Carbohydrate Polymers 311 (2023) 120782

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Progress and opportunities in Gellan gum-based materials: A review of preparation, characterization and emerging applications

D. Gomes [a,b,c], J.P. Batista-Silva [a], A. Sousa [a], L.A. Passarinha [a,b,c,d,*]

[a] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, 6201-506 Covilhã, Portugal
[b] UCIBIO–Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica 2829-516, Portugal
[c] Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA, 2819-516 Caparica, Portugal
[d] Laboratório de Fármaco-Toxicologia-UBIMedical, Universidade da Beira Interior, Covilhã, Portugal

ARTICLE INFO

Keywords:
Drug delivery
Electrospinning
Gellan gum
Tissue engineering
Ionotropic gelation
Polyelectrolyte complexation

ABSTRACT

Gellan gum, a microbial exopolysaccharide, is biodegradable and has potential to fill several key roles in many fields from food to pharmacy, biomedicine and tissue engineering. In order to improve the physicochemical and biological properties of gellan gum, some researchers take advantage of numerous hydroxyl groups and the free carboxyl present in each repeating unit. As a result, design and development of gellan-based materials have advanced significantly. The goal of this review is to provide a summary of the most recent, high-quality research trends that have used gellan gum as a polymeric component in the design of numerous cutting-edge materials with applications in various fields.

1. Introduction

Natural polymers are defined as materials that occur naturally or are extracted from plants or animals. Nowadays, they became one of the most versatile materials, having application in all spheres of human life. In particular, microbial exopolysaccharides (EPS) are emerging as useful components of human-made products in the chemical, pharmaceutical, cosmetics, and food industries due to their physical properties and easiness to obtain through industrial fermentation. EPS are water-soluble polymers that may be ionic or non-ionic and are mostly used as emulsifiers, gelling agents, film formers and stabilizers (Bajaj, Survase, Saudagar, & Singhal, 2007; Prajapati, Jani, Zala, & Khutliwala, 2013). Gellan gum (GG), a microbial exopolysaccharide produced by *Sphingomonas elodea* and *Shingomonas paucimobilis*, is one of the polymers most used due to its versatile properties and because it is a low-cost biomaterial manufactured on an industrial scale with high reproducible quality (Prajapati et al., 2013). After gellan gum was approved by FDA in 1992 to be used as a food additive, the interest in this polysaccharide for biomedical, pharmaceutical and other fields has vastly increased due to its biocompatibility and biodegradability. Over the past few decades, remarkable progress has been made in the design and development of gellan-based materials for different purposes. This led to a proliferation of methods to produce several materials in order to serve the desired application and so it is pertinent to scrutinize some questions to keep up with the evolution of these gellan-based materials. Particularly, one might question (i) what are the common methods for the synthesis of gellan-based materials? (ii) Which method is more suitable for each material and how to overcome its limitations? (iii) What are the purposes and the main characterization techniques applied? and lastly (iv) what are the main applications of gellan-based materials? Herein, we provide an overall review of the literature on gellan-based materials to deliver answers to the aforementioned questions and emphasize the importance of gellan-based materials for the future.

1.1. Gellan gum: structure, type and properties

Gellan gum is a linear and anionic polysaccharide that consists of a tetrasaccharide unit of two residues of β-D-glucose, one of β-D-glucuronate and one of α-L-rhamnose, which corresponds to approximately 60 % of glucose, 20 % of rhamnose and 20 % of glucoronate (Giavasis, Harvey, & McNeil, 2000). There are two types of gellan gum, native gellan gum, known as high acyl gellan gum and deacetylated gellan gum,

* Corresponding author at: UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal.
E-mail addresses: diana.gomes@ubi.pt (D. Gomes), joao.pedro.silva@ubi.pt (J.P. Batista-Silva), angela@fcsaude.ubi.pt (A. Sousa), lpassarinha@fcsaude.ubi.pt (L.A. Passarinha).

https://doi.org/10.1016/j.carbpol.2023.120782
Received 15 December 2022; Received in revised form 27 February 2023; Accepted 28 February 2023
Available online 6 March 2023

known as low acyl gellan gum (Fig. 1 – A1 and A2). The major difference is on the two acyl groups, glycerate and acetate, that native gellan gum has bound to the same β-D-glucose residue. Glycerate is found at every repeated tetrasaccharide unit, while acetate is found at 2 repeated tetrasaccharide units. These acyl groups can be removed from native gellan gum by a clarification step based on alkaline treatment producing deacetylated gellan gum. The removal of the acyl groups has a severe impact on the rheological properties of the resulting gels (BeMiller,

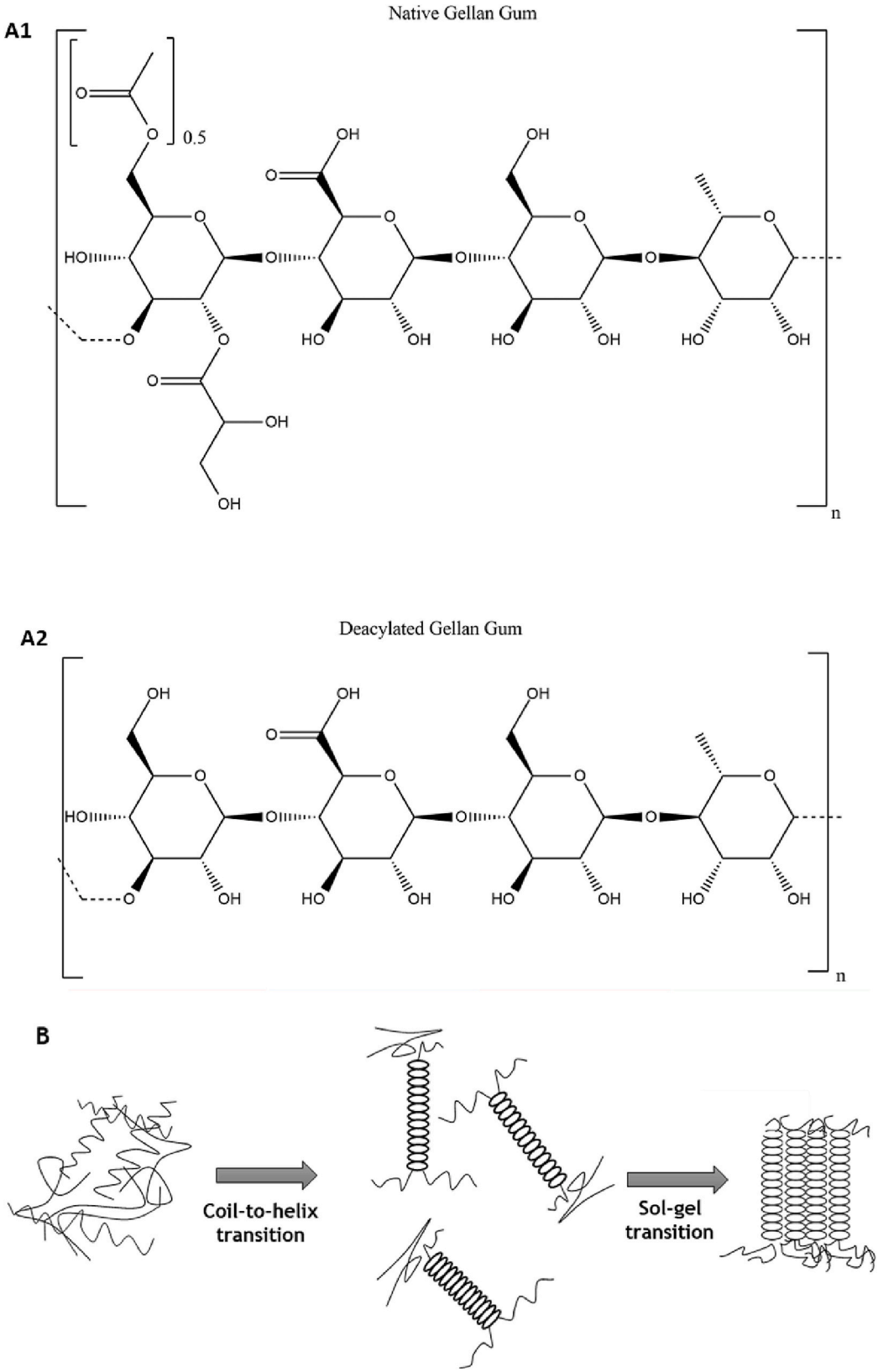

Fig. 1. Chemical structure of high acyl (A1) and low acyl (A2) gellan gum; Gelation process of gellan gum (B).

2019). For example, soft, elastic, deformable gels made with native gellan gum shift to harder (rigid), brittle gels with greater thermal stability and optimal gelling conditions, once there is less hindrance for bridge formation between the gellan gum chains (Bajaj et al., 2007; Fialho et al., 2008). Gellan gum gels are shear sensitive, meaning they can change their structure and viscosity when subjected to shear stress. In fact, deacetylated gellan gum showed Newtonian behavior with a range of concentration below 0.9 %, but plastic flow behavior above 1.0 % at 25 °C (Tako, Sakae, & Nakamura, 1989). Additionally, gellan gum solutions have a high viscosity, making it an effective thickener for liquids and suspensions.

Gellan gum has the ability to produce firm and translucent gels that are stable at low pH (3.0–5.0). This gelling property depends on the presence and type of cations in solution, pH, temperature and polymer concentration during the gelation process. The thermally reversible gelation is one of the most important rheological properties of gellan gum, which usually happens when a gellan gum solution is heated and then cooled (Miyoshi & Nishinari, 1999).

Through this physical alteration, the GG molecules go from a roughly coiled shape to well organized double helices. In this way, when heated and dispersed in water, gellan molecules will initially resemble random coil polymers. On cooling, these coils will form van der Waals forces and hydrogen bonds with the nearby chain, resulting in double-helical structures. Second, in the presence of cations that promote gel formation, the double helices assemble to form the cation-mediated junction zone. Once the ions reduce the repulsive interactions between the identical electrostatic charges from the gellan molecule, these cross-linkages will result in the development of strong gel channels (Morris, Nishinari, & Rinaudo, 2012; Zia et al., 2018). Gellan gum main characteristics include gelling, malleability and versatile texture that allow adjustable gel elasticity, biocompatibility, biodegradability and excellent thermal and acid stability (Fialho et al., 2008; Zia et al., 2018). As a result, this polysaccharide is being researched and used in a variety of fields, including the food, biotechnology, cosmetics, pharmaceutical, and biomedical ones (Osmalek, Froelich, & Tasarek, 2014; Prajapati et al., 2013).

1.2. Gellan gum: production, isolation and purification

*Sinphogomas paucimobil*is is a gram-negative, aerobic, rod-shaped bacteria that usually forms yellow pigmented colonies (Fialho et al., 2008). Each polysaccharide has a suitable growth media and other factors (pH, temperature, agitation rate, nitrogen source and dissolved oxygen) that can be manipulated in order to increase the production yield. For gellan production, it can be used a simple media just containing a carbon source, nitrogen source and inorganic salts, or can be used a complex media supplemented with vitamins that can enhance the cell growth (Prajapati et al., 2013).

In comparison with other hydrocolloids, the gellan gum production is more expensive, so in the literature is possible to find several reports about the use of industry by-products as substitution of nitrogen and carbon source in order to reduce the biosynthesis costs (Banik, Santhiagu, & Upadhyay, 2007; Huang, Zhu, Li, Zhang, & Ji, 2020; Nampoothiri, Singhania, Sabarinath, & Pandey, 2003). For example, Banik and co-workers applied Plackett-Burman design to study the effect of various nutrient supplements in gellan production using molasses, a by-product of sugar industry instead of glucose, sucrose, lactose or starch as carbon source (R.M. Banik et al., 2007). Raghunandan and co-workers, used biodiesel-derived waste glycerol (WG) as main carbon source for gellan production and they obtained high yields for both bacterial strains (*Sphingomonas pseudosanguinis* and *Sphingomonas yabuuchiae)* 51.6 and 52.6 g/L. In this work they produced gellan at a low-cost and at the same time were able to perform the bioremediation of WG (Raghunandan, Kumar, Kumar, Permaul, & Singh, 2018). Also, Fialho and colleagues compared the use of glucose, lactose and cheese whey (by-product of cheese production) as carbon source and found that the use of different carbon sources leads to the formation of gellan with different properties (variations in acyl substitution level, polymer rheological properties, and susceptibility to degradation). Nevertheless, the yield obtained for diluted cheese whey was 7 g/L, which was similar to the one obtained for lactose, 9 g/L (Fialho et al., 1999).

Regarding the complex nitrogen source, usually it is applied yeast extract, peptone (organic source) or ammonium nitrate, sodium nitrate (inorganic sources) (Fialho et al., 2008; Huang et al., 2020). As described for carbon source some researchers investigated by-products like corn steep liquor (CSL) and soybean pomace as low-cost nitrogen source for gellan production (Huang et al., 2020; Jin et al., 2003). It was compared several nitrogen sources, where CSL was able to replace peptone, yeast extract and soybean materials in gellan production, obtaining a promising tool for industrial applications (Huang et al., 2020). Moreover, it was tested the use of soybean pomace as substitution of bacto-peptone in gellan gum production by *S. paucimobilis* NK2000. The authors found that soybean pomace as nitrogen source was able to produce more gellan than bacto-peptone, thus soybean can be used for the production of gellan gum and at the same time contribute to the reduction of the environmental contamination caused by a disposal of agro-industrial by-products (Jin et al., 2003).

Another vital parameter for gellan production is the temperature, which in most fermentations is carried out at 30 °C (Bajaj, Saudagar, Singhal, & Pandey, 2006; Jin et al., 2003; Nampoothiri et al., 2003). According to Martin and Sá-Correia, the maximum yield is achieved at 20–25 °C, and after 30 °C, the yield significantly decreases (Martins & Sá-Correia, 1994). The pH has an important role in the gellan production and also in the cells growth. So, Bajaj and Nampoothiri research teams studied its influence within a range of 4 to 10, where the highest yield of gellan and biomass was achieved at pH of 7.0 (Bajaj et al., 2006; Nampoothiri et al., 2003). It was also recommended that gellan production should be carried out at a pH between 6.5 and 7.0 (Giavasis et al., 2000). Considering the agitation rate, it was reported that low levels of agitation weren't enough to homogenize the broth and high stirring rates (600–800 rpm) caused the formation of stagnant layer and heterogeneous broth that led to limitations in terms of heat and mass transfer. So, the use of 250 rpm is recommended for mixing the gellan broth (Giavasis et al., 2000).

