL of the different concentrations of GTE and GSE-containing alginate films against a control without GTE and GSE. (A) White column represents the control alginate film without extract infected with MNV, with ∼5 logs $TCID_{50}$/mL. From left to right: log $TCID_{50}$/mL values of 0.5GTE, 0.75GTE, 0.5GSE, and 0.75GSE alginate films infected with MNV. (B) White column represents the control alginate film without extract infected with HAV, with ∼5 logs $TCID_{50}$/mL. From left to right: log $TCID_{50}$/mL values of 0.75GTE, 0.5GTE, 0.75GSE, and 0.5GSE, alginate films infected with HAV. Reproduced with permission from ref 54. Copyright 2018 Elsevier.

Alginate oligomers exhibited no antiviral action on the infection and replication of human immunodeficiency virus type-1 (HIV-1), human T-cell leukemia virus type-1 (HTLV-1), and hepatitis B and C virus (HBV and HCV).[60] However, they showed potential inhibition capacity of the VSV-G-pseudotyped HIV-1 (HIV-1(VSV)).

2.2. Calcium and Zinc Alginate. When used as an encapsulation technique for human liver cell line (HuH-7 cells), calcium alginate microspheres demonstrated antiviral activity against several viruses when they were added to the supernatant, namely strain Sindbis virus (SINV), poliovirus type 1 (PV-1), and HSV-1 (Figure 4).[61]

As depicted in Figure 4, a dramatic reduction in the infectious titer of more than 2-fold was observed for HSV-1 and 3-fold was achieved for PV-1 and SINV. The use of calcium alginate hydrogel beads also prevented the release of HCV viral agents when the hepatic cells were previously infected and encapsulated. Calcium alginate-based hydrogels have also demonstrated antiviral capacity against influenza virus (IFV)[62] and against the first discovered virus, tobacco mosaic virus (TMV).[63] Even though calcium alginate is extensively proposed for a wide range of industrial applications, it lacks antibacterial activity.[64] Alternative alginate-based materials with intrinsic antibacterial capacity such as zinc alginate have thus been proposed in the biomedical field even for use against multidrug-resistant pathogens.[65,66] Calcium and zinc alginate fibers showed antiviral activity on Vero cells with IFV.[62] However, the authors of this study did not specify the IFV type and strain used in the experiments.

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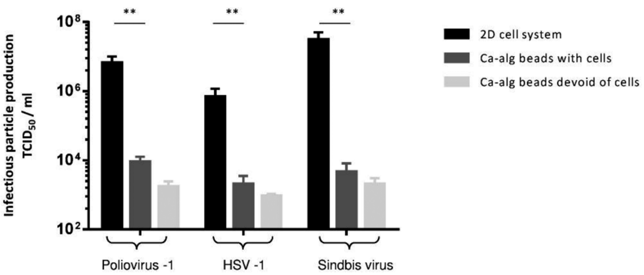

Figure 4. Protective properties of calcium-alginate microspheres against Poliovirus type 1, herpes simplex virus type 1 (HSV-1), and Sindbis virus. Empty calcium-alginate microspheres were used as a control. Reproduced with permission under a Creative Commons CC BY 4.0 License from ref 61. Copyright 2014 PLoS One.

2.3. Alginate-Based Composites and Nanocomposites. Few recent studies have focused on the antiviral activity of alginate-based composites such as alginate in combination with other materials or compounds. However, it has recently been reported that adding lipids and the GTE and GSE natural extracts to alginate hydrogels produced edible films by emulsion, which were tested for their antiviral capacity against MNV and HAV.[54] Interestingly, it was found that alginate films with GTE showed slightly more efficient viral inhibitory effects against HAV and MNV than films with GSE. In a related study, the researchers obtained the same results when the alginate/oleic films containing GTE developed for the preservation of red berries were tested against MNV and HAV.[59] This indicates that alginates have a potential role in the field of food preservation, which will however require further investigation before it can be successfully applied. On the other hand, an alginate-based impression material containing the didecyldimethylammonium chloride disinfectant showed *in vitro*

Table 1. Studies Analyzing Antiviral Properties of Alginate-Based Materials against 17 Types of Viruses[a]

