Polymers and Biopolymers with Antiviral Activity: Potential Applications for Improving Food Safety

(Review)

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<td>TCID$_{50}$</td>
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Abstract

Gastroenteritis and hepatitis, caused by human noroviruses (HuNoVs) and hepatitis A virus (HAV), respectively, are the most common illnesses resulting from the consumption of food contaminated with human enteric viruses. Food-grade polymers can be tailor designed to improve food safety, either as novel food-packaging materials imparting active antimicrobial properties, applied in food contact surfaces to avoid cross-contamination, or as edible coatings to increase fresh produce’s shelf life. The incorporation of antimicrobial agents into food-grade polymers can be used to control the food microbiota and even target specific foodborne pathogens to improve microbiological food safety and to enhance food quality. Enteric viruses are responsible for one fifth of acute gastroenteritis cases worldwide and the development of food-grade polymers and biopolymers with antiviral activity for food applications is a topic of increased interest, both for academia and the food industry, even though developments are still limited. This review compiles existing studies in this widely unexplored area and highlights the potential of these developments to improve viral food safety.
Introduction

Foodborne pathogens are a matter of increasing concern for consumers, industries and public institutions (WHO, 2015). Food, in fact, represents a vehicle of disease transmission caused by a wide range of pathogenic microorganisms, most notably pathogenic bacteria and enteric viruses (EFSA, 2016). Despite accounting for the major causes of foodborne outbreaks in high-income countries (EFSA, 2015; Shah et al., 2017), human enteric viruses have received comparatively less attention than other foodborne pathogens, such as Salmonella, Listeria, Escherichia coli, or Campylobacter. To date, almost 150 different types of human enteric viruses are known, which cause a variety of illnesses in humans, mainly gastroenteritis (such as human noroviruses (HuNoVs), sapoviruses, rotavirus, and astroviruses). They may also cause diverse additional disorders, such as hepatitis caused by hepatitis A virus (HAV) and hepatitis E virus (HEV), poliomyelitis (poliovirus), or meningitis (enteroviruses), even if reported to a lesser extent. In addition, they confer a high risk of morbidity and mortality in vulnerable populations, such as immunocompromised patients, children, the elderly and pregnant women (Rodríguez-Lázaro et al., 2012). Among them, HuNoVs and HAV have been determined to be the viruses of greatest concern from a food safety perspective (EFSA, 2016). Additionally, HEV has recently been identified as an emerging pathogen in Europe due to its potential for zoonotic transmission through the consumption of pork meat products (EFSA, 2011a, 2017).

Globally, foodborne hazards cause approximately 600 million illnesses annually, mainly due to infectious agents causing diarrheal diseases (550 million), with HuNoVs being responsible for 120 million cases attributed to water and food (WHO, 2015). Although the incidence rate of HAV infection has been on the decline in high-income countries, in part due to immunization availability, high-profile outbreaks continue to be reported,
with 14 million cases and over 28,000 deaths attributed to food and waterborne hepatitis
A worldwide every year (WHO, 2015).
Since these viral pathogens are mainly transmitted by the fecal-oral route, contaminated
food products, such as shellfish, leafy greens, and berries, are the main food products
associated with viral foodborne outbreaks. Contamination by food handlers or cross-
contamination through contaminated surfaces is mainly associated with ready-to-eat
products, such as salads and, bakery or delicatessen items, which are prepared or
handled raw or after the foods have been cooked. Contamination can also occur during
production, as for shellfish, usually harvested from waters affected by the discharge of
treated and untreated sewage (McLeod, Polo, Le Saux, & Le Guyader, 2017; Nappier,
Graczyk, & Schwab, 2008) or berries and leafy greens, contaminated in the fields by
pickers or through polluted irrigation waters (Le Guyader et al., 2004; López-Gálvez et
al., 2016; Randazzo, López-Gálvez, Allende, Aznar, & Sánchez, 2016), posing a higher
health risk because both foodstuffs are frequently eaten raw (Ethelberg et al., 2010). For
HuNoV outbreaks, shellfish represents the most commonly identified food vehicle in
Europe (EFSA, 2016), while leafy greens and fruits are the most frequently associated
foods in the United States (Hall, Wikswo, Pringle, Gould, & Parashar, 2014). In
addition, different studies have reported the transfer of viruses from the environment to
foods during preparation and handling (Kotwal & Cannon, 2014; Rönqvist et al.,
2014). In this sense, it is worth to report that recently a standard method has been
established for the quantification of HAV and HuNoVs in several foodstuffs (soft fruit,
leaf, stem and bulb vegetables, bottled water, bivalve molluscan shellfish) or food
surfaces. The specifications include concentration procedures, RNA extraction and
target sequences to be detected by RT-qPCR (ISO 15216-1:2017, 2017).
The low infectious dose of most human enteric viruses (Atmar et al., 2014; Teunis et al., 2008), together with their high stability in the environment, make them extremely infectious and highly transmissible. As non-enveloped viruses, human enteric viruses tend to be more resistant to inactivation than foodborne bacteria to commonly used food manufacturing processes (Sánchez, 2015). Overall, mild food manufacturing processes show only marginal effects on the viral load, but when the processes are combined, the synergistic effects may enhance the level of human enteric virus inactivation (Kim, Lee, Kim, & Park, 2015; Seo, Lee, Lim, & Ko, 2012). An interesting future approach might be research into the effect of combining food processes on human enteric viruses.

In such a scenario, although the prevention of viral contamination by good hygienic, agricultural, and manufacturing practices remains the main strategy in pursuit (Codex Alimentarius, 2012), the use of polymers and biopolymers to develop antiviral materials with potential in food applications is envisaged as a promising alternative, which could help to avoid cross and/or recontaminations in line with the principles of the hurdle technology.

When browsing the extensive literature on antimicrobial materials, it is clear that biomedical applications dominate over food applications. In the food field, active packaging is an innovative solution to meet the consumer demands of fresh ready-to-eat food products together with the market trends of current food global trade, as it extends the shelf life and improves the safety of the food (López-Rubio et al., 2004). Many studies have successfully used active packaging to control foodborne pathogenic bacteria by applying of different antimicrobials, such as metals, chemicals, different essential oils, enzymes, and bacteriocins (Brandelli, Brum, & dos Santos, 2017; Maisanaba et al., 2017; Sardarodiyan & Mahdian, 2016). Additionally, some food-
packaging materials with antimicrobial activity are commercially available, such as Zeomic\textsuperscript{TM}, AgIon\textsuperscript{TM}, Novaron\textsuperscript{TM} and Cleanaid\textsuperscript{TM} (Sung et al., 2013). Despite the extensive research carried out to evaluate the efficacy of antimicrobial packaging on foodborne pathogenic bacteria (Brandelli et al., 2017; Maisanaba et al., 2017; Sardarodiyan & Mahdian, 2016) and molds (Nguyen Van Long, Joly, & Dantigny, 2016), only limited information is available for their use against human enteric viruses (Table 1).

This review analyzes the published literature on the current state of knowledge regarding the development of polymers and biopolymers with antiviral activity for food applications and the future perspectives of their application to enhance microbial food safety.