After a successful fermentation, the recovery of gellan gum is a crucial step, where two different methods are available. The 1st method was described by Kang and co-workers and leads to gellan gum with divalent cations, while the 2nd method was described by Manna and colleagues and leads to gellan gum with the monovalent cations (Kang, Veeder, Mirrasoul, Kaneko, & Cottrell, 1982; Manna, Gambhir, & Ghosh, 1996). The major difference between these methods consists in the replacement of the filtration step (1st method) by a centrifugation step in the 2nd method. Briefly, in the 1st method the fermentation broth is heated to 95 °C and then cooled to 80 °C in a water bath. Then, the pH is adjusted to 10 for 10 min using basic solutions, namely KOH or NaOH, and cells are removed by filtration with 0.2 μm filters. To obtain a clarified and deacetylated gellan, the filtrate is precipitated by 2 volumes of 99 % isopropanol (Kang et al., 1982). In the 2nd method the fermentation broth is heated to 100 °C during 15 min in a water bath and then cooled to 80 °C. The pH is adjusted to 10 for 10 min using 2 M NaOH and then neutralized at pH 7.0 using 2 M H_2SO_4. This broth is dissolved in distilled water and centrifuged at 38000 *g* for 1 h to remove the cells, and the supernatant is again centrifuged. The gellan precipitation is achieved by using 4 volumes of propanol obtaining a clarified and deacetylated gellan (Manna et al., 1996). The recovered gellan of both methods is dried at 55 °C for 1 h (Giavasis et al., 2000). The purification of gellan gum described by Jin and co-workers consists in the washing of the isopropanol precipitated material with acetone and ether, followed by its dissolution in deionized water and dialysis for 2–3 days against the deionized water. Then, the solution is lyophilized to recover gellan gum (Jin et al., 2003). It was also performed a study that evaluated multiple procedures to recover and purify gellan gum from

the fermentation broth. The gellan recovered from the different processes was analysed by FTIR and NMR, where the best results in terms of purity were achieved with the following sequence: filtration, washing with acetone and ether, dissolution of permeate with distilled water and precipitation with acetonitrile (Coelho et al., 2019).

2. Typical methods applied to synthesize gellan-based materials: advantages and limitations

Given that the gellan gum market is expected to expand from 43.92 million dollars in 2018 to 54.47 million dollars in 2025 (Forecast, 2021), it can be concluded that this polysaccharide has become increasingly relevant across a variety of industries. Furthermore, GG has been adopted as one of the most important commercialized bacterial exopolysaccharides because to its unique features, adaptability, stability, biocompatibility, and biodegradability, as well as the wide variety of uses and the large and growing number of patent filings (Prajapati et al., 2013; Zia et al., 2018). To obtain materials with specific characteristics, GG can be easily modified by either physical or chemical treatments. Physical modification involves subjecting gellan gum to temperature, shear, and pH changes, which can be used to alter solubility, gel strength, and viscosity. It is also used to make heat-resistant gels. Chemical modification includes chemical compounds to crosslink the gellan gum molecules, increase gel strength and improve the clarity of the gel. The advantages of modifying gellan gum include improved gelling properties, increased viscosity, and improved stability. In addition, it can be used to create desired textures and longer shelf-life. However, modifying gellan gum can increase cost and decrease solubility. Additionally, it can also cause undesirable flavors and color changes in some products, as well as, cause foaming or clouding. Reviews published by several authors (Palumbo, Federico, Pitarresi, Fiorica, & Giammona, 2020; Zia et al., 2018) explore in greater detail the physical and chemical modifications of gellan gum and its applications. The interest in gellan for different purposes has been increasing and so several methods are being applied to create gellan-based materials according to their final applications. For example, gellan can be applied in tissue engineering as a scaffold or hydrogel, in drug delivery systems as micro/nanoparticles and also in food as a film or coating. In the next subsections we will discuss the methods most used to formulate gellan-based materials, their advantages and limitations.

2.1. Electrospinning

The electrospinning process was developed to obtain continuous fibers with diameters in the sub-micrometer to nanometer range, where the electric force is used to draw the polymer solution. First, the polymer is dissolved into a solvent and upload to a syringe with an attached needle. This equipment allows the solution extrusion as droplet at the tip of the syringe under the application of high voltage, which creates an electric charge on the droplet. When the electrostatic repulsion overcomes the surface tension, the Taylor's cone is formed and a charged jet is extended in a straight line. As the jet is stretched, the solidification and deposition of solid fibers occurs on the collector, Fig. 2 (Bhardwaj & Kundu, 2010; Jain, Shetty, & Yadav, 2020). Electrospinning is a simple method with a low-cost setup that allows the control of the fibers morphological structure by studying different parameters such as, temperature, polymer concentration, voltage, flow rate and distance from the tip to the collector (Xue, Wu, Dai, & Xia, 2019). Spun fibers have small pores, a great surface area, adjustable porosity and it is possible to manipulate the nanofiber composition according to the desired aim. Thus, electrospun fibers are being applied in tissue engineering, biomedical, pharmaceutical and biotechnology fields (Bhardwaj & Kundu, 2010).

Although the gellan natural polymer presents great properties for application in the biomedical field, the studies performed with electrospun gellan nanofibers are scarce. The lack of reports is related to the

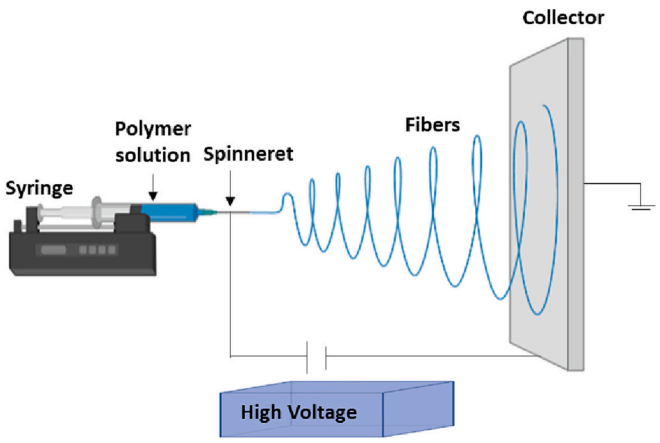

Fig. 2. Schematic representation of electrospinning process. A polymer solution is subjected to a high electrical voltage, which draws the liquid out into a thin fiber that solidifies upon landing on a collection surface.

difficulty in performing the electrospinning of a gellan solution, due to the complex gelling behavior, low shear viscosity and inability to form several chain entanglements in water, sufficient to sustain the integrity of the jet (Aadil, Nathani, Sharma, Lenka, & Gupta, 2019). Nevertheless, these constraints can be surpassed by the addition of a supporting polymer.

Aadil and *co*-workers were capable of producing gellan gum nanofibers with poly(vinyl alcohol) (PVA), once the addition of PVA moderated the repulsion between the negative charges of gellan molecules, by hydrogen bond interactions and its high molecular weight improved the electrospinnability of the solution (Aadil et al., 2019). The PVA-gellan nanofibers were used as scaffolds for tissue engineering to mimic the structure of extracellular matrix (ECM) and tissues at nanoscale. Thus, to obtain the smallest fiber diameter the authors suggest using low concentration of gellan since a concentration above 2 % leads to the formation of branched and beaded fibers. The degradation study revealed that after 14 days, the scaffolds lost 36 % weight (Aadil et al., 2019).

The addition of crosslinking step after the nanofibers production allows to overcome the degradation rate, enhance fibers stability and other mechanical properties. Vashisth and colleagues produced PVA-gellan nanofibers by electrospinning and applied four different crosslinking methods - physical, chemical, ionic and vapor crosslinking – to understand the effects in terms of stability and biocompatibility (Vashisth & Pruthi, 2016). Fig. 3 shows the electrospinning process and the different crosslinking methods applied to reinforce the nanofibers (Vashisth & Pruthi, 2016).

Chemical crosslinking with methanol and glutaraldehyde occurs through the hydroxyl groups of gellan and PVA. Although methanol leads to substantial changes in the structure of the nanofibers, the use of glutaraldehyde maintains the nanofibers morphology. Ionic crosslinking with $CaCl_2$ allows the formation of electrostatic interactions between the carboxylic groups of gellan, chains which forms a stronger structure and shrinkage of nanofibers. Regarding the nanofibers' properties, the methanol, heat and glutaraldehyde treatment increased the tensile strength due to the presence of inter-linkage bonds between gellan-gellan and gellan-PVA chains. The heat crosslinking enhanced cells growth and proliferation without affecting the nanofibers morphology. Basically, the best method to improve the PVA-gellan nanofibers stability and properties is the heat treatment (Vashisth & Pruthi, 2016). This system was applied in another work to encapsulate ofloxacin as a gastroretentive drug delivery system, since the spun nanofibers possess high surface area to volume ratio, which can improve the drug release in a sustained way (Vashisth et al., 2017). Also, previous studies showed superior biocompatibility of gellan-based nanofibers when compared to

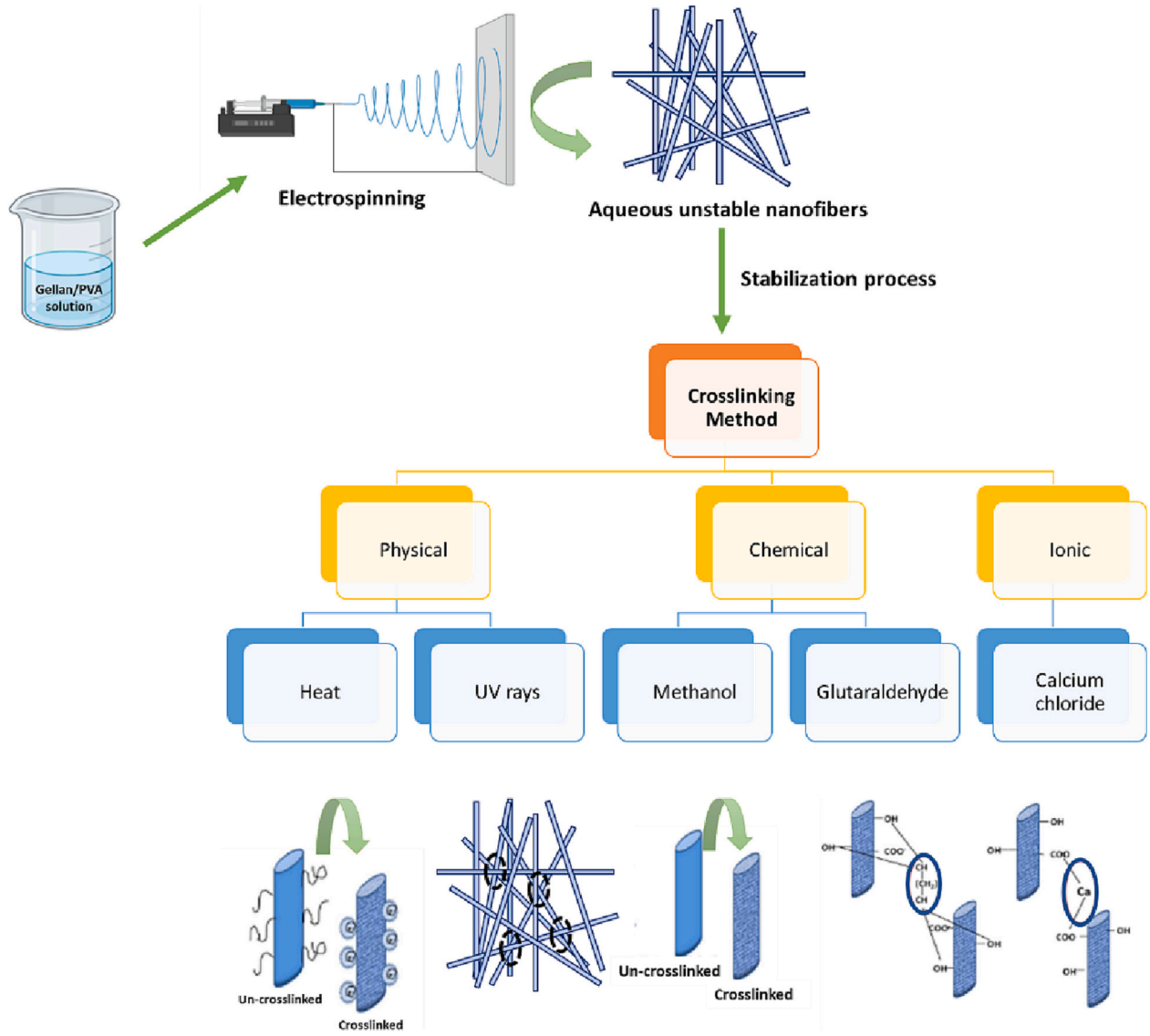

Fig. 3. Representation of electrospinning process and different crosslinking methods used to reinforce the nanofibers.

gellan-based films or hydrogels (Vashisth et al., 2016). It was concluded that the PVA-gellan nanofibers increase the ofloxacin bioavailability and *in vivo* efficacy due to the mucoadhesive property (Vashisth et al., 2016).