Alginic acid/sodium alginate and their derivatives	Source and Manufacture	Toxicity	Antiviral activity	Tested viruses	Tested cell lines	Year	Ref.
Sodium alginate (M_W > 12 kDa)	*Macrocystis pyrifera*	Not tested	No (ED_{50} = 83.3 μg/mL)	VSV	Human amnion (Wish) cells	1987	49
Alginic acid	Fluka, Switzerland	No (CC_{50} = *400 μg /mL)*	Yes (MIC_{50} = 1 μg/mL)	RAV	CER cells	1993	46
Alginic acid	Fluka, Switzerland	No (*up to 1 mg/mL*)	Slight inhibition of virus multiplication (15–20%)	RV	Vero cells	1997	47
Sodium alginate (M/G from 0.41 to 1.05 and different M_W at M/G=0.8)	Bioreactor grade from Kibun Food Chem. Ltd. (Tokyo, Japan)	Not tested	Yes (higher when M/G = 0.41, increased with the M_W and decreased with increasing alginate concentration)	TMV	Xanthi NN tobacco leaves	1999	75
Sulfated polymannuroguluronate (M_W = 8 kDa)	Extracted from brown algae, depolymerized by enzymatic hydrolysis, followed by sulfation	Not tested	Yes (interfering viral entry)	HIV-1	CD4+ T cells	2003	76
Sodium alginate	*Fucus gardneri*	No (*up to 33 μg/mL*)	Yes (95 % inhibition at 10 μg/mL)	PVX	*Chenopodium quinoa*	2004	48
Sulfated polymannuroguluronate (M_W ~ 10 kDa)	Extracted from brown algae, depolymerized by enzymatic hydrolysis, followed by sulfation	Not tested	Yes (decreased vulnerability of the Tat protein by attenuating calcium overload)	HIV-1	Rat pheochromocytoma PC12 cells	2008	77
Guluronic acid-rich sodium alginate (26 ± 5 kDa)	*Sargassum tenerrimum*	No (CC_{50} *≥ 1000 μg /mL)*	Yes (IC_{50} = 15 μg/mL)	HSV-1	RC-37 cells	2010	52
Sodium alginate (M/G =1.44 and M_W=21 ± 5 kDa) and two sulfated derivatives	*Sphacelaria indica*	No (*CC_{50} ≥ 1000 μg/mL)*	Yes (IC_{50} = 0.6-10 μg/mL)	HSV-1	RC-37	2011	51
Mannuronic-acid- rich alginate (M/G = 1.88)	*Sargassum trichophyllum*	Not tested	No	HSV-2	Vero or MDCK cells	2011	50
Alginic acid (32 ± 5 kDa and M/G = 0.80)	*Laminaria angustata*	No (CC_{50} *≥ 1000 μg/mL)*	Yes (IC_{50} = 25 μg/mL)	HSV-1	RC-37 cells	2012	53
Sodium alginate films with lipids and phenolic extracts (i.e. GTE and GSE)	Films of alginic acid sodium salt from brown algae (medium viscosity) by dissolution in water, mixing with the compounds and solvent casting	Not tested	Yes (~2 log TCID50/mL reduction)	MNV and HAV	RAW 264.7 cells (for MNV) and FRhK-4 cells (for HAV)	2018	54
Sodium alginate coatings with oleic acid and GTE	Films of alginic acid sodium salt from brown algae (medium viscosity) by dissolution in water, mixing with the compounds and solvent casting	Not tested	Yes (1.5-2 log TCID50/mL reduction)	MNV and HAV	RAW 264.7 cells (for MNV) and FRhK-4 cells (for HAV)	2019	59
Alginate oligomers	Prepared from sodium alginate (31132-75, 1000-cps; Nacalai Tesque Inc.,Kyoto, Japan) using the method described by Yokose et al.[78]	No (*in vitro*)	No (HBV, HCV, HTLV-1) IC_{50} = 266 μg/mL for HIV-1 R9 Yes (HIV-1(VSV))	HIV-1, HBV, HCV, HTLV-1, HIV-1(VSV)	HeLa cells	2019	60
Zinc and calcium alginate		**Toxicity**	**Antiviral activity**	**Tested viruses**	**Tested cell lines**	**Year**	**Ref.**
Calcium and zinc alginate fibers	Prepared with sodium alginate (Qingdao Bright Moon Seaweed Group. Co., Ltd)	No (*in vitro*)	Yes	IFV	Vero and HeLa cells	2011	62
Micrencapsulation in calcium alginate hydrogel microspheres (M_W=80,000-120,000 Da, M/G=2.03)	Sodium alginate medium viscosity from *Macrocystis pyrifera* (Sigma-Aldrich) dissolved in sterile saline solution and crosslinked with calcium chloride	Not tested	Yes (4, 3, 3 and 2 fold infection reductions for HCV, SINV, PV-1 and HSV-1, respectively)	HCV, SINV, HSV-1 and PV-1	HuH-7 cells	2014	61
Calcium alginate-lentinan-amino-oligosaccharide hydrogel	Dissolution of sodium alginate (viscosity of 200 ± 20 mPa·s) from Aladdin (Shanghai Aladdin Biochemical Technology Co., Ltd, China) in water, mixing with LNT, crosslinking with Ca^{2+} and coated with amino-oligosaccharides	No (*in vivo fish model*) Promoted plant growth	Yes (induced plant resistance against TMV)	TMV	Leaves of *Nicotiana benthamiana*	2019	63
Alginate-based composites and nanocomposites		**Toxicity**	**Antiviral activity**	**Tested viruses**	**Tested cell lines**	**Year**	**Ref.**
Alginate impression material impregnated with didecyldimethyl ammonium chloride	Commercial Blueprint and Blueprint Asept products (De Trey, Dentsply, Konstanz, FRG). Blueprint Asept contains 0-5% didecyldimethyl ammonium chloride and Blueprint lacks the disinfectant	Not tested	Yes (log reduction of 1.0-1.7 PFU for HSV-1) No (log reduction of 0.0-0.2 PFU for PV-1)	HSV-1and PV-1	BHK cells (for HSV-1) and HeLa cells (for PV type 1)	1989	67
Complex of alginate with rhamnolipid PS-17	Rhamnolipid biosurfactant PS-17 and its complex with the polysaccharide alginate produced by the *Pseudomonas* sp. S-17 strain isolated from a sample of agricultural soil from the Western Ukraine.	Low (MTC = 640 μg/mL)	Yes (IC_{50} = 435 μg/mL for HSV-1 and IC_{50} = 482 μg/mL for HSV-2; Δlog CCID50 from 0.17 to >5.5)	HSV-1 and HSV-2	MDBK cells	2008	68
Alginate dental impression material formulations with MgO	Experimental materials fabricated by dissolving in water and mixing Comercial materials prepared according to the manufacturer.	Not tested	Yes (0.5-4.0 log TCID50 reductions)	HSV-1	Vero cell line	2015	69
Alginate films with and without CNFs (M_W = 379.5 kDa; M/G=1.34)	Sodium alginate (Panreac AppliChem, Darmstadt, Germany) - based hydrogel films crosslinked with calcium chloride by solvent casting	No (100% viability of human keratinocytes)	Yes (log reduction up to 1 PFU)	T4 bacteriophage	Human keratinocyte HaCaT cells	2020	72