**Polymers and biopolymers as carriers of active compounds**

In the last decades, the study of polymers of interest for food-related applications has mainly aimed at improving the technological performance of packaging materials to extend the shelf life of the food products and improve food safety. However, their use has been customized providing them an active role for inhibiting the growth of spoilage and pathogenic foodborne bacteria and, more recently, of human enteric viruses, thus imparting antibacterial and antiviral properties of interest for food safety purposes. This can be achieved by temporarily trapping biocidal substances within the polymer (Su et al., 2015) or by covalent bonding (Thomassin, Lenoir, Riga, Jerome, & Detrembleur, 2007). In such a context, the stability and/or release of the active compounds incorporated, which is based on their interactions with the polymeric material and on the environmental conditions of its application (pH, temperature, relative humidity, etc.), are key for guaranteeing their efficacy.
The large variety of materials and compositions available and the possibility of blending, chemically modifying them, or using nanotechnology tools to improve their performance have made polymers and biopolymers the materials of choice as carriers of active compounds for this type of application (López-Rubio, Gavara, & Lagaron, 2006). Albeit there are several factors that may affect the stability and/or release kinetics, including the inherent characteristics of the active compounds to be incorporated (such as molecular weight, polarity, or initial concentration) (Hu, Chen, & Wang, 2012; Suppakul, Sonneveld, Bigger, & Miltz, 2011), the chemical composition of the packaged food product (Gherardi, Becerril, Nerin, & Bosetti, 2016; Han, Castell-Perez, & Moreira, 2008), or the ambient conditions (Chalier, Ben Arfa, Guillard, & Gontard, 2009; Chen, Wang, Hu, & Wang, 2012; Han et al., 2008; Kurek, Guinault, Voilley, Galić, & Debeaufort, 2014), the polymeric/biopolymeric materials themselves play a key role in the design of novel antiviral materials.

Several factors need to be considered when designing materials with antiviral properties. The main one is the intended application of this material, as synthetic and bio-based plastics like polypropylene (PP) or polyhydroxyalkanoates (PHAs) may be used if the material is to be used as a food contact surface or food-packaging material, while natural biopolymers like polysaccharides (starch, chitosan, cellulose, etc.), proteins (soy protein, zein, etc.) and lipids (such as beeswax) with “generally recognized as safe” (GRAS) status should be selected for applications as edible coatings on fresh food products.

The most relevant factors to be considered for material selection and design can be divided into the following:

(i) Intrinsic material characteristics: these include the chemical composition and polarity (which will determine their compatibility with the active
compounds) and molecular weight (Mw), which is not usually considered but it has been reported to affect release properties (Fernández-Pan, Maté, Gardrat, & Coma, 2015; Lavin, Zhang, Furtado, Hopkins, & Mathiowitz, 2013).

(ii) Processing conditions: the processing conditions used for material development will have a direct impact on several relevant properties, such as crystallinity, thermal properties, thickness, or porosity. All these factors will affect the stability and/or release properties of the biocidal compounds incorporated although very few studies exist to date that take them into account (Efrati et al., 2014).

All the knowledge gained from the large number of existing studies on antimicrobial polymers can be leveraged for the development of antiviral materials for food applications, through the incorporation of compounds with proven antiviral properties within the polymeric/biopolymeric structures. However, it should be mentioned that the practical application of these materials as effective biocidal carriers in the food industry has been limited for many reasons, including the degradation of the bioactive agents, or their quick release from the materials (Campos, Gerschenson, & Flores, 2011). This again denotes the need to understand how the structural characteristics of the materials developed affect both the stability and release (if the substances are not immobilized) of the active compounds.

Although in the following sections specific examples about the strategies followed for the development of different types of antiviral materials are given, a general trend in this area is related to taking advantage of the inherent characteristics of different biopolymers to foster their interactions with the active compounds to control the release properties (Mascheroni, Capretti, Limbo, & Piergiovanni, 2012; Tawakkal, Cran, &
Processing methods have also been adapted to improve biocidal activity by making use of nanotechnology tools, either to protect the bioactive compounds, through micro- or nanoencapsulation (Gómez-Mascaraque, Sánchez, & Lopez-Rubio, 2016), or to develop multilayered/nanolaminate delivery systems with improved performance (Aloui & Khwaldia, 2016; Castro-Mayorga et al., 2017). In the specific case of edible coatings for fresh food products, nanoemulsions are being implemented, as they can be formulated with natural food-grade ingredients and their production process is easily scalable in the industry by high-pressure homogenization process (Donsì, Annunziata, Sessa, & Ferrari, 2011). The layer-by-layer methodology has also received great interest as a new tool to create multilayer nanocoatings to extend the shelf life of perishable foods (Fabra, Flores-López, et al., 2016; Mantilla, Castell-Perez, Gomes, & Moreira, 2013; Moreira et al., 2014; Sipahi, Castell-Perez, Moreira, Gomes, & Castillo, 2013).

Recent developments in active food packaging include the use of active labels, surface modification, or incorporation of the biocide agents either included in coatings or in the adhesives of multilayer structures (Akrami et al., 2015; Gherardi et al., 2016; Han et al., 2008; Narayanan, Neera, Mallesha, & Ramana, 2013; Otero et al., 2014). These systems can be used as a starting point for the implementation of food materials with antiviral properties.

**Methodologies applied to assess the antiviral activity of polymeric and biopolymeric materials for food applications**

Most studies to determine the antiviral activity of polymeric and biopolymeric materials have been performed by artificially adding a known amount of a virus to a given material, determining the reduction in the infectious titer after subjecting the spiked material to designated conditions and applying statistical procedures to determine the significance of virus decay (Fig. 1). Evidently, this implies the use of virus strains that...
can be propagated in cell culture and enumerated through infectivity, thus greatly restricting the range of strains that are able to be included in these studies due to the difficulties in developing *in vitro* cultivation systems to replicate the most relevant human enteric viruses, such as HuNoV or wild-type HAV strains. Virus detection by cell culture is mainly based on the formation of cytopathic effects, followed by the quantification of the viruses by plaque assay, the most probable number, or tissue culture infectious dose 50 (TCID$_{50}$). In this sense, a promising *in vitro* cultivation system for HuNoVs using stem cell–derived human enteroids has been recently developed, but there are limitations that need to be overcome before it can be routinely used (Ettayebi et al., 2016). Therefore, the infectivity of HuNoVs has been mainly inferred through cultivable surrogates, such as feline calicivirus (FCV), murine norovirus (MNV) and, more recently, Tulane virus (TV) (Hirneisen & Kniel, 2013). Even though these animal viruses have largely been used to study the survival rate of HuNoVs exposed to different inactivation processes, the appropriateness of such surrogates as models still raises questions and need to be confirmed (Bae & Schwab, 2008; NACMCF, 2016).

Additionally, the wide range of applications of antiviral materials hampers the development of a standard methodology. To date, the absence of a specific and official regulation to evaluate the antiviral activity of active polymeric materials for food applications makes it difficult to compare the results of the assays of different studies. To overcome this lack, standard protocols developed for bacteria are often modified and adjusted to the purposes and circumstances of the antiviral assessment (Fig. 1). In fact, the antiviral experimental trials commonly apply well-established procedures aimed to assess the antimicrobial activity on plastics, and other non-porous surfaces, against bacteria and molds, such as the ISO 22196:2011 and the Japanese Industrial Standard
JIS Z 2801:2000 (Castro-Mayorga et al., 2017; Fabra, Castro-Mayorga, et al., 2016; Martínez-Abad, Ocio, Lagarón, & Sánchez, 2013). Other protocols have been also applied that differ in the inoculation, exposure, and recovery techniques adopted. For example, Amankwaah (2013) determined the virucidal activity of GTE films by placing them in the bottom of 6-well plates and filling the well with the virus suspension. After exposure, virus suspensions were recovered and titrated (Amankwaah, 2013; Haldar, An, Álvarez de Cienfuegos, Chen, & Klibanov, 2006). Similarly, Warnes and Keevil (2013) applied a previously proposed protocol optimized for bacteria (Warnes, Green, Michels, & Keevil, 2010) to assess the antiviral activity of copper coupons. In this work, virus recovery was performed with the help of glass beads, commonly used for bacteria.