Recently, native gellan gum has been combined with polymers like PVA to improve its spinnability some authors have tried to establish an electrospinning procedure using octyl and dodecyl derivatized GG, namely GG-C_8 and GG-C_{12} with potential applications as biomedical devices or drug delivery systems (Palumbo et al., 2021). In this work, derivatized gellan gum was mixed with PVA (1:1 ratio) and experimental design was applied to study the influence of flow rate, voltage and needle-to-collector distance on the electrospinning. It was noted that polymers concentration above 5 % (*w/v*) would not allow to produce membrane due to the high viscosity and that the applied voltage was the main parameter controlling the integrity and diameter of the obtained fibers. Overall, the GG-C_8/PVA mixture was more easily processed than the GG-C_{12}/PVA blend, yielding fibers about 200 nm. In addition, the authors found that the GG-C_{12} spinalibility was negatively affected by more robust hydrophobic interactions that caused a reduction in the flexibility. In conclusion, only GG-C_8 demonstrated to have good spinnability (Palumbo et al., 2021). The same research group produced an asymmetric membrane with bioactive factors using the GG-C_8 as a hydrophilic dermal layer and polyurethan polycaprolactone (PU-PCL) as hydrophobic epidermal layer for wound healing (Federico et al., 2021). Electrospinning of polysaccharides in aqueous dispersions is a safe strategy to avoid denaturation of bioactive factors. First, GG-C_8/PVA and the growth factor FGF-2 were electrospun with the defined parameters, as flow rate, needle-collector distance and electric field. In the second step, the PU-PCL copolymer loaded with ciprofloxacin (CPX) was electrospun, so that its nanofibers were deposited on the GG-C_8/PVA layer and Ciprofloxacin hemisuccinate particles were added previously in this mixture. *In vitro* experiments showed that the ionotropic properties of the GG-C_8 polymer could be used to control the release of the antibiotic ciprofloxacin (CPX) and the growth factor FGF-2, as well as their biological activity. The release of FGF-2 and chemoattractant activity can be significantly increased if the membrane is crosslinked with $CaCl_2$. With this ionotropic dependent release in mind, the system proposed here could allow for appropriate control in wound healing treatment (Federico et al., 2021).

Gellan gum has also been combined with natural polymers like chitosan and pullulan to form fibers for biomedical applications (Göttel et al., 2020; Rostami et al., 2019). Rostami and co-workers developed a resveratrol loaded chitosan-gellan nanofibers (NFs) as a gastrointestinal delivery system (Rostami et al., 2019). Polysaccharides, like gellan and chitosan, minimize the side effects of the drug and maximize its release

in the colonic region. Besides, gellan has high stability in acid environments, resistance to enzymatic breakdown and improves spinability of chitosan. The chitosan-gellan nanofibers were obtained by electrospinning technique as described above. To deliver the resveratrol the authors took advantage of a pH-sensitive release mechanism that consisted of protonation of chitosan amine groups at low pH and deprotonation of gellan carboxyl groups at high pH. The results of the *in vitro* biocompatibility and cell cytotoxicity assays suggested that these NFs can be used as a biomaterial for nutraceutical and medical applications (Rostami et al., 2019). A different research group explored a combination of two microbial polysaccharides, gellan and pullulan to develop an ocular drug delivery system based on electrospun fibers (Göttel, Souza e Silva, et al., 2020). Pullulan was chosen to improve fibers formation due to its hydrophilicity, fast dissolution and good spinnability (Stijnman, Bodnar, & Hans Tromp, 2011). The fibers display high porosity (87.5 ± 0.5 %) and pore interconnectivity (99 %), leading to a significant increase in fluorescein residence time when compared to conventional eye drops. Solid fibers have numerous advantages when compared to traditional eye drops: the water-free, solid-state nature of the drug increases stability and protects it from hydrolysis. It was concluded that the curved nanofiber *in situ* gelling mat represents a promising alternative system for ocular drug delivery (Göttel, Souza e Silva, et al., 2020). Therefore, the same research group studied different formulation strategies to load Amphotericin B in gellan/pullulan fibers as a possible treatment for Keratomycosis (Göttel et al., 2020).

Finally, the electrospinning technique has also been combined with other methods, namely crosslinking as already mentioned, 3D printing and freeze-drying to produce scaffolds for tissue engineering applications (Federico et al., 2021; Zhu et al., 2021). The main reason to combine electrospinning with these techniques is to increase the mechanical resistance of fibers and also considering the final application of the developed product.

2.2. Polyelectrolyte complexation

The association complexes produced between oppositely charged particles are known as polyelectrolyte complexes (PECs) (*e.g.* polymer-polymer, polymer-drug). These are created by the electrostatic interaction of polyions with opposing charges and prevents the usage of chemical crosslinking agents, lowering their potential toxicity and other negative effects (Kaur & Kaur, 2018; Lankalapalli & Kolapalli, 2009). The essential steps in this procedure are indicated in Fig. 4. The first step consists of main complex formation that is guided by Coulomb forces. The second step is the intracomplex creation process and entails the creation of new bonds and/or the repair of polymer chain aberrations. The third phase is intercomplex aggregation, which entails secondary complexes aggregating mostly through hydrophobic interactions (Kaur & Kaur, 2018; Lankalapalli & Kolapalli, 2009).

The formation of PECs is influenced by several factors as ion site, charge density, polyelectrolyte concentration, pH, ionic strength and temperature (Kaur & Kaur, 2018). Kaur and collaborators studied the effect of pH and polymers ratio on the formation of PECs between anionic gellan gum and cationic guar gum to explore their potential in formulating pH-dependent drug delivery system (Kaur & Kaur, 2018). According to the authors, the pH of the polymeric solutions is the most critical factor in polyelectrolyte complexation, since it influences the ionization state/charge density of polymers and consequently the complexation reaction and properties of the formed PECs. In this work was concluded that the ratio 30:70 and 40:60 GG:CGG at pH 5.5 presented near neutral charge, high yield and low viscosity. In addition, the PECs prepared at this optimum pH showed different responses to specific pH buffers, which suggested that GG-CGG PECs exhibit a pH-responsive behavior and can be explored for colon targeted drug delivery systems (Kaur & Kaur, 2018). PECs have been used as efficient delivery systems in the pharmaceutical sector to enclose, protect, and release the bioactives. They have a number of benefits, including a high payload, improved encapsulation effectiveness, and controlled release

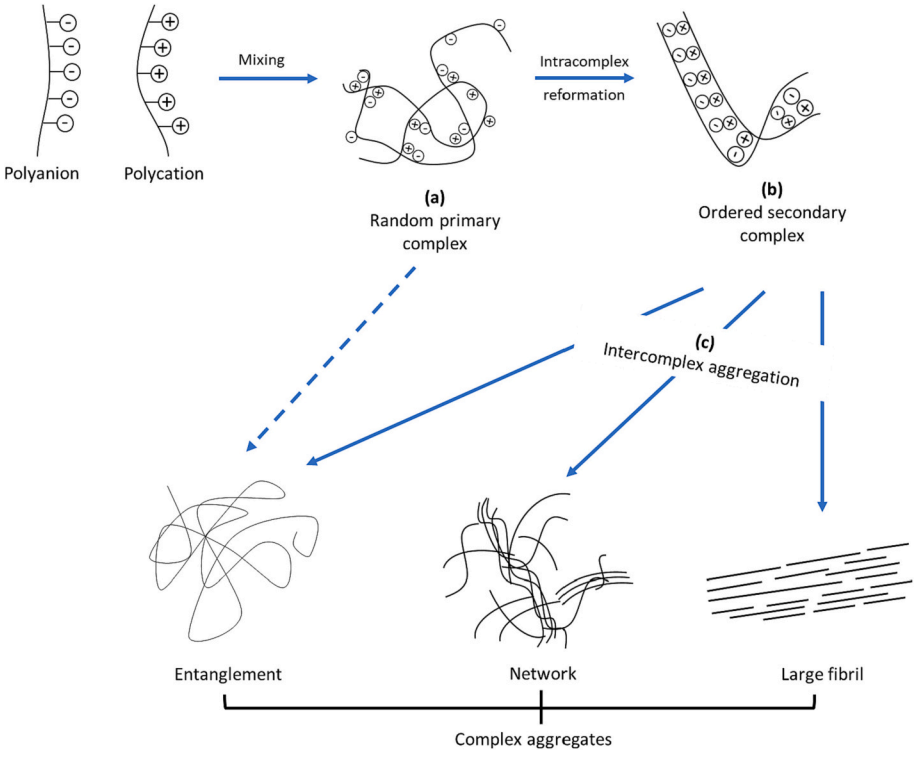

Fig. 4. Representation of PECs formation with the three major steps. (A) Primary complex formation; (B) Formation process within intracomplexes; (C) Inter complex aggregation process. The first step involves the formation of secondary binding forces, the second involves forming new bonds, and the third involves the aggregation of secondary complexes.

of materials. Mahajan and colleagues explored chitosan-gellan gum (CHT-GG) nanogels to encapsulate curcumin for intravenous administration (Mahajan & Patil, 2017). The crosslinking of positively charged chitosan and negatively charged gellan gum *via* coacervation process caused by electrostatic attraction between two oppositely charged polymers resulted in the rapid formation of curcumin-loaded chitosan-gellan gum nanogel. The curcumin-loaded nanogel displayed a positive zeta potential, good entrapment effectiveness, prolonged drug release, hemocompatible nature and no toxicity. These findings suggested that chitosan-gellan gum nanogel could serve as a curcumin carrier for use in the treatment of cancer (Mahajan & Patil, 2017). Another research group developed a versatile platform technology to self-assemble oppositely charged polysaccharides as chitosan and three negatively charged polysaccharides: alginate, gellan gum, and kappa carrageenan into multiscale fibrous hydrogels with controlled anisotropic microarchitecture (Patel et al., 2020). To obtain fibrous hydrogel, the authors tested different methods since bulk mixing until developing a continuous interfacial interaction between oppositely charged polysaccharides with a manual or automated collector, as shown in Fig. 5 (Patel et al., 2020).

In order to allow continuous fiber production *via* interfacial polyionic complexation, the researchers first optimized polymer-related parameters. Hydrogels can be tuned to present different anisotropic fibrous architectures (parallel *vs.* perpendicular) with additional ability to encapsulate small molecules and nanoparticles. The results demonstrated that interfacial mixing of the identical pairs of oppositely charged polysaccharides produced continuous interfacial fibers to a larger extent than bulk mixing of the same pairings (Patel et al., 2020). The automated fiber collector provided robustness to the hydrogel production process and eliminated user-dependent variation, allowing one-step encapsulation of small molecules and nanoparticles without the need for chemical modification or chemical crosslinkers (Patel et al., 2020). Considering that mucoadhesiveness is an important feature of oral drug delivery systems, nanocarriers based on gellan gum could have a higher diffusion and penetration into the mucus layer that covers the intestinal epithelium (Cardoso et al., 2021). Therefore, Cardoso and *co*-workers explored polyelectrolyte complexation between two polyanions gellan gum and retrograded starch (RS) and the positively charged protein bevacizumab (BVZ) to develop an oral delivery system (Cardoso et al., 2021). BVZ solution was dripped into GG/RS dispersion at different ratios under magnetic stirring to obtain non-crosslinked nanoparticles (NPs). A crosslinking step with aluminium chloride was applied to modulate the nanocarriers properties, and a 3^3 full-factorial design to optimize the production process of crosslinked nanocarriers with tailored properties (size, zeta potential, and association efficiency). The main results demonstrated that both polyelectrolyte complexation and ionic cross-linking processes ensured the structural integrity of BVZ loaded into non-cross-linked and cross-linked NPs. Hence, this technology is a suitable approach for developing nanocarriers with tailored features and a potential formulation for the oral delivery of BVZ (Cardoso et al., 2021).

It is well-known that the addition of methacrylate groups to gellan gum improves GG properties and their potential in several areas as tissue engineering and drug delivery systems (Palumbo et al., 2020; Zia et al., 2018). Vieira and *co*-workers encapsulated human adipose-derived stem cells in hydrogel beads through interfacial complexation of anionic methacrylated gellan gum (GG-MA) and cationic poly-L-lysine (PLL) (Vieira et al., 2021). Using a 30G needle mounted in a syringe pump GG-MA solutions were added dropwise to aqueous PLL solutions at concentrations of 0.1 and 0.1 % (*w/v*). These amounts produced robust spherical beads due to the fast electrostatic interaction occurred between the positively charged amino groups of PLL and the negatively charged carboxyl groups of GG-MA Investigations conducted *in vitro* and *in vivo* also demonstrated that the hydrogel beads served as an immunocompatible system for immune recognition. Overall, the modified beads revealed significant potential for hydrogel-mediated cell therapies, particularly in conditions requiring immunoprotection, like Type I diabetes and hepatic diseases (Vieira et al., 2021).

Based on all the studies discussed here, polyelectrolyte complexation is a low-cost, one-step protocol easily applied in the development of drug delivery systems and tissue engineering applications. Nevertheless, it is still necessary to perform additional *in vitro* and *in vivo* studies to validate biomaterials clinical application.