[a]Source and manufacture of the alginate-based materials, toxicity: 50% cytotoxic concentration (CC_{50}), antiviral activity: difference between virus titers of the drug-treated sample and the drug-free control sample (Δlog $CCID_{50}$); effective dose to protect 50% of the Wish cell monolayer from cytopathic effect (ED_{50}); half maximal effective antiviral concentration (EC_{50}); half-maximal inhibitory compound concentration (IC_{50}); MIC_{50} (minimal inhibitory concentration of compound (μg/mL) required to inhibit fluorescence by 50%, tested viruses, tested cell lines, year, and reference.

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antiviral action against HSV-1 with log reduction of 1.0−1.7 plaque forming units (PFU).[67] However, this impression material containing the disinfectant did not show any inhibition activity against PV-1. A complex of an alginate with rhamnolipid biosurfactant PS-17 exhibited inhibition capacity against HSV-1 and HSV-2.[68] In the field of dentistry, an alginate formulation with MgO has shown that pH changes through modification of magnesium ion concentration provide inhibitory action against HSV-1.[69] Finally, in the field of alginate-based composite hydrogels, an advanced hydrogel of calcium alginate−lentinan−amino-oligosaccharide (ALA) was produced by coating the surface of a calcium alginate hydrogel loaded with lentinan (AL) with amino-oligosaccharide by electrostatic action as an alternative strategy to traditional pesticides for controlling viral diseases in plants.[63] The ALA hydrogel continuously induced strong plant resistance to the TMV and significantly increased the release of Ca^{2+} to promote plant growth, particularly that of *Nicotiana benthamiana*. Lentinan (LNT) is a neutral polysaccharide pesticide capable of inactivating bacteria, fungi, and the TMV.[70,71] In the field of nanocomposite materials, alginate-based nanocomposite films produced with a low content (0.1% *w*/*w*) of carbon nanofibers (CNFs) have been studied very recently in terms of antiviral activity against the T4 coliphage viral model.[72] The results of the study showed that calcium alginate possesses antiviral activity against this nonenveloped virus and its inhibition capacity can be increased with the addition of the low percentage of CNFs. A previous study reported that incorporating this small amount of CNFs into calcium alginate films provided antibacterial activity against the life-threatening methicillin-resistant *Staphylococcus epidermidis*.[73] In addition, these nanocomposites showed enhanced physical properties such as mechanical properties, water diffusion and wettability, transparency, and similar biomedical behavior to that of pristine calcium alginate in terms of nontoxicity and cell adhesion.[22,64,74] The studies found in this review of the antiviral properties of alginate-based materials are summarized in Table 1.

3. ALGINATE-BASED MATERIALS AGAINST VIRUSES

In this review, 21 published papers were selected as studies of alginate-based materials and their antiviral activity. However, several studies of them have analyzed the antiviral properties of alginate-based materials against various viruses in the same study. These 21 papers thus contained a total of 32 studies of 18 different viruses, as shown in Figure 5.

As can be seen in Figure 5, the antiviral properties of alginate against HIV-1[60,76,77] and HSV-1[51−53,61,67−69] are the most frequently studied, followed by HCV,[60,61] HSV-2,[50,68] TMV,[63,75] PV-1,[61,67] and MNV and HAV.[54,59] Alginate-based materials have also been tested against HBV,[60] SINV,[61] RAV,[46] PVX,[48] IFV,[62] VSV,[49] HTLV-1,[60] RV,[47] T4,[72] and HIV-1(VSV).[60] Of these 32 studies, 25 showed viral inhibition, which suggest that alginate-based materials have significant potential as alternative antiviral agents. The viruses tested in contact with alginate-based materials are shown in Table 2 with all of their characteristics such as genus, family, type according to the Baltimore classification,[79] whether enveloped or not, viral affection, and disease/action.