As a general procedure applied for antiviral polymeric materials, the evaluation considers a viral inoculation step onto the material, a waiting/contact time while the active compound in the polymeric material exerts its expected antiviral activity, and, finally, a recovery step with a neutralizer solution to stop the inhibiting action or by swabbing (Bright, Sicairos-Ruelas, Gundy, & Gerba, 2008; Martínez-Abad et al., 2013). For comparative purposes, control materials must be prepared without the active compound and tested under the same experimental conditions to rule out the intrinsic antiviral activity of the polymers/biopolymers, as described for chitosan (Amankwaah, 2013; Davis, Zivanovic, D'Souza, & Davidson, 2012). Thus, the antiviral activity of the tested polymers is estimated by comparing the number of infectious viruses on the polymers containing active compounds and the polymers without the active compound at specific experimental conditions (normally, contact time and temperature). This approach cannot be used in the case of coatings, where the gel nature of the biopolymers normally employed complicates the assessment using the procedures applied for solid
To overcome this limitation, our group successfully adapted the ISO 14476:2013 (ISO 14476:2013, 2013) to test the antiviral activity of polymer gels. Specifically, pieces of each edible film (25 ± 5 mg) were inoculated with virus suspensions diluted in PBS and samples were incubated overnight at 37 ºC. Then, the effect of the active polymer gels was neutralized, and viruses were titrated in the corresponding cell line (Fabra, Falcó, Randazzo, Sánchez, & López, in press).

Thus, many parameters could differ among the different procedures, and they should be reported and clearly specified in the final report displaying the results. Amongst them, the main factors to be considered are: (i) the specifications of the active compound (such as the composition and relative concentrations) and how it has been incorporated within the polymeric material, (ii) the possible cytotoxicity effect against the tested cell lines, (iii) the effect of the neutralizer solution, (iv) the effect of working conditions tested (clean or dirty conditions), (v) temperature and humidity, (vi) the contact time with the active polymeric material, and (vii) the technique adopted for the virus recovery. As an example, while dirt conditions affect the disinfectant efficiency of antiviral solutions in surfaces (Li, Baert, & Uyttendaele, 2013), for food-packaging applications this parameter loses its importance, as the food matrix characteristics are the variables to be evaluated. Indeed, the assessment of the effectiveness of the antiviral materials for a specific food application could reflect important information for its final use under real conditions. In fact, it is known that food matrices might interfere with the antiviral activity of several compounds (Li et al., 2012; Sánchez, Aznar, & Sánchez, 2015), limiting its inactivation effect. Therefore, generally, greater amounts of active compounds need to be used in real conditions to get the same effect as in the in vitro tests (Goyal & Cannon, 2016). In this sense, some concerns have arisen due to the correlation between such high reported log reduction in laboratory tests and the real
decrease in the risk associated with foodborne viral transmission (Goyal & Cannon, 2016). For instance, reduced antiviral activity was observed when silver nanoparticles incorporated in coupons were used in surface waters, likely due to the interaction between silver nanoparticles with nonspecific particles in the highly turbid surface waters (Park et al., 2014).

New developments in the methodologies applied to assess the antiviral activity against HuNoVs

Apart from using cultivable surrogates, different methodologies to assess the antiviral activity of some compounds against HuNoVs have been reported (DiCaprio, 2017) (Fig. 1), such as:

(i) Using the HuNoV’s ability to bind saliva or porcine gastric mucin (PGM) (Tan & Jiang, 2005; Tang et al., 2010), which allows for the selective recovery of potentially infectious HuNoVs (Dancho, Chen, & Kingsley, 2012; DiCaprio et al., 2016). Saliva and PGM contain multiple human histo-blood group antigens that have been recognized as receptors or co-receptors for HuNoVs (Tian, Brandi, & Mandrell, 2005). Currently, PGM and saliva-binding enzyme-linked immunosorbent assays (ELISA) have been used to evaluate the antiviral activity of grape seed extract (GSE) (Li et al., 2012) and green tea extract (GTE) (Falcó et al., under review) against HuNoVs and virus-like particles (VLPs) of HuNoVs.

(ii) Using VLPs of HuNoVs, which are expressed in baculovirus-infected insect cells and have the same morphological, antigenic, and glycan-binding properties as HuNoVs. Furthermore, the protruding (P) domain of the major structural protein of HuNoV capsid VP1 forms subviral particles, the P
particles (Carmona-Vicente, Allen, Rodríguez-Díaz, Iturriza-Gómar, & Buesa, 2016; Carmona-Vicente, Vila-Vicent, et al., 2016). Both VLPs and particles have been used to investigate the effects of GSE and GTE by ELISA and electron microscopy (Falcó et al., under review; Li et al., 2012).

(iii) Using HuNoV suspensions pretreated with nucleic acid intercalating dyes, such as conventional intercalating dyes (i.e. propidium monoazide, PMA, and ethidium monoazide, EMA) and newly developed ones (i.e., PMAxx, and PEMAX) (Elizaquível, Aznar, & Sánchez, 2014; Randazzo, López-Gálvez, et al., 2016). This approach is based on the ability of intercalating dyes to penetrate only damaged or altered capsids and intercalate covalently into a viral genome after exposure to strong visible light, thus interfering with PCR amplification. Thus far, the inactivation of HuNoV GI and GII with epigallocatechin gallate, a natural compound, has been investigated by PMAxx-Triton pretreatment (Falcó et al., 2017).

Development of food-grade polymers and biopolymers with antiviral potential

Antimicrobial-packaging applications are directly related to food safety as well as to shelf life extension by preventing the growth of spoilage or pathogenic microorganisms. With this aim, biopolymer matrices can serve as an excellent carrier of antimicrobial agents, as noted in the above section. In fact, several works have been carried out in the last several decades concerning the antimicrobial efficacy of essential oils, natural extracts, and bacteriocins incorporated into biopolymer matrices (Cardoso et al., 2017; Honarvar et al., 2017; Moghimi, Alihmadi, & Rafati, 2017; Rezaeigolestani et al., 2017). Nevertheless, although their bactericide and fungicide properties have been largely studied, little information exists in the literature about how biopolymers could
act as carriers of virucide compounds and how they behave in a food package or edible coating. Thus, biopolymers can serve as an excellent vehicle of antiviral compounds in many fields within the food area, such as food packaging, food contact surfaces, and edible coatings.

Natural extracts (e.g., GTE, GSE), essential oils, or their main compounds (e.g., carvacrol, cinnamaldehyde) and nanometals (e.g., silver, copper) with demonstrated virucidal activity can be postulated as potential candidates to develop antiviral biopolymers (D'Souza, 2014; Li et al., 2013; Ryu et al., 2015).