2.3. 3D bioprinting

Scaffolds are 3D porous, fibrous, or permeable biomaterials frequently conceived as temporary structures having the desired geometry and physical, chemical, and mechanical properties (Ma, 2004; Müller et al., 2020). This approach is usually applied in the tissue engineering field and exploits specific and complex physical and biological functions, *in vitro* or *in vivo* by mimicking the extracellular matrix (ECM). Traditional scaffold fabrication methods, such as gas foaming, freezing-drying, fiber adhesion, particle/salt leaching, emulsification, or

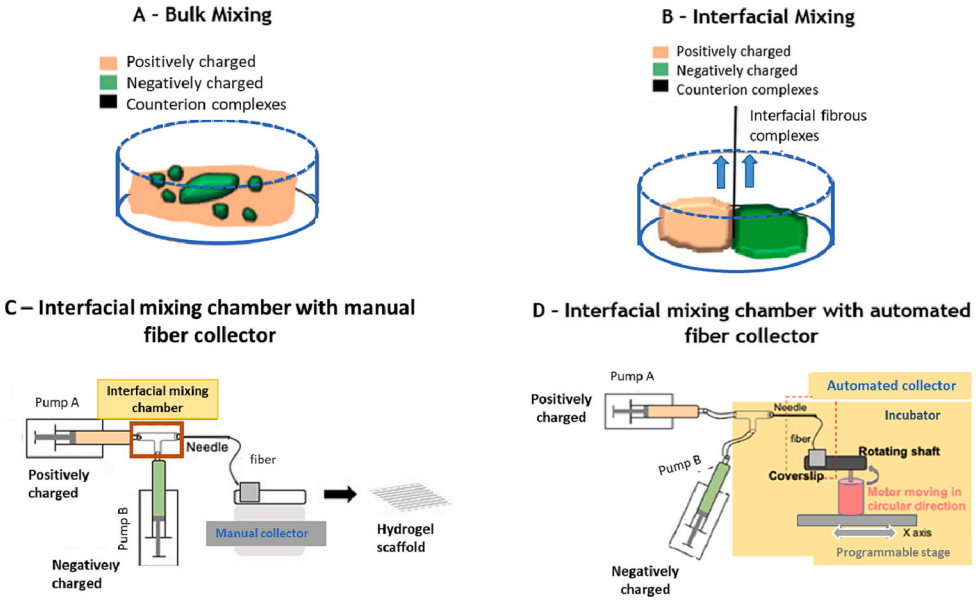

Fig. 5. Different methods to produce fibrous hydrogels using polyelectrolyte complexes adapted from (Patel et al., 2020). A - oppositely charged polysaccharide solutions are mixed in large quantities on a plate shaker, which causes one of the solutions to become encapsulated inside the electrostatically cross-linked membranous interface. B - illustrates how the interfacial mixing of diametrically opposed polysaccharide solutions leads to the development of fibrous complexes. C - Schematic of interfacial mixing setup with manual collector. D - Set up for automated fiber collection using two different types of motors, one moving in a circular motion to collect and cut fibers on a glass slide and the other (programmable stage) moving along a horizontal axis to arrange fibers parallel to one another.

electrospinning, cannot control the scaffold's porosity, pore interconnectivity, or spatial distribution of pores. As a result, accurately mimicking the extracellular microenvironment while meeting the requirements of personalized customization is difficult. 3D bioprinting technology is an ideal solution for producing complex fine structures and allowing for individual customization (Müller et al., 2020; Zhu et al., 2021).

3D bioprinting methods include extrusion, inkjet and laser assisted bioprinting. Extrusion-based 3D printing creates bioengineered 3D constructs with high structural control *via* layer-by-layer deposition (Müller et al., 2020; Pitarresi, Martorana, Palumbo, Fiorica, & Giammona, 2020) and is a pressure-driven technology. As shown in Fig. 6, the bioink is extruded through a nozzle, driven by pneumatic or mechanical pressure, and deposited in a predesigned structure (Kačarević et al., 2018; Pugliese, Beltrami, Regondi, & Lunetta, 2021; Rider, Kačarević, Alkildani, Retnasingh, & Barbeck, 2018).

Micro-nozzle sizes can be used to print highly viscous bioinks. Control over cell deposition, cell distribution rate, and process speed have significantly increased the application of this technology for scaffold fabrication. The ability to print with extremely high cell densities is the primary advantage of extrusion bioprinting. Despite its versatility and advantages, it presents some drawbacks when compared to other technologies. To keep the filamentous structure after deposition, only highly viscous materials can be extruded and the resolution is very limited, as the minimum feature size is typically >100 μm, which is lower than the resolution of other bioprinting techniques (Ma, 2004; Rider et al., 2018). For extrusion-based bioprinting, the rheological behavior of the hydrogel ink is critical. Hydrogels are mostly non-newtonian fluids, and for that reason their viscosity varies with shear rate. Shear thinning has a direct impact on print quality because it allows for the establishment of a plug-like flow, granting a greater control over starting and stopping the extrusion process (Agarwal et al., 2020; Murphy & Atala, 2014). Extrusion bioprinting is a promising technique for producing biomimetic structures that can be used to replace tissues and organs.

Another technique of 3D bioprinting is inkjet printing, a non-contact printing method, which is based on the use of individual droplets to pattern a substrate like in desktop inkjet printers. This technique enables precise cell positioning, allows the fabrication of structures with irregular shapes and high spatial resolution (50 and 300 μm). Nevertheless, cell aggregation within the bioink can alter droplet formation and

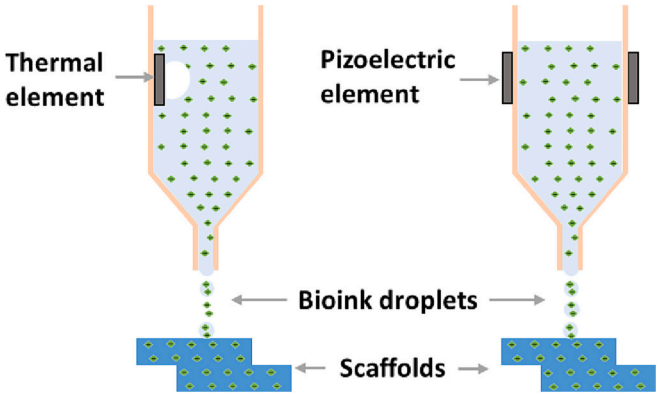

Fig. 7. Schematic of inkjet-based bioprinting. In thermal inkjet, the bioink is forced through the micro-nozzle *via* heat-induced bubble nucleation. Acoustic waves generated by the piezoelectric actuator force the bioink through the micro-nozzle.

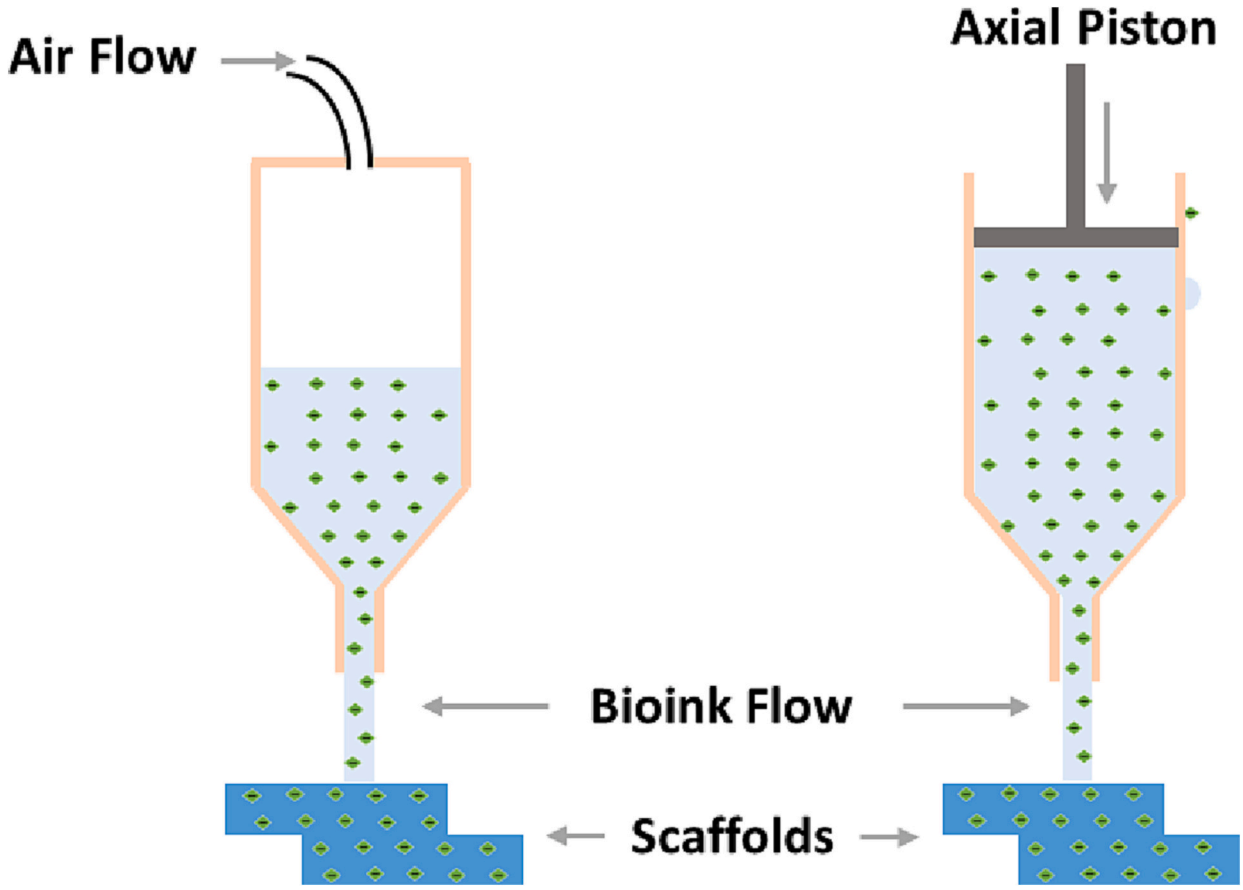

Fig. 6. Schematic of extrusion-based bioprinting: pneumatic-based (left) and mechanical based (right). Micro-nozzles are used to extrude struts under mechanical or pneumatic pressure. Structures with excellent mechanical qualities and print fidelity can be created using extrusion-based approaches.

trajectory, resulting in poor print quality. Fig. 7 depicts how cells and biomaterials are patterned into a desired pattern using droplets ejected *via* thermal or piezoelectric methods (Murphy & Atala, 2014).

In thermal inkjet printing, a heated element is applied to nucleate a bubble that will propel the bioink through the nozzle, resulting in the expulsion of a droplet. As for the piezoelectric method, it is the acoustic waves that eject the bioink from the nozzle. This mechanism restricts the use of highly concentrated and viscous bioinks because their viscosity dampens the applied acoustic/pressure waves, preventing droplet ejection (Agarwal et al., 2020). Overall, the inkjet bioprinting is very appealing due to its high resolution and cell viability with a great potential in tissue engineering and regenerative medicine (Kačarević et al., 2018; Pugliese et al., 2021; Rider et al., 2018).

Finally, laser-assisted bioprinting (LAB) is a scaffold-free method composed by a laser pulse; a donor-slide (or ribbon), which supports and propels the printing material; and a receiver-slide, which collects and supports the printed material, Fig. 8. The donor-slide is made of a layer of clear glass, a thin layer of metal, and a layer of bioink (Murphy & Atala, 2014). Basically, the laser pulse vaporizes the metal layer beneath the hydrogel, forming a bubble that propels the bioink to the receiver-slide, Fig. 3. This scaffold-free technique provides accurate multicell positioning with high cell viability, resolution ranging from 10 to 50 μm and viscosity within the midrange, 1–300 mPa s^{-1} (Kačarević et al., 2018; Malda et al., 2013; Rider et al., 2018). However, LAB systems are quite expensive and limited by the scale of tissue scaffolds it can produce.

In all the 3D bioprinting techniques, the printed material is called bioink. According to Groll and co-workers, an aqueous solution of polymer/hydrogel must have living cells as a component to be considered a bioink (Groll et al., 2018). On the other hand, if the aqueous solution of polymers/hydrogel does not have cells is only considered a biomaterial ink (Jongprasitkul, Turunen, Parihar, & Kellomäki, 2022). Hydrogels appear as one of the most promising basis for bioinks since they have the ability to establish models close to the ECM with a high water content, supporting cell survival and homogeneous distribution within the 3D structure. Additionally, hydrogels enable the formation of structures with several shapes and mechanical properties and biological molecules, as cells and peptide sequences can be easily incorporated (Lozano et al., 2015; Pitarresi et al., 2020).

Gellan gum has many advantages over other hydrogels as a bioink, including shear thinning behavior, high gelling efficiency at physiological temperature, good processability, biocompatibility and low production cost. Pitarresi and collaborators synthesized three gellan gum-*graft*-poly(D,L-lactide-*co*-glycolide) copolymers (GGm-PLGA a, b and c) to generate 3D printed scaffolds (Pitarresi et al., 2020). The modulation of grafting ratio influences the mechanical and physical properties of the scaffolds, as well as their degradation time. The authors claim that the physicochemical properties of novel polysaccharide-graft materials can be easily fine-tuned by adjusting the GG/PLGA ratio according to the desired application (Pitarresi et al., 2020). Another research group combined graphene oxide (GO) with gellan gum to improve the mechanical properties of the system and developed a high-fidelity GG/GO bio-ink for extrusion 3D printing (Zhu, Yao, et al., 2021). The optimal formulation for the scaffold consisted of 1.5 wt% GG and 1.2 wt% GO and curcumin was loaded by an immersion method. The GG/GO/Cur scaffold was pH responsive and suppressed the tumor growth *in vitro* through curcumin release in tumor cells' acidic environment. At the same time, the scaffolds could promote osteoblast attachment and proliferation. This dual-functional scaffold combines tumor therapy and bone regeneration and could be a promising therapeutic approach for the regeneration of tumor surgery-induced bone defects (Zhu, Yao, et al., 2021).

Costa and colleagues produced a hybrid construct for fibrocartilaginous regeneration by coprinting a cell-laden gellan gum/fibrinogen (GG/FB) composite bioink together with a silk fibroin methacrylate (Sil-MA) bioink in a layer-by-layer fashion (Costa et al., 2020). The GG/FB composite constructs revealed enhanced structural stability and mechanical properties by ionic interaction and thrombin treatment. The results showed that the GG/FB bioink provides a proper cellular microenvironment, whereas the Sil-MA bioink provides excellent biomechanical behavior and structural integrity. Altogether these findings support the versatile and promising alternative to produce advance fibrocartilaginous tissue by using a 3D bioprinting technique (Costa, Park, et al., 2020).

A different research group studied the rheological and mechanical properties of gelatin-methacryloyl (gelMA) supplemented with gellan gum for cartilage bioprinting using an extrusion-based method (Mouser et al., 2016). The authors found that the addition of gellan gum to gelMA hydrogels improves filament development and deposition by inducing yielding behavior, increases the construct rigidity/firmness, and promotes matrix production of embedded chondrocytes. The most suitable formulation for 3D printed cartilage constructs was obtained with 10 % gelMA and 0.5 % gellan gum with posterior UV crosslinking. This combination revealed the greater potential of gelMA/gellan blends for cartilage bioprinting where the yield stress was identified as a dominant factor for bioprintability (Mouser et al., 2016).