Table 2 shows that there are nine positive-sense single-stranded RNA viruses belonging to the same Baltimore group IV as the new SARS-CoV-2 coronavirus, which have been studied against alginate-based materials. Four of these nine viruses (HIV-1, RV, HCV, SINV) are also enveloped like SARS-CoV-2, and most of the studies performed in contact with alginate-based materials have shown viral inhibition capacity against them (see Table 1 and Figure 5). These preliminary studies indicate that the use of alginate-based materials for the treatment and prevention of the SARS-CoV-2 pathogen seems to be a very promising strategy. However, further alginate-based material research focused on this direction is necessary to confirm these results.

4. ANTIVIRAL MODE OF ACTION OF ALGINATE-BASED MATERIALS

The mechanism of action of alginate-based materials is uncertain.[75] However, results obtained with alginic acid showed that the antiviral mechanism of this compound can be attributed to the capacity of this anionic biopolymer to bind to RAV viral envelopes.[46] Thus, alginic acid interfered with the initial stage of the RAV infection in CER cells (i.e., viral adsorption) and showed 50% inhibition of the nucleocapsid synthetic process.[46] Although the results obtained with this assay were insufficient to draw conclusions about the effect of the polymer structure on their antiviral activity, the researchers speculated that anionic polysaccharides such as alginates could increase the negative charge of the viral envelope glycosylated G protein and the ionic receptor sites of eukaryotic cells, which were also negatively charged. Furthermore, SA exhibited a strong inhibitory effect against TMV.[75] When an alginate was added to the inoculum mixture, the quantity of lesions observed on *Xanthi tobacco* leaves was significantly reduced and the inhibition effect improved as the alginate concentration rose (see Figure 6), being greater when the alginate had a lower M/G ratio of 0.41.[75] These results suggest that viral inhibition depends on the mechanical properties of the biopolymer chain.[80,81]

It was also observed under electron microscopy that adding an alginate to the TMV suspension caused the viral particles to be in the form of great raft-like aggregates, which may be the reason behind alginate's effect on infectivity.[75] The alginate's antiviral activity, which increased with M_W, could be related to the blocking of the decapsulation of the TMV protein on the cell membrane surface. In good agreement with these results, Pardee et al. reported that alginate extracted from *Fucus gardneri* was capable of inhibiting PVX (>95%), and the electron micrographs showed also that the mode of viral inhibition could be attributed to viral aggregation.[48] In that study, many extracts from marine algae were tested against PVX, but only those obtained from *Fucus gardineri* completely inhibited local lesions on Chenopodium quinoa at 10 μg/mL and even showed an antiviral effect at 1 μg/mL (94% ± 3%). These results were consistent with those reported by Sano et al.[75] who indicated that the antiviral mode of action could be related to this aggregation that decreased the functional content of viral particles in solution or interfered with viral uncoating during infection. On the other hand, sulfated polymannuroguluronate (SPMG) inhibited the binding between the HIV-1 receptor in the human body, CD4+ T lymphocytes, and the envelop glycoprotein 120 (gp120), which is very critical in the initiation of the viral entry process of this RNA virus into the lymphocytes.[76]

An analysis of the potential targets for SPMG regarding the inhibition of the entry process showed that SPMG mainly linked to gp120 through the V3 loop region within the molecule, but also that

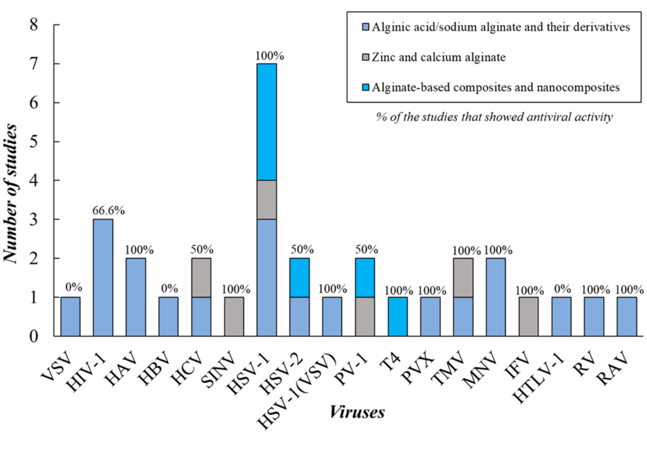

Figure 5. Studies of alginate-based materials against viruses indicating the percentage of studies that showed antiviral activity. The results are classified according to the type of alginate-based materials: alginic acid/sodium alginate and their derivatives, zinc and calcium alginate, alginate-based composites and nanocomposites shown in Table 1.

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Table 2. Information on Viruses Tested in Contact with Alginate-Based Materials[a]