The development of new packaging functionalities (i.e., antiviral) is possible because the processing equipment and conditions are the same as those currently being used (casting, melt-compounding) for other applications. Nonetheless, the effectiveness of the antiviral compounds could even be increased by developing multilayer structures (Fabra, Castro-Mayorga, et al., 2016), encapsulating the active compound (Gómez-Mascaraque et al., 2016) with potential application on shellfish depuration (McLeod et al., 2017), or even modifying the biopolymer surface to increase the antiviral activity.

More detailed information about the potential and specific areas of interest will be detailed below (Fig. 2).

**Antiviral materials for food contact surfaces**

Even if human enteric viruses are primarily transmitted through direct contact person-to-person, or through contaminated water or food (de Graaf, van Beek, & Koopmans, 2016; Sánchez, 2015), an increasing number of outbreaks in developed countries originated from cross-contaminated surfaces (fomites). In fact, their low infectious dose (Teunis et al., 2008), prolonged stability in the environment, and resistance to chemical inactivation (Cheesbrough, Green, Gallimore, Wright, & Brown, 2000; Kuusi et al., 2002), make human enteric viruses highly transmissible through environmental fomites,
including food contact surfaces. Common areas and facilities, e.g., hospitals, cruise ships, restaurants, and communal kitchens, represent exposure sites with a high health risk associated with viral outbreaks (EFSA, 2016; Hall et al., 2014; Hedlund, Rubilar-Abreu, & Svensson, 2000). Both non-porous (aluminum, china, glazed tile, glass, latex, plastic, polystyrene, and stainless steel) and porous (cloth, different types of papers, and cotton cloth) surfaces have been reported to harbor enteric viruses (Abad et al., 2001; Boone & Gerba, 2007). Thus, different guidelines have been proposed to limit and control the occurrence of pathogens on food products by increasing hygienic measures along the entire food chain (Codex Alimentarius, 2012; WHO, 2015). Specifically, for food industries and food handling services, it is extremely important to control the viral contamination of surfaces, both in food-processing lines and in manipulation counters. For food contact surfaces, the ideal material should exert its antiviral activity throughout its use. Therefore, the polymeric material should act by direct contact and not by migration of the active compounds, as is normally the case for most natural compounds. Another aspect to consider when selecting the material to be used is that it must be nontoxic and designed to withstand the environment of their intended use and the action of food. Moreover, a smooth and non-porous surface is preferable to avoid the presence of bacterial and/or viral reservoir niches. Additional features should be the material’s ease of cleaning and washing and its resistance to chemical detergents. The first attempt to develop antiviral materials for food contact surfaces was successfully carried out by Martinez-Abad and collaborators (Martinez-Abad et al., 2013). Specifically, the authors applied silver-infused polylactide films for the inactivation of FCV and tested their antiviral performance in food application. The active renewable food- packaging material based on polylactic acid (PLA) incorporating silver ions (from 0.1 to 10 g/Kg) was obtained by a solvent casting technique, and its
antimicrobial efficacy was evaluated by using the JIS Z 2801 standard (Japanese Standards Association, 2012). The antiviral activity of silver-PLA films was dose-dependent, where increasing concentrations of silver showed increased reduction in viral titers. FCV, often reported as the most sensitive HuNoV surrogate compared to others, such as MNV or Tulane virus, was less susceptible than *Salmonella*, suggesting a higher resistance of viruses to antimicrobial compounds than bacteria (Russell, 2003).

In particular, reductions of approximately 2 and more than 4.4 log TCID<sub>50</sub>/mL were shown by 0.1 and 1% of silver-PLA films after 24 h contact, respectively, while for *Salmonella*, reductions of more than 6 log CFU/mL were reported under the same experimental conditions (Martínez-Abad et al., 2013). The authors also demonstrated that after consecutive washings of the silver-PLA material, it efficiently kept its antiviral activity, thus providing a long-lasting antiviral effect, highlighting its suitability for use as food contact surfaces.

Another attempt to develop antiviral surfaces was made by Park et al. (2014), who developed a novel micrometer-sized magnetic hybrid colloid (MHC) activated with variously sized AgNPs and evaluated its efficacy for inactivating bacteriophage φX174, MNV, and adenovirus serotype 2 (AdV2). The infectivity of phage φX174 and MNV was reduced by more than 2 log after exposure to 4.6×10<sup>9</sup>Ag<sub>30</sub>-MHCs/mL (silver content 400 ppm) for 1 h at 25 °C while in a previous study *Escherichia coli* reductions of more than 6 log CFU/mL were reported (Park, Park, Ko, & Woo, 2013).

Since it is of particular interest to develop active surfaces with enhanced virucidal activity at lower loadings due to legislative restrictions, Castro-Mayorga et al. (2017) have recently developed novel routes to guarantee the dispersion and stability of silver nanoparticles (AgNPs) in biopolymer matrices. Furthermore, the effect that the stabilized AgNPs had on transparency and mechanical properties was negligible due to
the low AgNPs loadings. Concretely, they applied poly (3-hydroxybutyrate-co-3-
hydroxyvalerate) (PHBV) materials enriched with AgNP to inactivate HuNoV
surrogates, FCV and MNV. Interestingly, the active surface was obtained by depositing
a coating of thermally post-processed electrospun PHBV18 (18% mol valerate)/AgNP
fiber mats over compression molded PHBV3 (3% mol valerate) films, showing
excellent antimicrobial properties even at 0.027%. Moreover, the homogeneous
distribution of AgNP into the coating and onto the PHBV3/PHBV18 layer was
confirmed by scanning electron microscopy (SEM) and energy dispersive analysis of X-
rays (EDAX) analysis. The antiviral activity of AgNP materials, tested by adapting the
ISO 22196:2011 norm (ISO 22196:2011), showed complete inactivation for FCV only,
following 24 h exposure at 37 °C and 100% RH. In the same conditions, MNV
infectivity was reduced by only 0.86 log (Castro-Mayorga et al., 2017) and no viable
counts of *Salmonella enterica* and *Listeria monocytogenes* were recorded (Castro-
Mayorga, Fabra, & Lagaron, 2016).

A different antiviral material (plastic coupons based on zeolites containing silver/copper
ions) was evaluated by Bright et al. (2008), who reported its effectiveness against feline
infectious peritonitis virus and FCV. Zeolites (sodium aluminosilicate) are porous
minerals that can be activated with metal ions, exchanging those for other cations
present in the environment, finally resulting in an interesting progressive release of the
active compounds. The antiviral effectiveness of zeolites activated with silver/copper
ions and incorporated into plastic coupons was evaluated by recovering the target virus
from the active surface by a swabbing procedure. Bright et al. (2008) reported more
than a 5 log reduction for FCV infectivity after 24 h at 23 °C on plastic coupons
impregnated with 10% zeolite powder containing 6.5% copper and 3.5% silver ions.
The silver amount into these coupons was approximately 3.5 ppm, and excluding the
additional antiviral effect of copper (Warnes & Keevil, 2013), such concentration resulted in a greater inactivation in comparison with the AgNP-containing material tested by Castro-Mayorga et al. (2017). However, although the reduction rates reported for FCV by Martínez-Abad et al. (2013) using silver-PLA materials (>4.4 log) were greater than those reported by Castro-Mayorga et al. (2017) using AgNP-PHBV materials (1.42 log) under the same experimental conditions (24 h at 25±1 °C, 100% RH), the silver concentration within the polymer matrices was significantly different, 10 ppm and 0.27 ppm, respectively. Nevertheless, the specific surface of nanoparticles as well as the dispersion and stability of AgNPs in the polymer are key aspects that determine the antiviral activity of the active surfaces. However, although it could then be inferred that PHBV3/PHBV18/AgNP may have higher antiviral activity against FCV than the above-mentioned publications, further studies should evaluate the efficacy of such system in experiments mimicking real application conditions.