Wu and colleagues developed a double hydrogel combining gellan gum and poly(ethylene glycol) diacrylate (PEGDA) by extrusion-based

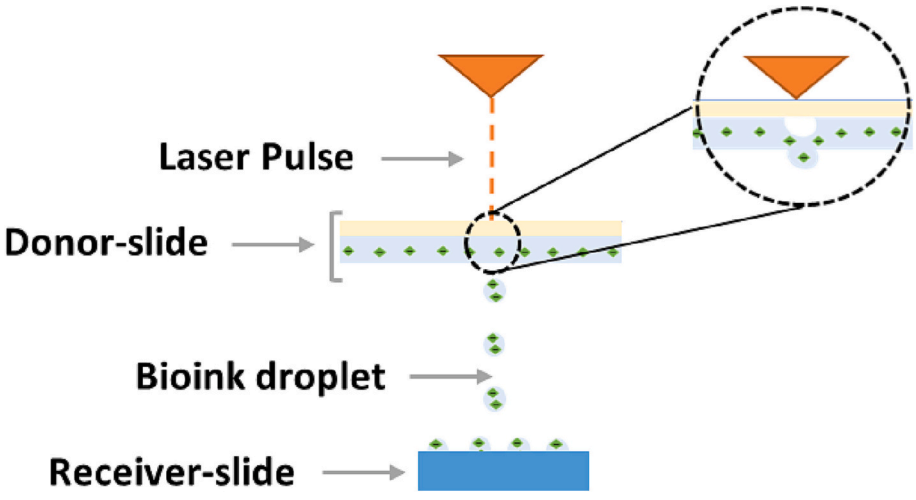

Fig. 8. Schematic of laser-assisted bioprinting. Droplets of bioink are propelled towards the substrate by bubble nucleation brought on by laser radiation. A scaffold, polymer sheet, or biopaper can serve as a receiver-slide.

3D bioprinting with cells for tissue engineering applications (D. Wu et al., 2018). The best combination consisted of 10 % PEGDA and 1.5 % GG followed by UV crosslinking to achieve an interpenetrating polymer network structure that was strong and stretchable. GG/PEGDA hydrogel was stable, non-toxic, and biocompatible, making it an excellent candidate to produce human-on-a-chip systems as well as biomimetic complex organs (D. Wu et al., 2018). The same research group combined poly(lactic acid) PLA with the double network hydrogel GG/PEGDA and electrospinning fibers to construct an intervertebral disc scaffold (Zhu, Tan, et al., 2021). The mechanical strength of the composite scaffold is comparable to that of natural intervertebral discs, and it is cytocompatible. *In vivo* studies revealed that the artificial IVD scaffold can maintain disc height and promote proteoglycan deposition. This tissue-engineered stem cell-loaded IVD scaffold has the potential to regenerate a degenerated or injured IVD (Zhu, Tan, et al., 2021).

Bone tissue engineering may require that biomaterial inks contain ceramic particles which can lead to inconsistent printing results (Müller et al., 2020). Muller and co-workers decided to optimize the 3D extrusion-based bioprinting process of the ink composed by gellan gum methacrylate (GG-MA), hyaluronic acid methacrylate (HAMA) and hydroxyapatite (HAp) particles. First, a reproducible large batch synthesis of GG-MA was established, followed by the ink preparation obtained by mixing 4 % w/v GG-MA and 1.5 % w/v HAMA with different amounts of hydroxyapatite particles. After UV-crosslinking, the links exhibited shear thinning behavior and appropriate stiffnesses. The addition of HAp to the GG-MA/HAMA mixture improved MSC adhesion to the ink as well as cell viability (Müller et al., 2020).

Considering that photocrosslinkable bioinks have gained popularity in 3D bioprinting because of their versatility and ease of use, Jongprasitkul and colleagues developed a two-step crosslinking method, which made unprintable GGMA into fiber-forming, stackable biomaterial ink capable of forming 3D hydrogel constructs *via* extrusion-based 3D bioprinting (Jongprasitkul et al., 2022). First, a pre-crosslinking approach *via* coordination bonding of carboxylate groups to calcium ions provided enough stability to keep the shape before photocrosslinking with UV. In the presence of an ionic crosslinker, GGMA transformed from a liquid precursor to a weak extrudable hydrogel, which was then photocrosslinked to transform it into a true hydrogel with good shape fidelity. The two-step crosslinking technique produced a biomaterial ink with good printability and allowed for the printing of genuine 3D constructs. As a result, pre-crosslinked GGMA may be useful for a variety of applications, namely in tissue engineering (Jongprasitkul et al., 2022).

Lozano and co-workers utilized a newly developed handheld reactive bathless printing process (3D printing) to easily create free-formed 3D structures for dissociated primary cortical neural cells based on peptide-modified GG, Fig. 9 (Lozano et al., 2015). Cortical neurons were printed using two different ionic crosslinkers, 1 M $CaCl_2$ or (5×) DMEM, and it was discovered that both crosslinkers supported similar cell viability after 5 days. The low-cost, simple extrusion printing technique and bio-ink (RGD-GG) could be used to create contained, layered, and viable 3D cell structures, with the potential to organize cortical neuron subtypes in layer structures. These brain-like structures have the potential to provide a more accurate representation of 3D *in vivo* environments, which could have applications ranging from cell behavior studies to understanding brain injuries and neurodegenerative diseases to drug testing (Lozano et al., 2015).

The ability of GG fast gelation shows its suitability for application in reactive inkjet printing. Also, its high transparency and biocompatibility suggests GG as an appropriate material for corneal application (Duffy, Liang, Williams, Wellings, & Black, 2021). Therefore, Duffy and *co*-workers printed porous hydrogel structures based on the reactive inkjet printing of poly-ε-lysine and gellan gum (Duffy et al., 2021). The immediate reaction between two inks creates a unique honeycomb-like topography and the best ink was achieved with 20 wt% pεK and 0.35 wt% GG. After 10 days in culture, printed hydrogels endorsed the growth of a monolayer of corneal epithelial cells, which demonstrated significant cell attachment. With the high resolution of inkjet printing, it is possible to replicate a complex structure such as that of the human cornea; however, more optimisation of printing pK/GG is required to achieve this goal (Duffy et al., 2021).

Amiri and colleagues explored the structural and biological properties of 3D-printed polyurethane (PU) scaffolds that had been dip-coated with gellan gum (GG), hyaluronic acid (HA), and glucosamine (GA) for tissue engineering applications (Amiri et al., 2022). The optimum GG concentration was 3 % (w/v) while maintaining porosity at 88.1 %. The GG-HA-GA surface coating on the PU scaffolds increased the compression modulus, the water uptake, the degradation rate, and the contact angle. The ternary surface coating stimulated cell proliferation, viability, and adhesion and also increased the levels of collagen II and aggrecan gene expression. As a result, the fabricated scaffold is a promising scaffold for meniscus tissue engineering. *In vivo* experiments with long-term monitoring are still required to assess the clinical validation of these hybrid scaffolds for meniscus regeneration (Amiri et al., 2022).

2.4. Solvent-casting method

According to the Royal Society of Chemistry the solvent casting method consists of "a process for forming thermoplastic polymer samples by dipping a mould into a solution of the polymer and drawing off the solvent to leave a polymer film adhering to the mould". This is an old and very simple way to obtain films, but it is also used to produce other materials as scaffolds (Kim et al., 2019; Razali, Ismail, & Mat Amin, 2020). Gellan-based materials produced by casting method are mainly applied in wound healing (Razali et al., 2020), food packaging (Balasubramanian, Kim, Lee, & Lee, 2019; Kim, Thangavelu, et al., 2019) and bone tissue engineering (Kim et al., 2020; Kim, Thangavelu, et al., 2019) due to GG low cost, availability, biodegradability and good film forming properties.

Mirón-Mérida and colleagues used a coffee parchment waste (CP) in combination with gellan gum to develop a film as an alternative in bioactive food packaging (Mirón-Mérida, Yáñez-Fernández, Montañez-Barragán, & Barragán Huerta, 2019). After glycerol addition, films were produced by casting on polytetrafluoroethylene (PTFE) plates for 24 h at 60 °C, followed by deposition for 24 h at 20 °C in a desiccator. GG/CP films demonstrated growth inhibition against *Fusarium sp., Colletotrichum gloeosporioides, and Fusarium verticillioides* and comparable mechanical qualities regardless of the extract concentration. Although the

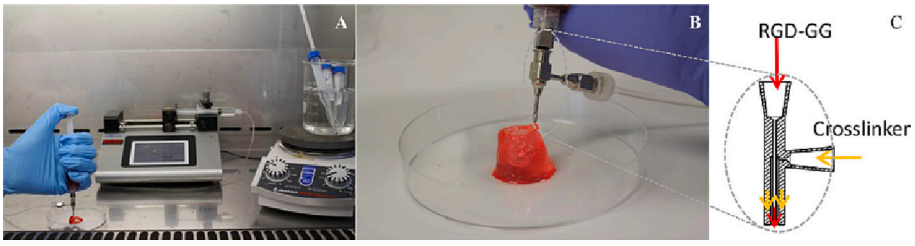

Fig. 9. Hand-held bathless printing process (adapted from (Lozano et al., 2015)).

addition of the CP extract resulted in certain structural alterations, the antifungal qualities and general performance of GG/CP extract films make them appropriate for additional uses in food packaging (Mirón-Mérida et al., 2019). Following this line of investigation, Balasubramanian and team incorporated titanium dioxide (TiO_2) nanoparticles into a ternary blend of natural polysaccharides' film to improve their functional properties as packaging material (Balasubramanian et al., 2019). The hydrogel based composite film was prepared from *k*-carrageenan (*k*-C), xanthan gum (X) and gellan gum (G) by solvent casting method. The transparent films produced with these gellable materials have a soft texture, outstanding elasticity, and great break strength. The utilization of the k-C/X/G and TiO_2 nanocomposites as UV-shielding packaging materials in the food and non-food industries has a high potential, since they successfully blocked UV radiation and demonstrated some microbiological activity against *Staphylococcus aureus* (Balasubramanian et al., 2019). Besides TiO_2 being an interest as a UV filter due to its optical and electrical properties, it is also explored in drug/gene delivery, cell imaging, cancer therapy and synthesized polymer nanocomposites as reinforcement materials (Balasubramanian et al., 2019; Razali et al., 2020). Razali and co-workers combined TiO_2 nanotubes (TiO_2-NTs) and gellan gum film to develop a wound dressing material (Razali et al., 2020). The applied methodology consisted of combining 1, 5, 10, 15 and 20 *w*/w% (percentage weight relative to GG) of TiO_2 nanotubes to a gellan gum solution for 2 h at 70 °C, followed by pouring on a petri dish and dried at 50 °C for 24 h. The mechanical characteristics of gellan gum were improved by the addition of TiO_2-NTs, which were uniformly disseminated throughout the gellan gum matrix films. *In vivo* experiments showed that the GG + TiO_2-NTs accelerated wound healing compared to wounds that received no therapy, and the films could be removed without causing any damage to the freshly repaired tissue. According to this study, GG + TiO_2-NTs films present great potential as a material for wound dressings (Razali et al., 2020).

Considering GG versatility, excellent capability for alteration and optimization to suit a particular application, Kim and colleagues combined demineralized bone powder (DBP) with GG to obtain scaffolds for bone regeneration (Kim, Thangavelu, et al., 2019) and osteochondral lesions (Kim, Cho, et al., 2020). The procedure was based on casting solvent method followed by lyophilization/freeze-drying. Gellan gum was dissolved with a small amount of crosslinker ($CaCl_2$), followed by the addition of DBP solution, which after cooling to 60 °C were poured into a petri dish. After 30 min the fabricated hydrogels were punched out using a biopsy punch and lyophilized to obtain a sponge (Kim, Cho, et al., 2020; Kim, Thangavelu, et al., 2019). On both studies, the best composition consisted of 1 % DBP/GG, which displayed outstanding compressive strength, porosity, degradation, biocompatibility, and osteochondral regeneration. In addition, several techniques confirmed chondrocyte differentiation and bone regeneration, which was supported by *in vivo* studies in rats and rabbits (Kim, Cho, et al., 2020; Kim, Thangavelu, et al., 2019). This scaffold can be a superior substitute for the enhanced and modified surgical graft for applications, involving osteochondral/bone tissue regeneration. Nevertheless, it is necessary to study the scaffold on larger animals before applying in humans.

2.5. Ionotropic gelation or ionic crosslinking

Natural polyelectrolytes have specific anions and cations and by combining with opposing ions, these ions form meshwork structures that cause gelation through crosslinking. Natural polymers function as a release rate retardant in addition to coating/deliver drugs (Patil, Chavanke, & Wagh, 2012). Ionotropic gelation is a process that creates hydrogels or beads, by allowing polyelectrolytes, as gellan gum, to crosslink in the presence of cations. It is the most used technique for producing gellan-based particles due to its simple use and nontoxicity (Iurciuc et al., 2018; Lyu et al., 2019; Meneguin, Beyssac, Garrait, Hsein, & Cury, 2018; Vieira et al., 2019). By adding a drug-loaded gellan solution to an aqueous solution of divalent or trivalent cation solution, the gelation approach produces particles. In the drug-loaded polymeric droplets, the cations spread and create a three-dimensional lattice of ionically crosslinked moieties. Under mild conditions, biomolecules can also be inserted into these gelispheres to maintain their three-dimensional structure. When applying ionotropic gelation method it is important to have in mind the following factors, since they greatly affect the final product. Polymer and crosslinking electrolyte concentration, temperature, pH of crosslinking solution and drug concentration need to be adjusted for each formulation due to their impact on reaction rate, drug entrapment efficiency, shape and size of beads (Meneguin et al., 2018).