virus name	abbreviation	genus	family	type, Baltimore group	enveloped	infects	disease/action	ref
Human immunodeficiency virus type 1	HIV-1	Lentivirus	Retroviridae	IV ((+)ssRNA)	Yes	Humans	AIDS	60, 76, 77
Hepatitis A virus	HAV	Hepatovirus	Picornaviridae	IV ((+)ssRNA	No	Humans	Hepatitis A	54
Hepatitis B virus	HBV	Orthohepadnavirus	Hepadnaviridae	I (dsDNA)	Yes	Humans	Hepatitis B	60
Hepatitis C virus	HCV	Hepacivirus	Flaviviridae	IV ((+)ssRNA)	Yes	Humans	Hepatitis C	60, 61
Sindbis virus	SINV	Alphavirus	Togaviridae	IV ((+)ssRNA)	Yes	Humans	Sindbis fever	61
Herpes simplex virus type 1	HSV-1	Simplexvirus.	Herpesviridae	I (dsDNA)	Yes	Humans	Herpetic disease	51−53, 61, 67−69
Herpes simplex virus type 2	HSV-2	Simplexvirus	Herpesviridae	I (dsDNA)	Yes	Humans	Genital ulcer disease	50, 68
Poliovirus type 1	PV-1	Enterovirus	Picornaviridae	IV ((+)ssRNA)	No	Humans	Polio	61, 67
Rabies virus	RAV	Lyssavirus	Rhabdoviridae	V ((−)ssRNA)	Yes	Humans and animals	Rabies	46
Potato virus X	PVX	Potexvirus.	Alphaflexiviridae	IV ((+)ssRNA)	No	Potatos	Mild or no symptoms	48
Tobacco mosaic virus	TMV	Tobamovirus	Virgaviridae	IV ((+)ssRNA)	No	Tobacco and *Solanaceae*	TVX	63, 75
Murine norovirus	MNV	Norovirus	Caliciviridae	IV ((+)ssRNA)	No	Mice	Gastroenteritis	54
Influenza virus	IFV	Not specified	Orthomyxoviridae	V ((−)ssRNA)	Yes	Humans and animals	Flu	62
T4 macrophage	T4	Tequatrovirus	Myoviridae	I (dsDNA)	No	*Escherichia coli*	Replication in *E. coli*	72
Vesicular stomatitis virus	VSV	Vesiculovirus	Rhabdoviridae	V ((−)ssRNA	Yes	Humans	flu-like illness	49
Human T-cell leukemia virus type-1	HTLV-1	deltaretrovirus	Retroviridae	VI (ssRNA-RT)	Yes	Humans	ATL, HTLV-1-associated myelopathy, uveitis and others	60
Rubella virus	RV	Rubivirus	Matonaviridae	IV ((+)ssRNA)	Yes	Humans	Rubella	47
Severe acute respiratory syndrome coronavirus 2	SARS-CoV-2	Betacoronavirus	Coronaviridae	IV ((+)ssRNA)	Yes	Humans	COVID-19	Not studied

[a]Virus name, abbreviation, genus, family, type according to the Baltimore classification[79] (dsDNA: double-stranded DNA virus; (+)ssRNA: positive-sense single-stranded RNA viruses ; (−)ssRNA: negative-sense single-stranded RNA viruses; ssRNA-RT: single-stranded RNA viruses with a DNA intermediate in their life cycle), enveloped virus or not, spectrum of infection, disease/action, and references. Information on the SARS-CoV-2 is also included in this table as a comparative reference.

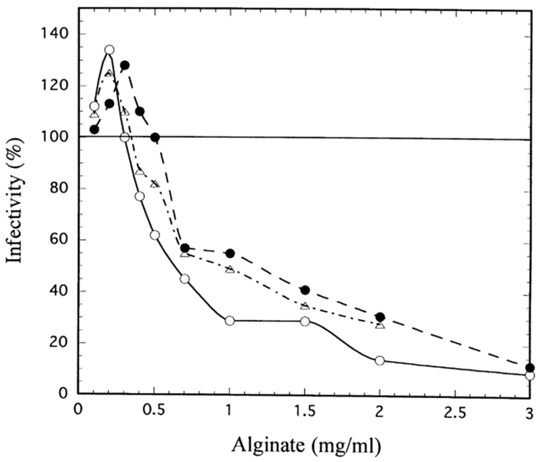

Figure 6. Infectivity in % of sodium alginate (Alg 500G) with different M/G ratios: 0.41 (solid line), 0.8 (chain line), and 1.05 (dotted line). Reproduced with permission from ref 75. Copyright 1999 Elsevier.

the SPMG could bind to gp120 through other sites of the protein.[76] In fact, the V3 loop located within gp120 is a highly charged region of the protein and has been shown to attract anionic molecules.[82,83] The surface plasmon resonance (SPR) assay showed that one SPMG molecule bound to three to four 28-amino acid peptides within the V3 loop with high affinity, which was also demonstrated by the digital docking of the SPMG octasaccharide backbone and the V3 loop region (Figure 7).

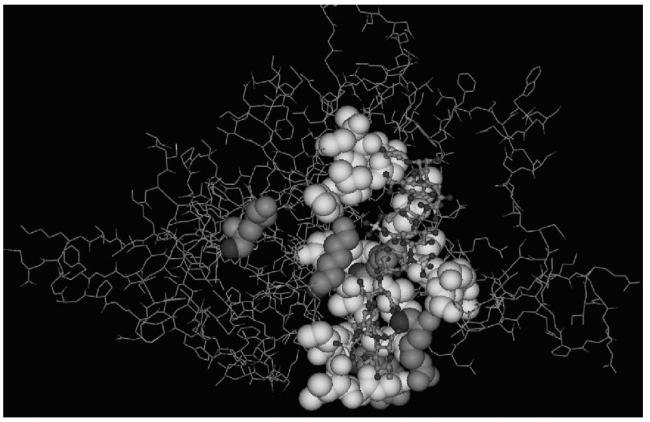

Figure 7. V3 loop of gp120 with the octasaccharide unit of SPMG backbone: computer docking modeling. The binding was mainly attributed to the electrostatic force. Reproduced with permission from ref 76. Copyright 2003 Elsevier.