Studies evaluating copper antiviral activity incorporated into surfaces have been reported for several viruses. For example, Noyce, Michels, and Keevil (2007) reported that influenza A virus particles inoculated onto copper surfaces showed nearly a 4 log decrease following 6 h of incubation at 22 °C at 50 to 60% relative humidity. Using a surrogate of HuNoV (Warnes & Keevil, 2013; Warnes, Summersgill, & Keevil, 2015), reported rapid MNV inactivation on dry copper and copper alloy (60-80% Cu) surfaces, demonstrating that it was due to the loss of viral capsid integrity. Similarly, Manuel, Moore, and Jaykus (2015) evaluated the reduction of HuNoV genogroup II.4 exposed for 60 min to pure copper surface as 4 log units by RT-qPCR, observing, in addition, a proportional reduction effect related to the percentage of copper in the alloys.

The antiviral effect of polymeric matrices containing copper has been also reported by Borkow and Gabbay (2004), who evaluated the antiviral efficacy of latex gloves
containing copper that reduced human immunodeficiency virus (HIV-1) infectivity in a
dose-dependent manner (Borkow & Gabbay, 2004).

The antiviral activity of glass coated with thin films of titanium dioxide (TiO$_2$), copper
oxide (CuO), and hybrid CuO/TiO$_2$ prepared by atmospheric chemical vapour
deposition (Ap-CVD) was investigated by Ditta et al. (2008) using the inactivation of
bacteriophage T4 as a model for inactivation of enteric viruses. The inactivation rates
were reported to be higher by CuO and CuO/TiO$_2$, suggesting that photocatalysis and
toxicity of copper acted synergistically to inactivate bacteriophage T4.

Castro-Mayorga, Fabra Rovira, Cabedo Mas, Sánchez Moragas, and Lagarón Cabello
(2018) recently developed and characterized two active copper-based systems
performing interesting antiviral activity. In their study, the antiviral activity of
biodegradable PHBV melt mixed nanocomposites containing 0.1 and 0.05% of CuO
was compared to bilayer structures consisting of a bottom layer of compression molded
PHBV3 (3% mol valerate) coated with an active electrospun fibers layer made with
microbial mixed culture-derived PHBV18 (18% valerate) and CuO nanoparticles
(0.05%). Remarkably, the antiviral assay carried out with MNV adapting the ISO
22196:2011 showed 1.83 and 3.19 log TCID$_{50}$/mL reductions for 0.1 and 0.05% neat
PHBVs films, respectively, while no infectious viruses were recovered when in contact
with the coated structure for the same experimental conditions (24 h at 25 °C, 100%
RH). Therefore, it was demonstrated that by incorporating CuO into an electrospun
coating, the CuO loading could be reduced.

As reported for other antimicrobial materials, higher antibacterial activity against
Salmonella enterica and Listeria monocytogenes was recorded under the same
experimental conditions (Castro-Mayorga et al., 2018).
Even if evidence of zinc virucidal activity has been reported against rhinoviruses (Hulisz, 2004), respiratory syncytial virus (Suara & Crowe, 2004), vaccinia virus (Katz & Margalith, 1981), herpes simplex virus (HSV) (Arens & Travis, 2000), and HIV-1 (Haraguchi, Sakurai, Hussain, Anner, & Hoshino, 1999), it has been scarcely investigated against human enteric viruses either in suspension or applied into materials. Some inference regarding its antiviral activity has been drawn for brass (zinc-copper alloy) using MNV (Warnes et al., 2013). In particular, Warnes and Keevil (2013) suggested that zinc did have some antiviral effect, which was synergistic with copper and resulted in an increased efficacy of brasses with lower percentages of copper.

To date, the antiviral activity of gold-based surfaces has not been reported, and the elevated cost of this metal limits its application in food contact surfaces. Nevertheless, Broglie et al. (2015) reported the rapid inactivation of HuNoV due to Au/CuS core/shell NPs evaluated on HuNoV GI.1 VLPs as a model viral system and using an absorbance based ELISA.

Regulatory issues

Interestingly, the use of metal nanoparticles has attracted considerable attention, even though nanotechnology is still out of most legislation frames. In this context, the application of nanostructured materials demonstrated enhanced antimicrobial activity at low concentrations due to the high surface-to-volume ratio, thus representing a promising tool to improve the functionality of polymers used in antimicrobial food antimicrobial food contact surfaces. In the last years, nanoparticles composed of metals, metal oxides, metal salts, and metal hydroxides have been developed, the metal nanoparticles of zinc oxide and silver being the most promising against both foodborne...
bacteria (Mauriello, 2016; Moritz & Geszke-Moritz, 2013) and human enteric viruses (Castro-Mayorga et al., 2017).

In the US, and especially in Japan, the use of silver zeolites and silver zirconium phosphate resins is well established with several commercial brands incorporating silver in textiles or as coatings in different products (Appendini & Hotchkiss, 2002; Quintavalla & Vicini, 2002) with a maximum silver content of 3%. However, in the food area, only silver nitrate is regulated with a maximum limit of 0.017 mg/kg in foodstuffs and 0.1 mg/kg for drinking waters (FDA, 2010). As far as nanosilver is concerned, colloidal solutions are accepted in the US and commercialized as nutrition supplements. In the EU, the EFSA provisionally accepts the use of silver in food contact materials with a maximum of 5% silver in the form of silver zeolites or silver zirconium phosphate glasses, and silver migration is restricted to a maximum 0.05 mg/kg in food (EFSA, 2006) while 5 mg Cu/Kg food is the permitted migration limit established by the current EU regulation (European Commission, 2011) for a hypothetical package surface of 6 dm²/Kg food, although there is not a specific regulation for NPs. As of now, the EU legislation requires a market authorization of nanomaterial applications in foods based on a safety assessment by the EFSA of the potential health risks that may be associated with nanomaterials in foods (EFSA, 2011b).

**Antiviral food packaging materials**

Currently, the increasing consumers’ demand for fresh, minimally processed, and ready-to-eat (RTE) products has challenged industries to extend the shelf life of food products together with guaranteeing their safety. Furthermore, the changes in food production (i.e., global trade) and food processing (i.e., cross-contamination) have posed novel
risks that need to be controlled. As a result, food-packaging materials have been
developed with the aim of controlling pathogenic and spoilage bacteria, yeast, and
molds in food, exerting an inhibition of their growth (bacteriostatic activity) or a killing
effect (bactericidal activity) (Yildirim et al., 2018). In this sense, the physicochemical
properties of the polymer constituting the selective barrier to gas transport together with
the antimicrobial properties of different active compounds have been pointed out as key
factors of a hurdle technology applied to extend foods’ commercial shelf life. The use of
biopolymers as food-packaging materials is of high interest given their excellent film-
forming, non-toxic, odorless, tasteless, biodegradable, and edible properties. In the case
of food-packaging materials with antiviral activity, the main goal is the inactivation of
human enteric viruses that may be present in the food due to contamination from both
raw materials or during processing procedures.