In the case of GG ionotropic gelation, calcium and aluminium are the cations most applied to produce hydrogel beads ranging from nano to microscale (Adrover et al., 2019; Dey, Ghosh, & Giri, 2020; Jana, Pramanik, Nayak, & Sen, 2022; Modi et al., 2022). Two research groups explored gellan-based beads crosslinked with calcium ions to achieve drug delivery systems with improved stability in the gastrointestinal tract (Adrover et al., 2019; Dey et al., 2020). Dey and co-workers extruded a quercetin and gellan gum solution into a calcium chloride solution leading to hydrogel beads formation due to ionic crosslinking (Dey et al., 2020). The authors found that the entrapment, swelling and drug release profiles depend on the amount of crosslinker and quercetin used. Quercetin-loaded GG beads displayed a pH sensitive behavior and improved intestinal stability when compared to free quercetin. Considering that quercetin displays anticancer activity further studies are necessary to validate this material and see if the interaction between quercetin and GG does not comprise its applicability (Dey et al., 2020). Adrover and team also applied GG crosslinked with calcium to obtain hydrogel beads but studied the effect of adding clay laponite on the sustained drug release (Adrover et al., 2019). The presence of laponite in the bead formulation enhanced the drug entrapment effectiveness of two model drugs, theophylline and vitamin B12 (Adrover et al., 2019). Since both drugs were completely released in the first two hours of residence in Simulated Gastric Fluid without laponite, the results indicate that laponite may be a useful addition in the development of GG beads for sustained release of drugs (Adrover et al., 2019). Although both works performed an extensive characterization of the drug delivery systems, additional studies are required to validate their potential clinical application. Ideally the drug delivery system should present a size at nanoscale in order to be applied *in vivo* and understand its effectiveness and efficacy. Therefore, Modi and colleagues applied ionotropic gelation method to obtain tacrolimus-gellan gum nanoparticles (TGNPs) for the effective management of dry eye disease (DED) (Modi et al., 2022). Tacrolimus was dissolved in methanol and added dropwise to an acidic GG solution, which was after extruded into aluminium chloride (ALC) solution under continuous stirring. The results revealed that concentrations of GG and ALC influenced the particle size, zeta potential and % entrapment efficiency of the TGNPs. An increase in nanodispersion size was seen when crosslinker concentration was increased and higher GG concentrations led to microparticles formation. TGNPs showed improved tacrolimus entrapment efficiency (74.2 %) and loading capacity (36.1 %) with extended drug release over and increased precorneal retention. Furthermore, pharmacological experiments demonstrated that TGNPs were successful in the treating of DED symptoms, and the safety of the formulation for ocular usage was also validated. Therefore, the topical administration of TGNPs may hold promise for effective DED therapy (Modi et al., 2022). Interpenetrated polymer network (IPN) based microparticles/beads of GG have gained research interest to improve the properties of ionically crosslinked beads (Jana et al., 2022). Jana and team blended GG and carboxymethyl tamarind gum (CTG) followed by ionotropic gelation with ALC as crosslinker to obtain sustained drug release systems (Jana et al., 2022). DFS-loaded CTG-GG IPN microbeads demonstrated prolonged sustained release over an 8 h period, and *in vivo* studies suggested sustained anti-inflammatory action. These findings demonstrated the capability of CTG-GG IPN microbeads loaded with DFS to sustain long-lasting anti-

inflammatory activity (Jana et al., 2022). Ionotropic gelation method is a promising approach for developing drug delivery systems with controlled release because it is simple to scale up, does not utilize dangerous chemicals, and is inexpensive.

2.6. Emulsification

Emulsions are easily classified as either oil-in-water (O/W) or water-in-oil (W/O) emulsions or systems, according to the continuous phase. An oil-in-water (O/W) emulsion is defined as oil droplets dispersed in an aqueous phase, whereas a water-in-oil (W/O) emulsion is defined as water droplets dispersed in an oil phase (McClements, 2007). For example, to create a water-in-oil emulsion (W/O), an aqueous biopolymer solution such as gellan gum is homogenized with an oil phase containing an oil-soluble emulsifier. The size of water droplets can be modified by adjusting either the homogenization conditions (pressure and number of passes) or the solution composition (oil-to-water ratio, emulsifier-water ratio). The inner water phase is then gelled using a mechanism appropriate for the biopolymer used, such as temperature change, cross-linking agent addition, or pH/ionic strength change. Finally, the biopolymer particles can be obtained by centrifuging/filtering the W/O emulsion, collecting the particles, and washing them to remove any remaining oil. In terms of particle formation, this technique is extremely useful for industrial applications because it can be easily scaled-up when compared to nozzle-based methods (Giri, 2016).

In the case of gellan gum, the emulsions are mainly applied in drug delivery and encapsulation of target molecules. Dhanka and colleagues applied a simple water-in-oil emulsion for encapsulating methotrexate (MTX) into GG microparticles (Dhanka, Shetty, & Srivastava, 2018). The developed microparticles were spherical in shape and had a rough surface morphology. MTX was encapsulated into GG microparticles with high encapsulation efficiency (84.8 %) and loading capacity. *In vitro* release study of MTX-GG MPs showed 84 % drug release within 24 h and the microsphere system was hemocompatible with human red blood cells and with L929 fibroblast cells. The potential *in vivo* injectable application of MTX-GG MPs was emphasized by the authors (Dhanka et al., 2018).

Several works have been combining the emulsification technique with other methods, namely crosslinking and ionotropic gelation to obtain GG particles (Coelho et al., 2019; Kuhn, e Silva, Netto, & da Cunha, 2019; Park et al., 2021). The main reason to combine emulsification with these techniques is to increase the hardness and stability of particles (Kuhn et al., 2019; Park et al., 2021). Kuhn and co-workers developed microbeads of whey protein (WPI) and gellan gum to encapsulate flaxseed oil and protein hydrolysate (FPH) as a way to avoid the functionality and bioavailability loss before their release in the gastrointestinal system (Kuhn et al., 2019). Microgels were resistant to gastric conditions, but they disintegrated under intestinal conditions in the presence of enzymes, releasing the encapsulated flaxseed oil. Thus, flaxseed oil and protein hydrolysate could be encapsulated and protected throughout digestion and released in the small intestine using GG microbeads (Kuhn et al., 2019). When devising or optimizing a protocol for obtaining a material the design of experiments (DoE) tool is excellent since it allows to evaluate the effect of single or multiple changes to a process or design limiting the risk of a wasted effort. Considering the vast literature available on the production of gellan gum particles, Coelho and her research team applied the DoE tool to create GG microspheres with reduced diameter using a W/O emulsion followed by ionic crosslinking (Coelho et al., 2019). The authors applied a Central Composite Design (CCD) in the formulation of gellan microspheres, considering 3 inputs (gellan concentration, stirring velocity, and temperature), which revealed to be the most critical factors that affect the gellan microspheres formulation. The authors used crosslinked GG microspheres to capture model proteins, which was proven successful (Coelho et al., 2019) and demonstrated the potential applicability in the direct capture of proteins (bovine serum albumin, lysozyme and catecol-*O*-methyltransferase) from microbial complex lysates (Gomes et al., 2021).

Overall, the emulsification technique is widely applied to obtain gellan-based particles but is usually combined with other methods to form particles with higher stability and enhanced mechanical resistance. Microfluidic technology allows to fine control the template dimensions and offers high flexibility regarding the materials that can be used, making droplets with smaller size and lower polydispersity (Costa, Gomes, Ushikubo, & Cunha, 2017). Therefore, several researchers have been exploring the combination of this technology with emulsification to obtain gellan-based particles with great properties. Additionally, this method has the benefit of creating droplets with less energy consumption than traditional techniques, which reduces the rate at which temperature- and pressure-sensitive chemicals deteriorate. In order to prepare microgels in microfluidic devices, a biopolymer droplet must first be generated (emulsification); next, a chemical or physical crosslinking agent must gel the droplet. The appropriate configuration of the microfluidic device, the characteristics of the liquid phases (interfacial tension and viscosity), and the processing conditions allow for precise control of the droplet size during the emulsification step (flow rate of both phases). The choice of the biopolymer, the gelling/crosslinking agent, and the residence period of the droplets are also relevant inputs in the gelation step's difficulty in creating microgels (Costa et al., 2017; Michelon, Leopércio, & Carvalho, 2020; Santos, Costa, Michelon, Costa, & Cunha, 2020).

Gellan microgels incorporated with *jabuticaba* extract were produced by externally gelating monodisperse W/O emulsions (Santos et al., 2020). The authors' findings demonstrated how to manufacture and control the microgel diameter and polydispersity by adjusting the flow rate of continuous phase at a fixed flow rate of the dispersed phase. Microgels' diameters ranged from 185 μm to 342 μm. The low gellan content and the osmotic pressure difference during storage were the main causes of the low stability of microgels loaded with jabuticaba extract. Despite this, the suggested microfluidic approach may be expanded to include other hydrophilic molecules and applied to include and protect anthocyanin extracts (Santos et al., 2020). Another research group used a similar approach to encapsulate a hydrophilic dye, rhodamine B (Costa et al., 2017). This work investigated different design of microchannels for encapsulating hydrophilic substances in gellan microgels made by the external gelation process employing planar microfluidic devices. In the first design, gellan solution was previously mixed with the hydrophilic dye and then loaded to the same microchannel, whereas for the second strategy gellan solution and the dye were introduced separately and collided each other at the microchannel junction. Gellan microgels were created, ranging in size from 63 to 113 μm on average, with a homogeneous, spherical shape. After ten days of storage, gellan microgels remained stable. Therefore, the characteristics of the biopolymer and the disperse phase, as well as the particle applications and process circumstances, will all play a role in the decision of the encapsulating design of hydrophilic compounds in microfluidic devices (Costa et al., 2017).

Gellan-based particles produced by microfluidic technology are also capable of encapsulating hydrophobic compounds. Michelon and his team studied the process to obtain microcapsules by ionotropic gelation of gellan gum from monodispersed oil-in-water-in-oil (O/W/O) double emulsion templates using glass capillary microfluidic devices (Michelon et al., 2020). The range of microcapsule diameter increased along with the capillary's diameter, so, devices with smaller injection and collecting capillary tip widths can produce smaller capsules. For encapsulating, delivering, and controllably releasing hydrophobic active chemicals in a water-based medium, the proposed technology may be applied in a wide variety of situations (Michelon et al., 2020).

Table 1 provides a summary of the advantages and drawbacks of the methods applied to obtain gellan-based materials, discussed in this section. Overall, some of the described methods can stand alone to produce gellan-based materials with good features and the potential to

Table 1
Advantages and drawbacks of the preparation methods of gellan-based materials that have been described in Section 2.

Methods to obtain GG-based materials	Advantages	Limitations
Electrospinning	Easily scaled up for industrial production. The fine fibers have a high surface area-to-volume ratio, making them suitable for various applications such as tissue engineering, drug delivery, and filtration. Simple process.	Requires specific conditions such as high concentration and high voltage. Can form gel-like structures that can clog electrospinning needles. The uniformity of the fibers produced can be difficult to control. The fibers can have poor mechanical properties, making them unsuitable for some applications where high strength is required.
Polyelectrolyte complexation	Materials with improved stability and mechanical properties compared to simple physical mixtures of the polymers. Improved water-holding capacity and water-retention. Ability to form gel-like structures with tunable properties. Increased surface area and improved release properties for active ingredients.	Requires specific conditions such as pH, ionic strength, and concentration. Can be difficult to dissolve and disperse in water and organic solvents. The kinetics of polyelectrolyte complexation can be slow, making it difficult to form complexes in a timely manner.
3D Bioprinting	Allows for the creation of customized, patient-specific tissue structures and implants. Rapid production of complex tissue structures. Potential to reduce the costs associated with traditional tissue engineering methods. Improved accuracy by precisely control the orientation and organization of cells during printing.	Limited solubility in water and organic solvents, requiring the use of specialized solvents. Requires specific conditions for gelation and stability, such as pH and ionic strength. The process is still in its early stages and the technology is not yet advanced enough to create functional, complex tissues. The technology has not yet been perfected to allow for large-scale production of functional tissue structures. The cost is still high.
Solvent-casting	Simple and inexpensive method of preparing thin films and coatings. The method is versatile and can be used with a wide range of polymers. Easy to scale up. Good control over film thickness, which can be easily adjusted by changing the concentration of the polymer solution.	The method involves the use of solvents, which can leave behind residue in the final product. The resulting films may have poor mechanical properties, such as low tensile strength and brittle fracture. The time required for the solvent to evaporate and the film to dry can be long, leading to slow production times.
Ionotropic gelation or Ionic crosslinking	Ability to form gel-like structures with tunable mechanical properties. Fast process, allowing for the rapid formation of gels in seconds to minutes. The method is versatile and can be used with a wide range of polymers.	Ionotropic gelation is sensitive to pH, and variations in pH can affect gel properties. Ionotropic gels may have limited stability and can break down over time, especially in the presence of enzymes or changes in temperature or pH. Some of the agents used in ionotropic gelation, such as salts and cross-linking agents, may be toxic and limit their use in medical applications.
Emulsification	Create uniform dispersions. Increased surface area by the formation of small droplets. Enhance the absorption and bioavailability of drugs, nutrients, and other substances.	Emulsions are often thermodynamically unstable and can separate over time, requiring the use of stabilizing agents. High viscosities are difficult to handle and pump. Sensitive to shear forces and can break down or change properties in high shear environments.

be applied in clinical or industrial scenarios. Nevertheless, several studies reported combining different methods to tailor the materials' properties with the desired aim and fulfill its limitations.

3. Gellan-based materials characterization techniques

Gellan-based materials are receiving a growing attention being used in a wide range of areas from biomedical field to environmental protection, to food and cosmetics industry. With this great diversity, there is the need to understand the properties of different materials. The characterization of materials is known as the process of studying the morphological and functional properties of various substances, with the aim to learn critical information, such as their level of resistance and dependability, as well as their potential applications. As result, the material analysis facilitates the characterization of physical, chemical, and mechanical properties, which could be used to predict the materials performance.