It was also shown that SPMG significantly reduced the vulnerability of PC12 cells to HIV Tat protein by protecting these cells.[77]

The antiviral studies performed with guluronic acid-rich SA (26 ± 5 kDa) suggested that the antiviral activities of these biomolecules were exerted directly by interfering with anti-HSV virion envelope structures or masking viral structures, which are required for cell adsorption thus blocking viral entry[52] as had been observed previously for diverse compounds,[84,85] although further clarification is required. The antiviral activity of SA (B) isolated from *Sphacelaria indica*,

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composed of 41% G and 59% M blocks, and two sulfated versions (BS1 and BS2) was tested against HSV-1 and their antiviral mechanism were investigated in cells pretreated with these compounds before infection.[51] The HSV-1 were incubated with acyclovir both before and after infection to determine alginate's inhibitory effect during different stages of the replication cycle (Figure 8).

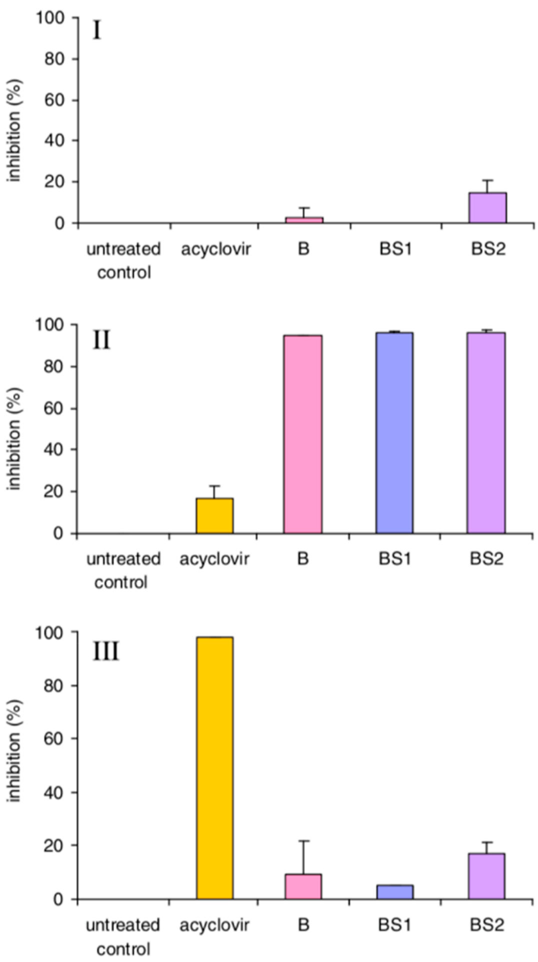

Figure 8. Inhibitory effect of sodium alginate (B) and its sulfated derivatives (BS1 and BS2), and acyclovir against HSV-1 through different stages of the viral replication cycle: (I) treated cells before infection; (II) HSV-1 preincubated with the aforementioned molecules before cell infection; (III) HSV-1 infected cells incubated for 3 days during viral replication with the molecules. Reproduced in part with permission from ref 51. Copyright 2011 Elsevier.

Figure 8I shows that there was no significant effect when the cells were treated with alginates before infection, while pretreating HSV-1 with different alginate types before infection dramatically inhibited plaque formation in all the alginate-based materials (Figure 8II). However, while none of the alginate molecules had a significant antiviral effect, as expected, acyclovir inhibited viral replication by 98.6% since it inhibits viral DNA as it is synthesized (Figure 8III). These results indicate that the alginates' antiviral activity against HSV-1 is produced by interference with virions or covering the viral parts needed for the viral entry into the host cells, as has been previously reported for a wide range of chemical compounds.[77,84,85] Sulfate content has been shown to affect the antiviral activity of the polysaccharides.[51] Thus, the incorporation of sulfate groups improved the macromolecules capability to inhibit HSV-1.[53] The results of a study of anti-RV alginic acid indicated that the inhibitory capacity of the compounds occurred during the early steps of RV replication.[47] Thus, these compounds blocked virus replication in the internalization or uncoating, which is a step subsequent to virus attachment. Calcium alginate microspheres showed capacity against other enveloped positive-sense single-stranded RNA viruses such as HCV and SINV in a dose- and incubation time-dependent manner that depended on chemical interactions between the gel and the virions.[61] Encapsulating HuH-7 cells prior to HCV infection and encapsulating previously infected cells did not produce any infectious HCV particles due to HCV's inability to enter the encapsulated cells. These results indicate that the negative charge density of calcium alginate gels may interact with components within the viral envelope inhibiting membrane receptors. However, an alginate oligomer showed no antiviral activity against HIV-1, HBV, HCV, HTLV-1, and HTLV-1 in its replication cycle.[60] Although, in this study, the alginate oligomer exhibited antiviral activity against VSV-G-pseudotyped HIV-1 in HeLa cells.