To date, limited information is available concerning food-packaging materials
specifically developed to control human enteric viruses (Table 1 and Fig. 2), and just
one study evaluated its efficacy in food products (Martínez-Abad et al., 2013). In this
study, a silver-infused PLA material with antiviral activity was successfully
manufactured and evaluated on lettuce and paprika (Martínez-Abad et al., 2013).
Generally, the authors reported lower FCV inactivation when films were applied on
food samples compared to food-contact surfaces, also showing variable results
depending on the food type. In fact, 1% silver-PLA films eliminated the infectivity of
FCV in lettuce. Less silver amount (0.1%) progressively reduced the FCV infectivity,
achieving the complete inactivation only at the end of the storage time (6 days). On the
contrary, silver-PLA films did not affect FCV infectivity on paprika, suggesting an
interfering effect dependent on the food matrix. Additionally, silver-PLA films applied
to food showed higher inactivation on *Salmonella* compared to FCV.
The use of ions incorporated into packaging materials showed some drawbacks, as physical or chemical factors may alter their properties resulting in compounds without antimicrobial activity (Castro-Mayorga et al., 2016; Ilg & Kreyenschmidt, 2011). For instance, sulphides or other silver complexes may occur due to the exposure of silver ions to mid-high temperature, light, or UV, not only losing the antimicrobial activity, but also producing a strong brownish or blackish coloration of the material (Kasuga, Yoshikawa, Sakai, & Nomiya, 2012), which finally limits its application for food packaging applications.

Apart from metals, several plant extracts have been incorporated into polymers with the idea of developing antiviral-packaging materials, even though these materials have not been evaluated in food matrices. For example, a pioneering study by Fabra, Castro-Mayorga, et al. (2016) evaluated the antiviral activity of a multilayer structure based on polyhydroxybutyrate (as outer layers) interlaided with electrospun zein fibers activated with cinnamaldehyde. In particular, the electrospinning process produced biodegradable multilayer structures by the application of electrostatic forces that draw polymer solutions or melts into ultrathin fibers, thus depositing them as mats of micro- or nano-scale fibers (Fabra, Busolo, Lopez-Rubio, & Lagaron, 2013). This process overcomes the main drawback of typical technologies where high temperatures are needed to mold biodegradable polymers, a factor that limits their application for producing food-packaging materials activated with thermally-sensitive compounds. This proof-of-concept study developed a multilayer system activated with cinnamaldehyde (2.60 mg/cm²) with antiviral activity against norovirus surrogates, but not for HAV. In particular, adapting the ISO 22196:2011, 2.75 log TCID₅₀/mL reductions and complete inhibitions (>2.48 log TCID₅₀/mL) were reported for MNV and FCV after overnight treatment at 37 °C and 100% RH, respectively. Changes in temperature conditions
resulted in significant reduced effectiveness of cinnamaldehyde multilayer systems, indicating that temperature is a major factor influencing the release or effectiveness of cinnamaldehyde, as previously reported for other natural compounds (Su & D'Souza, 2011).

Among the natural polysaccharides, chitosan showed strong antimicrobial, antifungal and antioxidative activities (Friedman & Juneja, 2010; Seyfarth, Schliemann, Elsner, & Hipler, 2008), together with excellent film- and coating-forming properties. As an example, the reduction or control of foodborne pathogenic bacteria could be enhanced by the addition to chitosan coating and films of natural active compounds, as essential oils (Randazzo, Jiménez-Belenguer, et al., 2016; Yuan, Chen, & Li, 2016) or propolis (Torlak & Sert, 2013) that act synergistically.

Although recently the antiviral properties of chitosan have been reported for virus suspensions (Chirkov, 2002; Davis et al., 2012; Davis, Zivanovic, Davidson, & D'Souza, 2015; Su, Zivanovic, & D'Souza, 2009), its antiviral activity when incorporated in food packaging remains poorly investigated. In Amankwaah’s study (2013), chitosan did not enhance the antiviral activity of GTE and GSE in film-forming solutions (FFS) of either film. GTE and GSE were incorporated into chitosan films and tested against norovirus surrogates. Specifically, 1.60 and 4.50 log PFU/mL reductions of MNV were obtained after 24 h contact with 5% and 10% GTE films, respectively. The film with the highest GTE concentration tested (15%) reduced MNV infectivity to undetectable levels (Amankwaah, 2013). Similarly, MNV infectivity was reduced by 0.92, 1.89, and 2.27 log PFU/mL after 4 h at 23 °C in contact with films with 5, 10, and 15% GSE, respectively. Higher reductions were recorded after 24 h; the 15% GSE film completely inactivated MNV (more than 4 log) (Amankwaah, 2013).

Promising developments
Although many other natural compounds have shown antiviral activity when tested *in vitro*, their application into packaging materials to inactivate human enteric viruses still remains unexplored. In fact, many of such compounds have been successfully tested against food pathogenic bacteria and demonstrated antimicrobial activity also when incorporated into food-packaging materials.

Of interest for future investigation to develop active antiviral materials are, for instance, clove, oregano, and zataria essential oils, as they have demonstrated interesting antiviral activity against norovirus surrogates (MNV and FCV) and HAV, as well as some main essential oil compounds, such as thymol and carvacrol (Elizaquivel, Azizkhani, Aznar, & Sánchez, 2013; Sánchez et al., 2015; Sánchez & Aznar, 2015). Moreover, essential oils have demonstrated antimicrobial properties when incorporated into packaging films (Kashiri et al., 2017; Maisanaba et al., 2017; Randazzo, Jiménez-Belenguer, et al., 2016; Requena, Jiménez, Vargas, & Chiralt, 2016) and antifungal activities (Mateo et al., 2017). Therefore, taking into account such encouraging findings, future research should investigate the antiviral performances of packaging films enriched with essential oils.

Furthermore, other natural plant-derived substances, such as aloe vera and Eriobotryae folium extracts, showed antiviral activity against MNV (Ng et al., 2017) and could be incorporated into food packaging materials in future studies.

In the last years, many algal-derived products have been investigated, and some of the algal polysaccharides, like carrageenan, fucan, laminaran, and naviculan, are candidates as natural antiviral agents in agricultural, biomedical, food, and pharmaceutical applications (Ahmadi, Zorofchian Moghadamtousi, Abubakar, & Zandi, 2015). For instance, HIV, papillomavirus, HSV, and influenza A virus have been successfully inactivated *in vitro* by carrageenan, a phycocolloid extracted from different red seaweed species (Pangestuti & Kim, 2014). Moreover, recent studies developed active food-
packaging films with antibacterial activity by including marine algal compounds (Luzi et al., 2017; Rodriguez-Martínez et al., 2016).

**Antiviral food coatings**

The growing consumer demand for minimally processed, easily prepared, and ready-to-eat “fresh” food products with minimal chemical preservatives pose major challenges for food safety and quality. Different methods have been evaluated to eliminate or reduce human enteric viruses in food products, but many of the effective food-processing technologies cause physicochemical changes in foodstuffs (Sánchez, 2015).