For example, the morphological changes that occur at the materials surface can be studied by scanning electron microscopy (SEM), field emission scanning electron microscope (FESEM), transmission electron microscopy (TEM) or atomic force microscope (AFM). It can be noticed from literature that SEM micrographs are the most used to assess the morphological changes on the polymer surface and failure characteristics (Šlouf, Vacková, Lednický, & Wandrol, 2014). Morphological characteristics usually assessed in gellan-based materials are (1) structural shape, (2) change in surface texture, (3) modification in the characteristics due to the addition of other materials, (4) porosity and (5) elemental distribution and composition. SEM is a technique that generates images of a sample by scanning it with an electron beam. The electrons interact with the atoms on the sample surface, resulting in a variety of signals that reveal information about the sample surface's morphology and composition. For SEM analysis, samples must be electrically conductive at the surface. In this way, polymeric materials are normally coated with an electrically conducting material, as gold or platinum (Muthukumar et al., 2020; Sinha Ray, 2013). In some cases, Energy dispersive X-ray (EDX) is coupled with SEM to give information about the elemental composition of the materials' surface. Nevertheless, to obtain high-quality images at nanoscale, AFM or TEM are preferred once SEM and FESEM have a resolution around 100 nm (Muthukumar et al., 2020). AFM is based on the displacement of a tip with a very small radius of curvature (~20 nm) along the surface of a sample to be analysed. This technique allows to obtain numerous information on the properties of materials as adhesion, roughness, elasticity, transport, magnetism and thermal conductivity. AFM can be operated in two basic modes: the contact and tapping mode. In the first, the tip of the cantilever is in continuous contact with the surface, while in the tapping mode the cantilever is vibrated above the sample surface because the tip only interacts intermittently with the surface. In general, the AFM technique provides non-destructive 3D information with high resolution without the need of vacuum or beam interaction. It does not require a lot of sample preparation, but there is a limitation in the height of the

samples (<20 μm) (Rydz, Šišková, & Andicsová Eckstein, 2019; Sinha Ray, 2013).

Dynamic light scattering (DLS), also known as photon correlation spectroscopy, and zeta potential have emerged as simple techniques for investigating particle (hydrodynamic) size and surface charge, respectively (Raval et al., 2019). DLS assesses the hydrodynamic size of particles using the light scattering mechanism of a laser passing through the colloidal solution and analysing the temporal fluctuations of the light scattered due to the Brownian motion of particles. DLS is sensitive, non-invasive, requires minimal sample preparation and avoids pre-experimental calibration, which makes it a powerful analytical tool, usually applied in the characterization of macromolecules and colloids in solution (Nimesh, 2013). However, some weaknesses consist of narrow resolution, which prevents the recognition of particles that differ from each other for a factor lower than 3, and the analysis of coloured or fluorescent samples (Ghezzi et al., 2021). Zeta potential is an important parameter to evaluate stability and determine the interaction of particles, being used to measure the net surface charge of micro or nanoparticles. In the case of medical or pharmaceutical formulations, a zeta potential greater than −30 mV or +30 mV allows a long-term stability since the particles are considered stable (Gupta & Trivedi, 2018). Considering all the above, the use of two different but complementary techniques provide an overall evaluation of particle size and morphology.

For molecular structure characterization, the main techniques applied are Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD) and Energy-Dispersive X-Ray Spectroscopy (EDX) coupled to SEM, since it is possible to understand which elements, functional groups and bonds constitute the material. Usually, for FTIR analysis, a sample is exposed to electromagnetic radiation in the infrared region of the spectrum. The wavelengths absorbed by the sample are determined by the chemical groups present (Ebnesajjad, 2014; Tomovska, Agirre, Veloso, & Leiza, 2014). It is widely used for qualitatively identifying molecular structure (*e.g.*, functional groups and bonds) based on target frequencies, and quantitatively determining sample constituent content based on band intensity (Pakzad, Fathi, & Mozafari, 2019). XRD is used to characterize materials' properties like crystal structure and crystallite size and strain. Usually, the sample is passed through to a beam of X-rays that are scattered, or diffracted, which allows to measure the intensity and scattering angles of the X-rays (Raval et al., 2019; Sinha Ray, 2013). This technique is vastly applied to study the material structure at an atomic level, despite having some limitations (difficulty in analysing the growing crystal in real time).

Thermal properties analysis is important to develop new polymer-based materials, and in the biomedical field, thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC) are the most applied. These techniques allow the understanding of how physical properties are affected by temperature change (Pakzad et al., 2019). The DSC is used to measure the amount of absorbed and released energy of materials as a function of time and temperature, while the sample is exposed to a controlled temperature program. The TGA allows the measurement of thermal stability by assessing sample weight changes, while its temperature is being increased (Ebnesajjad, 2014; Pakzad et al., 2019).

Mechanical properties, such as polymerization degree, crosslinking, polymer structure, and functionality, can influence the biocompatibility of polymeric biomaterials. Tensile testing can determine the most important mechanical parameters in biomedical applications (for example, tensile strength, compressive strength, and elastic or Young's modulus). The impact of these parameters will differ depending on the application of biomaterials. Rheometry, which measures viscosity and storage modulus, is commonly used to assess the viscoelastic behavior and rheological properties of polymeric biomaterials (Pakzad et al., 2019; Raval et al., 2019).

Typically, material characterization is an essential research tool when it comes to understand the properties and behaviours of gellan-based materials considering the final application. Briefly, Table 2 condenses some of the most applied characterization techniques in gellan-based materials.

4. Gellan-based materials in classic and emerging applications

Traditionally, GG was mainly used in the food industry by virtue of its excellent gelling properties, high transparency, and good flavor release (Fialho et al., 2008; Giavasis et al., 2000). The fact that GG exhibits thermal and acidic stability also allowed GG incorporating food products to undergo harsher processing operations (Yu, Kaonis, & Chen, 2017; Zia et al., 2018). The GG matrix can have several different functions depending on the type of food product where it is included. For instance, in dairy products, GG acts as a bulking and thickening agent (Bajaj et al., 2007). When applied in jams, jellies and pie fillings, GG enhances overall stability and performs a water-binding service, preventing moisture fluctuations and minimizing syneresis (Bajaj et al., 2007; Zia et al., 2018). For fabricated foods and confectionary and bakery products, GG functions as a structuring and texturizing agent, which can enhance organoleptic properties (Giavasis et al., 2000; Prajapati et al., 2013). On top of that, GG can partially replace starches, hindering their dulling effect on food flavor (Bajaj et al., 2007; Giavasis et al., 2000). The fact that GG could be employed in a wide array of food products with a diverse spectrum of roles, made it a very popular and efficient food additive. Nevertheless, GG found success in other applications, namely, in cosmetic, personal care and dental industry. The shear thinning rheology of GG gels made it ideal for the stabilization and suspension of emulsions in shampoos, conditioners, hair care products and air freshener formulas (Prajapati et al., 2013). In fact, GG could also be responsible for an improved lubrication feel in lotions and creams (Prajapati et al., 2013; Sajna, Gottumukkala, Sukumaran, & Pandey, 2015). It also plays a binding role in toothpaste formulations, exhibiting excellent flavor release (Prajapati et al., 2013). Furthermore, GG was employed in the biotechnology industry as an agar substitute, to produce solid culture media (Bajaj et al., 2007; Banik, Kanari, & Upadhyay, 2000). This behavior is extremely positive, since GG does not contain impurities, which were often present in agar and could be toxic to sensitive microorganisms (Prajapati et al., 2013). Several reports also confirmed that GG could be used as a culture medium for plant tissue cultivation, exhibiting higher levels of cell proliferation, rooting and embryogenesis rates when compared to agar media (Bajaj et al., 2007; Giavasis et al., 2000).

At the turn of the century, new applications for GG based materials emerged and proved the versatility and the potential of GG. Indeed, GG edible coating films were developed to reduce fat uptake during the frying process of food products (Albert & Mittal, 2002; Williams & Mittal, 1999). Another developed strategy was the encapsulation or immobilization of cells within GG beads. Moslemy and coworkers showed that the immobilization of activated sludge within GG beads significantly increased the biodegradation of gasoline hydrocarbons when compared to free cells (Moslemy, Guiot, & Neufeld, 2004; Moslemy, Neufeld, & Guiot, 2002). In addition, GG beads were also effective in the sorption of heavy metals, in particular nickel (Lázaro, Sevilla, Morales, & Marqués, 2003). Both these results reveal the potential of GG beads in the field of bioremediation. Around this time, researchers also focused on the development and optimization of GG beads (Kedzierewicz, Lombry, Rios, Hoffman, & Maincent, 1999) and *in situ* gelling systems (Kubo, Miyazaki, & Attwood, 2003; Miyazaki, Aoyama, Kawasaki, Kubo, & Attwood, 1999) for drug delivery applications, showing promising results. Nonetheless, these methods were fairly new and the total bulk of research on these topics was rather scarce.

In the last few years, natural polymers caught the attention of researchers since they have very desirable characteristics, such as, natural abundance, hydrophilicity, high swelling capacity, biodegradability, relatively cheap production methods and outstanding biocompatibility (Babu, Sathigari, Kumar, & Pandit, 2010; Dhanka et al., 2018; Prezotti,

Table 2
Characterization techniques for gellan-based materials.

Materials' Properties	Characterization Techniques	Importance	References
Structural and Morphological	Scanning electron microscopy (SEM)	Technique widely applied to study the shape, surface morphology, size, texture, and aggregation of micro/nanoparticles, as well as the pore size in the case of scaffolds.	(Iurciuc et al., 2018; Kim, Thangavelu, et al., 2019)
	Transmission electron microscopy (TEM)	Applied to characterize nanoparticles in terms of size, shape and morphology.	(Ismail, Amin, Majid, & Razali, 2019)
	Atomic force microscope (AFM)	Can be used to study films' structure and roughness.	(Mirón-Mérida et al., 2019; Razali et al., 2020)
	Fourier-transform infrared spectroscopy (FTIR)	Consists in the characterization of the molecular structures based on functional groups. Allows the understanding of which type of interactions occurs between different polymers/molecules.	(Dhanka et al., 2018; Gomes, Costa, Queiroz, Passarinha, & Sousa, 2021)
	Dynamic Light Scattering (DLS)	Analysis of size micro- or nanoparticles by measuring the hydrodynamic diameter.	(Dhanka et al., 2018)
	Electrophoretic light scattering (ELS)	Evaluation of surface charge of micro- or nanoparticles by measuring the changes in the electrophoretic mobility obtaining the zeta potential.	(Dhanka et al., 2018)
	Energy-Dispersive X-ray (EDX) coupled to SEM	Used to analyse the elemental composition of the material while observing through the SEM.	(Coelho et al., 2019; Gomes, Gonçalves, et al., 2021)
	X-Ray Photoelectron Spectroscopy (XPS)	Analyse the chemical and elemental composition of a materials' surface by measuring the kinetic energy of electrons.	(Liang et al., 2020)
	X-ray diffraction analysis (XRD)	Used for characterization of the materials' crystalline structure.	(Dhanka et al., 2018)
Thermal, Mechanical and Rheological	Thermogravimetric analysis (TGA)	Employed to examine changes in thermal properties of the materials and their stability.	(Bonifacio et al., 2020; Rostami et al., 2019)
	Differential scanning calorimeter (DSC)	Characterization of the thermal behavior (heat capacity as a function of time and temperature) of materials based on their physical properties.	(Paolicelli et al., 2018)
	Rotational technique (Rheometry)	Allows rheological characterization of a fluid, where the viscosity is calculated from torque and rotor speed measurements.	(Paolicelli et al., 2018)
	Tensile test	Consists in measuring the tensile properties (modulus of elasticity, yield strength and strain) of a material while it is being pulled.	(Paolicelli et al., 2018)
Thickness	ASTM test with Micrometer	Used to measure the thickness of dry films.	(Paolicelli et al., 2018)

Cury, & Evangelista, 2014). As one might expect, GG quickly rose to prominence and several novel approaches were developed. This in turn, branched out the range of applications for GG based materials, which will be discussed in the following subsections.

4.1. Applications in food

Even to this day, GG has been highly favored in the food industry, with new research being published quite regularly on the development of new GG based materials or the improvement of existing systems with modifications in production variables, to enhance rheological behavior (Chen et al., 2020; de Souza et al., 2016). Indeed, GG's advantage over existing hydrocolloids has been its capacity to create desirable gels at lower polymer concentrations and with less cationic crosslinker content. Moreover, by modulation the ratios of the native and deacetylated GG, it is possible to obtain gels with distinct physical features, which could be advantageous. For example, this could enable the tuning of textural properties of GG-incorporating food products (Stephen & Phillips, 2006). Still, the conjugation with other polymers has been a common approach to tackle GG's shortcomings (*e.g.*, mechanical strength). For instance, a composite hydrogel of GG and pullulan has also been shown to have good organoleptic properties (Richa, 2019). Furthermore, an interpenetrating network (IPN) of GG and maltodextrin has been proposed for application in low-fat products, as it can mimic the creaminess of the traditional products (Kanyuck, Norton-Welch, Mills, & Norton, 2021).