5. TOXICOLOGICAL ASPECTS OF ALGINATE-BASED MATERIALS

Most studies have reported that alginate-based materials are noncytotoxic. However, alginate and its related materials may exert cytotoxicity effects on host cells and in fact must be purified for certain biomedical applications such as cell encapsulation.[86] Nevertheless, we focused our attention on the toxicological studies performed with the alginate-based materials presented here that were tested against the 17 types of viruses analyzed in this review (Table 1). Thus, most of the studies shown in this review exhibited very low or did not provide any cytotoxicity of the alginate-based materials (see Table 1). Thus, a study showed that anti-RV alginic acid did not exhibit any cytotoxic effect at a concentration of 1 mg/mL in Vero cells.[47] However, another study of alginic acid showed effective antiviral activity against RAV at concentrations below the cytotoxicity threshold (CC_{50} = 400 μg/mL).[46] Antiviral alginate against PVX showed no indication of toxicity at a concentration up to 33 μg/mL.[48] Furthermore, an anti-HSV-1 guluronic acid-rich alginate derived from Sargassum *tenerrimum* lacked cytotoxicity at concentrations up to 1 mg/mL.[52] A sodium alginate with an M/G ratio of 1.44 and two sulfated versions inhibited HSV-1 in a dose-dependent manner with an IC_{50} of 10, 0,65 and 0,6 μg/mL, respectively, and a 50% toxic concentration (TC_{50}) ≥ 1000 μg/mL.[51]

Another study showed that anti-HSV-1 alginic acid also did not exhibit cytotoxicity at concentrations up to 1 g/mL.[53] In addition, alginate oligomers showed no cytotoxicity in MT-4 cells in a concentration up to 400 μg/mL.[60]

Zinc alginate can induce high toxicity due to the release of Zn^{2+} cations.[65,66] However, zinc alginate can be toxic for human cells depending on time and concentration.[24] Anti-IFV calcium or zinc alginate fibers showed good cellular biocompatibility and thus nontoxicity in African Green Monkey kidney cells (Vero) and human cervical cancer cells (Hela).[62] Anti-TMV calcium alginate−lentinan−amino-oligosaccharide hydrogel as pesticide carrier showed high safety to organisms because no fish died in these hydrogels in the toxicological study and promoted plant growth.[63] Calcium alginate with and without CNFs showed significant antiviral capacity and noncytotoxicity in human keratinocyte HaCaT cells (Figure 9).[72]

The anti-HSV-1 and anti-HSV-2 complex of alginate with rhamnolipid biosurfactant PS-17 exhibited maximal tolerated concentrations (MTC) of 640 μg/mL in contrast to 64 μg/mL for purified rhamnolipid PS-17 in the *in vitro* cytotoxicity tests performed in monolayer MDBK cell cultures.[68]

6. CONCLUSIONS

This review has demonstrated that alginate-based materials have antiviral activity against a wide range of 17 types of viruses, which are double-stranded DNA virus, positive-sense or negative-sense single-stranded RNA viruses, or single-stranded RNA viruses with DNA intermediate in their life cycle. These viruses can infect different organisms: humans by

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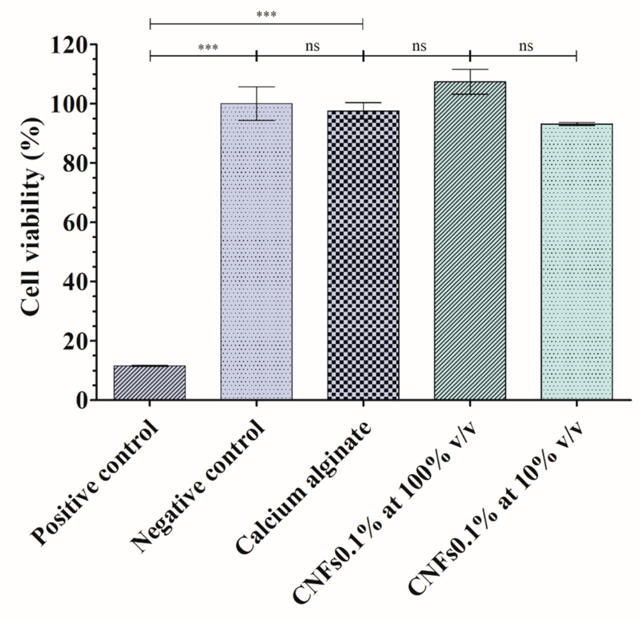

Figure 9. Cell viability (%) of human keratinocyte HaCaT cells: extract of the calcium alginate film, extract of the calcium alginate/CNFs film at two concentrations (100% and 10% *v*/*v*), negative control (culture medium), and positive control (toxic concentration of zinc chloride at 1000 μM). *** $p > 0.001$; ns: not significant. Reproduced with permission under a Creative Commons CC BY 4.0 License from ref 72. Copyright 2021 MDPI.

human immunodeficiency virus type 1, the hepatitis A, B, and C viruses, Sindbis virus, herpes simplex virus type 1 and 2, poliovirus type 1, rabies virus, rubella virus, and the influenza virus; mice by murine norovirus; bacteria by T4 coliphage; and plants by tobacco mosaic virus and the potato virus X. Many of these viruses are enveloped viruses that belongs to the same Baltimore group IV as SARS-CoV-2, which shows great promise for this family of materials in the treatment of the currently rapidly evolving COVID-19 disease. In addition, when the toxicity of these materials have been tested, it has shown to be very low or negligible. The antiviral mode of action is mainly attributed to viral aggregation and viral inhibition through interaction of alginate-based materials with components of the viral envelope. Therefore, these previous studies open future research lines in the area of alginate-based biomaterials with antiviral properties against SARS-CoV-2 and other clinically relevant viral pathogens.