In contrast, edible films and coatings have been postulated as an emerging technology because their efficiency is based on the controlled release of the antimicrobials retained in the biopolymer matrix by optimizing and restricting additive doses. Edible films and coatings can be defined as food-grade emulsions based on polysaccharides, proteins, and lipids, which can be applied to most foodstuffs by spraying, spreading, or dipping, to enhance food quality, stability, and safety. The use of antimicrobial (bactericide, fungicide, and virucide) agents into edible films and coatings present several advantages, such as the dosage adjustment, cost reductions, and greater product adherences on the foodstuffs (Aloui & Khwaldia, 2016; Dehghani, Hosseini, & Regenstein, 2018).

There are many antimicrobial natural compounds with recognized bactericide, fungicide, or virucide activity than can be incorporated into edible films and coatings to reduce the risk of foodborne contamination and inhibit the development of spoilage microorganisms. The incorporation of natural extracts (i.e., polyphenol compounds) or essential oils having antimicrobial properties in edible films and coatings represent a new route to control microorganisms and foodborne pathogens transmitted through food to consumers. When selecting the active agent to be incorporated in an edible coating,
not only its effectiveness against the target microorganism should be taken into account, but also its potential interactions with the hydrocolloid matrices and with the food components over which it will act (Sánchez-González, Cháfer, Hernández, Chiralt, & González-Martínez, 2011).

There are several works in the literature reporting on the bactericide and fungicide properties of natural compounds incorporated into edible films and coatings (Bermúdez-Oria, Rodríguez-Gutierrez, Vioque, Rubio-Senent, & Fernandez-Bolanos, 2017; Bosquez-Molina, Jesús, Bautista-Baños, Verde-Calvo, & Morales-López, 2010; Guo, Yadav, & Jin, 2017; Umagiliyage, Becerra-Mora, Kohli, Fisher, & Choudhary, 2017; Umaraw & Verma, 2017). However, to the best of our knowledge, there is no information about antiviral edible films and coatings. This new aspect could be of great interest, for instance, in minimally processed fruits and vegetables, which are obtaining increasing recognition as important vehicles for the transmission of human pathogens, including foodborne viruses (Lynch, Tauxe, & Hedberg, 2009). Therefore, there is a need to develop new strategies, such as the development of edible coatings to improve the virological safety of these products.

In this regard, potential natural compounds with demonstrated antiviral activity, such as carvacrol (Sánchez et al., 2015), cinnamaldehyde (Fabra, Castro-Mayorga, et al., 2016), GTE (Falcó et al., 2018; Randazzo, Falcó, Aznar, & Sánchez, 2017), and GSE (Joshi, Su, & D'Souza, 2015; Su & D'Souza, 2011; Su & D'Souza, 2013), can be added to hydrocolloid matrices to confer them antiviral activity. In fact, Fabra et al. (in press) have recently reported, for the first time, the antiviral properties of alginate-lipid edible films containing GTE or GSE against MNV and HAV, GTE being more efficient than GSE (Fig. 1 and 2).
It is worth mentioning that, in this particular case of antiviral edible coatings in which the coating can be eaten, the dose is the most restrictive aspect since even though most of the antimicrobial agents are GRAS, there are some limiting doses to avoid toxicity, which is the case of essential oils (Bakkali, Averbeck, Averbeck, & Idaomar, 2008).

According to EU legislation, edible coatings are included in the regulations for food additives, which says “in order to protect human health, the safety of additives for use in foods for human consumption must be assessed before they are placed on the community market” so in the development of edible films and coatings, only additives that appear in the community list of authorized substances can be used (EC 1331/208).

In addition, the ingredients used should not mislead consumers and sufficient information (i.e., toxicity assays) is needed to confirm that the additive used is safe for consumers (EU 234/2011).

In the US, edible coatings are considered a part of the food; as a consequence, their ingredients must be declared on a label under the Food and Drug Administration (FDA).

In fact, the FDA provides a list that must be used as part of the coatings and emulsions. This regulation permits the use of the listed components (mainly GRAS substances and other safe ingredients) at certain concentrations (Aguirre-Joya et al., 2018; Franssen & Krochta, 2003). These authorized substances appear in the Title 21 “Food and Drugs,” Chapter I “Food and Drug Administration, Department of Health and Human Services” in part 175 “Indirect Food Additives: Adhesives and Components of Coatings” (FDA, 2017). Furthermore, the US regulation also indicates that in the case of fruits and vegetables, consumers must be informed about the food product composition (including the coating ingredients).

**Encapsulation of antiviral compounds**
As previously mentioned, shellfish represents one of the most common food vehicles of viral contamination. Shellfish depuration is a commercial processing technology used worldwide, where shellfish are placed in tanks containing clean seawater and allowed to purge the contaminants for several days. Shellfish depuration rapidly removes bacterial pathogens, however the scientific community agrees on the inadequacy of commercial shellfish depuration processes for enteric viruses (McLeod et al., 2017). In this specific case, the incorporation of antiviral compounds within the water tanks is envisaged as the most promising approach. However, many natural antimicrobial compounds are sensitive molecules which can be affected by food processing conditions or interaction with food components, resulting in reduced antimicrobial effect. Micro- and nanoencapsulation processes, in which a compound is embedded within a protective matrix, have attracted increasing research interest for the protection of these sensitive compounds (Gómez-Mascaraque, Ambrosio-Martín, Fabra, Pérez-Masiá, & López-Rubio; Gómez-Mascaraque & Lopez-Rubio, 2016; Pérez-Masiá, Lagaron, & López-Rubio, 2014). Many studies have focused on the development of micro- or nanoparticle-based systems for increased antimicrobial stability and activity but, to date, information about their potential use in food products is rather limited (Castro-Rosas et al., 2017). For instance, encapsulation of antimicrobial compounds has demonstrated to enhance their stability during food-processing treatments, such as electron beam irradiation (Gomes, Moreira, & Castell-Perez, 2011), and the usefulness of these techniques to generate natural additives (Ko, Kim, & Park, 2012) or to formulate antibacterial disinfectants (Krogsgard Nielsen et al., 2017). Similarly, encapsulation of antivirals for food applications has been scarcely explored. Recent developments in encapsulation of antiviral compounds include the use of chitosan to enhance the protection for (−)-epigallocatechin gallate (EGCG, a green tea polyphenol) (Gómez-Mascaraque et al.,
2016) which was previously reported to be a very effective antiviral compound, reducing the titers of HAV and MNV in a dose-dependent manner at neutral pH. Microencapsulated EGCG showed the potential to prolong the antiviral activity of EGCG against MNV via gradual bioactive release combined with its protection against degradation in simulated physiological conditions. Therefore, these results highlight the potential of encapsulated natural antiviral compounds to be used in food applications. For example, GSE and GTE have been successfully used as natural sanitizers of fresh produce and food contact surfaces (Falcó et al., 2018; Li et al., 2012; Randazzo et al., 2017; Su & D'Souza, 2013), and encapsulation of these antiviral compounds may provide enhanced and prolonged antiviral activity as a consequence of the protection and more gradual release provided by the biopolymeric encapsulating matrices. In fact, different studies demonstrated the efficacy of alginate-based delivery particles to target shellfish tissues (Darmody et al., 2015; Prado-Alvarez et al., 2015), suggesting that encapsulation could represent a viable tool for the transport and delivery of antiviral compounds directly to the shellfish tissues.