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Author Contributions

Á.S.-A. conceived the idea of this work, wrote the manuscript, and conducted major editing and proof-reading. M.F.-M. helped to find information and figures of this review. R.W. edited and proof-read the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

Δlog CCID50, difference between virus titers of drug-treated sample and drug-free control sample
AAD, adipic acid dihydrazide
ACLSV, apple chlorotic leaf spot virus
AIDS, acquired immunodeficiency syndrome
AL, calcium alginate−lentinan drug-loaded
ALA, calcium alginate−lentinan−amino-oligosaccharide
Alg, alginate
ALV, alginate-lipid vesicle
ALTV, alginate-lipid-tremella vesicle
A-OA-GTE, alginate-oleic acid-green tea extract films
ASGV, apple stem grooving virus
ASPV, apple stem pitting virus
ATL, adult T-cell lymphoma
AV, alginate vesicle
BMSCs, bone marrow stem cells
BSA, bovine serum albumin
Ca^{2+}, calcium ion
$CaCl_2$, calcium chloride
$CaCO_3$, calcium carbonate
$CaSO_4$, calcium sulfate
CC_{50}, cytotoxic concentration
CER cells, chicken embryo-related cells
CNFs, carbon nanofibers
COVID-19, Coronavirus disease 2019
dsDNA, double-stranded DNA virus
EB/AO, ethidium bromide/acridine orange
EC_{50}, half maximal effective antiviral concentration
ED_{50}, effective dose to protect 50% of Wish cell monolayer from cytopathic effect
FRhK-4, monkey kidney cells
G, α-L-glucuronic acid
Gp120, envelop glycoprotein 120
GSE, grape seed extract
GTE, green tea extract
HAD, HIV-associated dementia
HAd5, human adenovirus type 5
HAV, hepatitis A virus
HBV, hepatitis B virus
HCV, hepatitis C virus
H9, human embryonic stem cell line
HeLa cells, human cervical cancer cells
HepG2. 2.15, human hepatoblastoma cell line

HIV-1, human immunodeficiency virus type 1
HSV-1, herpes simplex virus type 1
HIV-1(VSV), VSV-G-pseudotyped HIV-1
HSV-2, herpes simplex virus type 2
HTLV-1, human T-cell leukemia virus type-1
HuH-7 cells, human liver cell line
IC_{50}, half-maximal inhibitory compound concentration
IFN-1, interferon type 1
IFV, influenza virus
IgG, immunoglobulin G
IgM, immunoglobulin M
IHNV, infectious hematopoietic necrosis virus
IHNV G, infectious hematopoietic necrosis virus glycoprotein
IPN, interpenetrating polymer network
KD, dissociation constant
LNT, lentinan
M, (1−4)-β-D-mannuronic acid
MDBK, Madin-Darby bovine kidney cell line
M/G ratio, mannuronate to guluronate ratio
MIC_{50}, minimal inhibitory concentration of compound (μg/mL) required to inhibit fluorescence by 50%
MNV, murine norovirus
MSCs, mesenchymal stem cells
MT4, human cutaneous T-lymphocyte
MTC, maximal tolerated concentrations
M_W, molecular weight
Na^{2+}, sodium ion
NaCl, sodium chloride
NaOH, sodium hydroxide
NIPAAm, *N*-isopropylacrylamide
PAG, poly(aldehyde guluronate)
PAH, poly(acrylamide-*co*-hydrazide)
PCR, polymerase chain reaction
PEG, poly(ethylene glycol)
PEG-*co*-PCL, poly(ethylene glycol)-*co*-poly(ε-caprolactone)
PG, polyguluronate
PNIPAAm, poly(*N*-isopropylacrylamide)
PVX, potato virus X
PV-1, poliovirus type 1
qPCR, quantitative real-time polymerase chain reaction
RAW 264.7, murine macrophage cells
RC-37 cells, African green monkey kidney cells
RGD, cellular recognition peptide arginine-glycine-aspartic acid
RAV, rabies virus
RV, rubella virus
SA, sodium alginate
Semi-IPN, semi-interpenetrating polymer network
SI, selectivity index
s-IgA, intestinal secretory immunoglobulin A
SINV, sindbis virus
SPMG, sulfated polymannuroguluronate
SPR, surface plasmon resonance
(+)ssRNA, positive-sense single-stranded RNA
(−)ssRNA, negative-sense single-stranded RNA
ssRNA-RT, single-stranded RNA viruses with a DNA intermediate in their life cycle
Tat, transactivator of transcription protein
T4, T4 coliphage
TC_{50}, 50% toxic concentration
$TCID_{50}$, 50% tissue culture infectious dose
TI, therapeutic index
TMV, tobacco mosaic virus
UV, ultraviolet radiation
Vero cells, African green monkey cells
VSV, vesicular stomatitis virus

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