**Final remarks**

Food contamination by human enteric viruses is a serious health and economic problem. Currently, food manufacturing processes that may inactivate human enteric viruses cannot be applied without adversely affecting food quality. Therefore, the effective prevention of contamination, new food-processing strategies, new sanitation approaches, and consumer education could reduce enteric virus numbers and thereby decrease consumer risks of enteric virus infections. Among these strategies, one promising technology is the use of polymers and biopolymers with antiviral activity. To evaluate the potential of polymers or biopolymers with antiviral activity, some publications have explored their efficacy against HAV and HuNoV, mainly using
HuNoV surrogates. The use of different virus titers, inoculum-suspending matrices, and virus-recovery procedures complicates comparisons among studies, as documented in this review. Additionally, antiviral polymers have been mainly applied in *in vitro* experiments with different levels of success.

The use of metals or metal nanoparticles to render antimicrobial polymeric materials has significant potential applications. This is particularly the case in food contact and packaging applications. However, there are still a number of issues, such as regulatory issues and effectiveness at low dosages that need to be better addressed and resolved for this interesting technology to be widely used in industrial applications. The most promising research is oriented toward the mastering of nanoparticles, which seems to offer better stability, efficacy, and cost effectiveness.

Although there is increasing interest in the use of antimicrobial packaging and edible coatings, motivated by the increasing consumer demand for safe and stable foods, little information is available in the literature about how biopolymers could act as carriers of antiviral compounds in real food samples. Therefore, the development of biopolymers with antiviral activity and their applications in the food area is today an open field of research that needs to be fully addressed.

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**Author Contributions**
Randazzo and Falcó researched prior studies, interpreted the results and drafted the manuscript. Fabra compiled data and drafted the manuscript. López-Rubio and Sánchez conceived the original idea and drafted the manuscript. All authors contributed to the final manuscript.
Table 1. Active packaging and food contact surfaces with virucidal activity.

<table>
<thead>
<tr>
<th>Application</th>
<th>Active compound</th>
<th>Type of polymer or biopolymer</th>
<th>Virus</th>
<th>Concentration of the active compound</th>
<th>Test conditions</th>
<th>Inactivation (log reduction)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active packaging</td>
<td>Green seed extract</td>
<td>Chitosan</td>
<td>MNV</td>
<td>5; 10; 15%</td>
<td>23 °C, 24 h</td>
<td>1.9; 3.2; &gt;4.0</td>
<td>(Amankwaah, 2013)</td>
</tr>
<tr>
<td></td>
<td>Green tea extract</td>
<td>Chitosan</td>
<td>MNV</td>
<td>5; 10; 15%</td>
<td>23 °C, 24 h</td>
<td>1.6; 4.5; &gt;4.5</td>
<td>(Amankwaah, 2013)</td>
</tr>
<tr>
<td></td>
<td>Cinnamaldehyde</td>
<td>PHB</td>
<td>MNV</td>
<td>2.60 mg/cm²</td>
<td>37 °C, ON, 100% RH</td>
<td>2.7</td>
<td>(Fabra, Castro-Mayorga, et al., 2016)</td>
</tr>
<tr>
<td>Active packaging and food contact surfaces</td>
<td>Silver ions</td>
<td>PLA</td>
<td>FCV</td>
<td>0.1; 1%</td>
<td>24 °C, 24 h</td>
<td>2; &gt;4.4</td>
<td>(Martinez-Abad et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>Silver nanoparticles</td>
<td>PHBV</td>
<td>MNV</td>
<td>0.027%</td>
<td>37 °C, 24 h</td>
<td>0.86</td>
<td>(Castro-Mayorga et al., 2017)</td>
</tr>
<tr>
<td></td>
<td>Silver ions</td>
<td>Plastic coupons impregnated with zeolite powder</td>
<td>FCV</td>
<td>0.00035%</td>
<td>23 °C, 24 h</td>
<td>5</td>
<td>(Bright et al., 2008)</td>
</tr>
<tr>
<td>Food contact surfaces</td>
<td>Silver nanoparticles</td>
<td>Magnetic hybrid colloid</td>
<td>φX174</td>
<td>0.04%</td>
<td>25 °C, 1 h</td>
<td>&gt;2</td>
<td>(Park et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>Copper</td>
<td>PHBV</td>
<td>MNV</td>
<td>0.05%</td>
<td>25 °C, 24h, 100% RH</td>
<td>3.19</td>
<td>(Castro-Mayorga et al., 2018)</td>
</tr>
<tr>
<td></td>
<td>Copper</td>
<td>Copper surfaces</td>
<td>MNV</td>
<td>89%</td>
<td>RT, 30 min</td>
<td>5</td>
<td>(Warnes et al., 2015)</td>
</tr>
<tr>
<td></td>
<td>Copper</td>
<td>HuNoV GII.4</td>
<td>100%</td>
<td>RT, 1 h</td>
<td>4</td>
<td>(Manuel et al., 2015)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: PHB, Polyhydroxybutyrate; PHBV, 3-hydroxybutyrate-co-3-hydroxyvalerate; PLA, Polylactide; LOD, limit of detection.
Figure 1. Diagram of the methods used for assessing the antiviral activity of food-grade polymers or biopolymers.
Figure 2. Food application of polymers and biopolymers with antiviral activity.

Current developments

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Active compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>Silver</td>
<td>Martinez-Abad et al. 2013</td>
</tr>
<tr>
<td>PHBV</td>
<td>Silver and cooper nanoparticles</td>
<td>Castro-Mayorga et al. 2017 and 2018</td>
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</table>

Active packaging

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Active compound</th>
<th>Reference</th>
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<tbody>
<tr>
<td>PLA</td>
<td>Silver</td>
<td>Martinez-Abad et al. 2013</td>
</tr>
<tr>
<td>PHBV</td>
<td>Silver</td>
<td>Castro-Mayorga et al. 2017</td>
</tr>
<tr>
<td>PHBV</td>
<td>Cinnamaldehyde</td>
<td>Fabra et al. 2016</td>
</tr>
<tr>
<td>Chitosan</td>
<td>GTE and GSE</td>
<td>Amankwaah 2013</td>
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Coatings

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<th>Active compound</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Alginate</td>
<td>GTE</td>
<td>Fabra et al., in press</td>
</tr>
<tr>
<td>Alginate</td>
<td>GSE</td>
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Encapsulation

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<th>Active compound</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Chitosan</td>
<td>EGC</td>
<td>Gómez-Mascaraque et al. 2016</td>
</tr>
</tbody>
</table>

Future needs

- Legislation
- Application under real conditions
- Incorporation of new natural extracts
- Application in food (e.g., berries)
- Application in food (e.g., seafood, washing water)

Standardized procedures to assess the antiviral activity of the polymers/biopolymers
References


Amankwaah, C. (2013). Incorporation of selected plant extracts into edible chitosan films and the effect on the antiviral, antibacterial and mechanical properties of the material. (PhD), The Ohio State University, Retrieved from https://etd.ohiolink.edu/ap/1070::NO:10:P10_ACCESSION_NUM:osu1366220367


Codex Alimentarius, Codex Committee on Food Hygiene. CAC/GL 79-2012. Guidelines on the application of general principles of food hygiene to the control of viruses in food.


EU Commission (2011). Regulation on plastic materials and articles intended to come into contact with food (10/2011).


ISO 14476:2013. Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of virucidal activity in the medical area. Test method and requirements (Phase 2/Step 1).


JIS Z 2801. Antibacterial products: Test for antibacterial activity and efficacy.