In recent years, consumers are becoming increasingly aware of the potential health benefits of certain compounds in fruits and vegetables (*i.e.*, antioxidant or antimicrobial). This newfound knowledge and the pursuit of a healthier lifestyle led to an increase in the consumption of minimally processed fruits and vegetables (Ramos, Mellinas, Soliberrieta, Garrigós, & Jiménez, 2021). In fact, it has been proposed that people consume significantly more fruit if it has been peeled and sliced. This in turn, resulted in an increased demand by consumers for ready to eat, natural, fresh, nutritious, and convenient minimally processed fruits and vegetables without the addition of synthetic preservatives and colorants (Giannakourou & Tsironi, 2021; Ramos et al., 2021). However, the removal of preservatives and the addition of production steps leads to a quick deterioration rate and spoilage of fruits and vegetables (Giannakourou & Tsironi, 2021). In this setting, biopolymers have been appointed as a promising soft hurdle to minimize deterioration and maintain nutritional value and sensorial attributes (Giannakourou & Tsironi, 2021; Ramos et al., 2021). A blend of GG and cassava starch incorporating lecithin encapsulated thyme essential oil was found to be able to interact with the low surface energy of apple, tomato and persimmon skins (Sapper, Bonet, & Chiralt, 2019). The presence of thyme essential oil was able to modulate and positively influence the contact angle and surface tension values. In this manner, the composite film exhibited good spreadability on the surface of all fruits and confirmed the suitability of GG to be applied in edible fruit coatings (Sapper et al., 2019). Furthermore, a GG edible coating was applied to ready to eat mango bars. The presence of the coating minimized the occurrence of syneresis, while reducing the release and loss of volatile compounds during storage. This is rather important, since volatile compounds present in fruits are often responsible for their characteristic flavor and aroma (Danalache, Carvalho, Alves, Moldão-Martins, & Mata, 2016). Moreover, an edible coating comprised of GG and geraniol, a natural antimicrobial, was shown to be effective in reducing the count of mesophilic bacteria, psychrophilic bacteria, yeasts and molds. The coating provided an extra barrier which could increase total storage time from 5 to 7 days (Tomadoni, Moreira, Pereda, & Ponce, 2018). In addition, gallic acid modified cellulose nanocrystals loaded in a GG film were able to substantially slow down the ripening of *Agaricus bisporus* mushrooms, as seen by a reduced color change and diminished loss of firmness when compared to the control group (Paula Criado et al., 2020). This edible coating was also able to significantly reduce the respiration rate of *Agaricus bisporus* mushrooms, which in turn, slows down the maturation and prolongs shelf-life of the product (Criado, Fraschini, Shankar, Salmieri, & Lacroix, 2021). In addition to edible coatings, GG has been used as a base matrix in composite films for sustainable food packaging materials. Wey and *co*-workers developed a GG-chitosan polyelectrolyte composite film containing thyme essential

oil nanoemulsions that exhibited mechanical flexibility and UV shielding capacity. Besides that, the film showed effective antimicrobial activity, reducing total *E. coli* count nearly 3-fold (X. Zhang et al., 2021). Moreover, a GG and purple sweet potato (PSP) composite film was shown to be a promising active packaging material. The PSP anthocyanins exhibit natural antioxidant activity and on its own is a desirable feature to prolong shelf life. In addition, the composite film was able to undergo a color change in response to the growth of *E. coli*. The digestion of proteins during the growth of the microorganism released volatile compounds that caused a shift in color of the composite film. Thus, the GG-PSP film can detect and monitor the spoilage of foods and their suitability for consumption (Wei, Cheng, Ho, Tsai, & Mi, 2017). Recently, besides being used as a structuring agent in fabricated foods, GG was also found to be effective in retaining volatile compounds in structured fruit products. The percentage bioaccessibility of ascorbic acid, total antioxidant activity and total extractable polyphenols has been shown to be similar or improved after the production process of structured mango/caja, mango/cashew apple and mango/acerola mixed pulps (Leal et al., 2022). These evidences has also been observed for structured guava (Costa et al., 2020; Nascimento da Costa et al., 2021) and for a mixed leather of açaí, banana, peanut and guarana, utilizing GG has the prime hydrocolloid (Santos, Machado de Sousa, Rangel Moreira Cavalcanti-Mata, & Barros de Vasconcelos, 2021). The described phenomenon is uplifting as it proves the resemblance between structured fruits and fresh pulps regarding nutritional value. In turn, this feature allows for the year-round market presence of seasonal fruit or the extension of shelf-life of highly perishable fruit (Leal et al., 2022).

4.2. Applications in tissue engineering

With an increase in life expectancy and a shift towards an aging society, bone and cartilage defects, as well as chronic diseases have become an unprecedented problem with substantial costs to health care (Bonifacio, Cochis, Cometa, Gentile, et al., 2020; Cho et al., 2021; Lee et al., 2021). Classic Tissue Engineering (TE) approaches were somewhat lackluster as they could not find a solution for the long-term biocompatibility issues that arose and for the necessity of invasive surgeries for the removal or revision of implants (Nair & Laurencin, 2007). For this reason, in recent years, a large focus has been placed on producing scaffolds for tissue repair with natural biopolymers, largely due to their biocompatibility and biodegradability, tackling aforementioned concerns (Nair & Laurencin, 2007). Naturally, GG has been a focal point in modern TE. However, the weak mechanical strength in comparison to native tissue, poor bone induction ability and relatively smooth gel surface, which means a lack of binding sites for cells, has limited the application of GG as a single base matrix in the production of scaffolds (Lee et al., 2020; Xu et al., 2020). Nevertheless, in the last couple of years, researchers were able to circumvent these issues by developing new composite hydrogel-like scaffolds. The incorporation of other polymers (Aadil et al., 2019), nanoparticles (Ismail et al., 2019), demineralized bone powder (Aadil et al., 2019) or adhesion signaling peptides (Jiang et al., 2020) has proven to be effective in modulating GG overall features to closely mimic the mechanical values for target tissue and to create a 3D matrix similar to the extracellular matrix (ECM) (Trucco et al., 2021). In fact, the simplicity of production and the ease of manipulation of the GG chemical structure has allowed for the control of gelation temperature close to physiological levels, safely incorporating cells (ter Horst et al., 2019), growth factors (Dohle et al., 2020) and other bioactive compounds, such as hydroxyapatite (HA) and antibacterial agents (Xu et al., 2020). Furthermore, the 3D printability of GG has been confirmed (Yu et al., 2017).

Several reports have confirmed the potential of GG in osteogenesis. Ding and colleagues reported upregulated expression of osteogenic markers in stem cells and enhanced biomineralization due to a strong HA deposition from a composite bioactive glass, alginate and GG scaffold. This in turn, accelerated new bone formation *in vivo* (Ding et al., 2021). In the same way, the addition of demineralized bone powder to GG increased overall mechanical resistance similar to native bone values and promoted a rougher surface. As previously mentioned, a rougher morphology is ideal for cell attachment and differentiation and the results showed higher cell proliferation on the hybrid gel and heightened osteogenic differentiation (Cho et al., 2021).

Materials based on GG have also found application in cartilage repair. Manuka honey (MH)/GG composite hydrogel reinforced with halloysite nanotubes presented Young's compression and equilibrium modulus similar to native cartilage. The large quantity of methylglyoxal in MH conferred the hydrogel with antibacterial activity against *S. aureus* and *S. epidermidis* (Bonifacio et al., 2018). The replacement of halloysite nanotubes with mesoporous silica in the MH/GG hydrogel improved mechanical properties, morphological characteristics and chondrogenic differentiation (Bonifacio et al., 2020). The upregulation of chondrogenesis has also been verified for other GG systems. A GG hydrogel loaded with dexamethasone-cyclodextrin complexes resulted in higher expression of genes involved in the formation of new cartilaginous tissue while minimizing inflammation (Choi et al., 2020). Similar effects were shown for GG/lignocellulosic nanofibrils carrying melatonin loaded forsterite nanoparticles (Kouhi, Varshosaz, Hashemibeni, & Sarmadi, 2020). Furthermore, a bilayered hydrogel was developed for articular cartilage repair. The top portion was representative of superficial cartilage and consisted of GG with 10 % poly (ethylene glycol) diacrylate (PEGDA) with a coating of graphene oxide and the bottom portion represented deep cartilage with a larger percentage of PEGDA. The incorporation of graphene oxide conferred a lubrication property to the scaffold, which in turn, was resistant to wear and tear even after 100,000 cycles on a knee simulator (Trucco et al., 2021). Such a bilayer structure is often utilized in the repair of osteochondral lesions, where one part will be bone-like and the other cartilage-like. This configuration has been reported twice with a bilayer of GG/demineralized bone powder, showing adequate osteochondral regeneration and new tissue integration (Choi et al., 2020; Kim, Cho, et al., 2020).

GG has also been sought out to treat skin lesions. In fact, GG forms superabsorbent hydrogels which can absorb excess exudate and provide moisture to the injury. Based on this feature, GG films laden with titanium dioxide nanomaterials have been applied for wound healing, where GG is responsible for the exudate control and titanium nanoparticles confer an antimicrobial effect. In addition, an expedited wound closure rate was observed in rat models (Ismail et al., 2019; Razali et al., 2020). An IPN of GG and collagen has also shown to be a suitable domain to encapsulate mesenchymal stem cells. As they differentiate in the wound microenvironment, they release paracrine factors to reduce inflammation and promote wound closure (Ng et al., 2021). Moreover, GG/polyethylene glycol and GG/gelatin/glycol chitosan hydrogels have been employed as retinal pigment epithelium (RPE) cell delivery systems for the treatment of age related macular degeneration (Kim et al., 2019; Rim et al., 2020). The functionalization of GG hydrogels by dopamine has also been reported to be efficient in RPE delivery, by enhancing the expression of RPE specific genes (Lee et al., 2021). Additionally, GG and polylysine complex fibers, prepared by wet spinning, exhibited adequate antibacterial activity, absence of hemolysis and no adverse reaction was detected when applied as a wound suture material, which in turn, confirms the suitability of GG fibers to be utilized as suture lines (Peng et al., 2020). Other relevant advances of GG based materials in TE are listed in Table 3.

4.3. Applications in drug delivery systems

Besides the addressed characteristics of GG in former sections, they also present potent mucoadhesive capacity, rivaling or surpassing those of commonly used natural polymers in pharmaceutical applications (Boni, Prezotti, & Cury, 2016; Prezotti et al., 2014). This has made GG a very attractive material for drug delivery systems, since it can adhere to mucous membranes, prolonging retention time and sustaining the

Table 3
Recent Advances in GG based materials for TE applications.

Scaffold	Application	Cell type/*In vivo* model	Main finding	Ref.
GG/polyvinyl alcohol (PVA) nanofibers	Wound Dressing	Human Dermal Fibroblasts (3T3L1)	Enhanced cell adhesion and proliferation over conventional films	(Vashisth et al., 2016)
GG hydrogel loaded with virgin coconut oil microemulsion		Sprague-Dawley Rats	Scaffold exhibited superior wound healing after 2 weeks *vs* commercial Opsite wound dressing	(Muktar et al., 2021)
Polydopamine doped GG hydrogel	Bone Regeneration	Human Adipose derived Stem Cells (hASCs)	Polydopamine coating resulted in superior adhesion and proliferation of hASCs	(Pacelli et al., 2020)
GG/chitosan hydrogel		Bone Marrow Mesenchymal Stem Cells (BMSCs)	Hydrogel Blend promotes greater adhesion, proliferation and spreading of BMSCs stimulating repair	(Oliveira et al., 2020)
GG/Tuna skin gelatin membrane		Rabbit	GG/TSG membrane acts as a barrier preventing the invasion of soft tissue in the bone defect	(Jung et al., 2020)
GG/PEGDA/PEGMA/MDP hydrogel		Preosteoblasts (MC3T3-E1)	Composite hydrogel induces *in situ* mineralization, accelerating HA formation while being compatible with MC3T3-E1 cells	(Li et al., 2020)
GG/Silk fibroin hydrogel		hASCs	Hydrogel blend induced mineralization and osteogenic differentiation	(B. Kundu et al., 2020)
GG/Platelet-rich plasma hydrogel	Cartilage Repair	hASCs	Upregulated chondrogenic markers indicate induced chondrogenesis	(Kim et al., 2020)
GG/PEGDA hydrogel		BMSCs/Mice	Exposure of BMSCs seeded in GG/PEGDA to transforming growth factor accelerated chondrification of BMSCs	(Li et al., 2020)
GG/Glycol chitosan hydrogel		Chondrocytes isolated from rabbits	Incorporation of Chitosan enhanced Glycosaminoglycans synthesis and levels of cartilage related genes	(S. Lee et al., 2020)
GG/gelatin/HA hydrogel		Mouse Fibroblasts (L929)	Hydrogel exhibited great compressive resistance and elasticity while sustaining a self-healing feature	(Wang et al., 2020)
GG/Poly glutamic acid (PGA)/ glycerol hydrogel		L929	PGA increased mechanical and thermal stability while glycerol conferred flexibility to the hydrogel allowing the adhesion of fibroblasts	(Lin & Chiu, 2021)

delivery of therapeutics. Moreover, the functional groups embedded in GGs structure can be easily modified to control the drug release profiles to accomplish intended objectives (Prezotti et al., 2014). In fact, fine tune control of production variables and surface modifications has been shown to produce site-specific targeted GG delivery systems. Indeed, GG/PVA nanofibers have been shown to have gastroretentive properties, sustaining the release of ofloxacin for over 24 h (Vashisth et al., 2017). Likewise, a GG/chitosan hydrogel containing a cyclodextrin-curcumin complex was found to release most of the curcumin in simulated intestinal fluid when lysozyme was present (Oliveira et al., 2021). In turn, the blending of GG with other natural polymers shows promise of altering the bulk drug release to distinct portions of the gastrointestinal tract (Gadziński et al., 2020). On the other hand, GG based materials also tend to have great permeation features, whether it be a mucous membrane (Gangane & Kawtikwar, 2020) or skin permeation (Gehrcke et al., 2021). For this reason, GG drug delivery systems have found success in a plenitude of applications with distinct routes of administration.

Chen and coworkers developed an *in situ* gelling system composed of breviscapine nanosuspensions incorporated in GG. When in contact with the nasal fluids, the composite system underwent a sol-gel transition and significantly enhanced residence time, improving drug bioavailability (Chen et al., 2020). The intranasal administration of the drug could bypass the blood-brain barrier, as the drug targeting efficiency of the cerebrum, cerebellum and olfactory bulb was of 4006 %, 1999 % and 3290 %, respectively. Values over 100 % indicate the drug transport to the brain after intranasal administration, as opposed to intravenous administration (Chen, Liu, et al., 2020). Furthermore, hexadecyl GG nanoparticles were shown to immensely increase the drug solubility of simvastatin in water. Release of the statin from amphiphilic nanoparticles resulted in a total reduction of LDL cholesterol of 69.04 % *vs* 24.70 % for pure drug (P. Kundu, Datta, & Maiti, 2015). GG drug delivery systems have also been employed in the therapy of diabetes. Vildagliptin loaded GG/sodium alginate beads optimized by an experimental design tool showed great encapsulation efficiency and mucoadhesion, while sustaining the release of vildagliptin for over 12 h (Shirsath & Goswami, 2021).

Additionally, GG has been explored as a base matrix for the delivery of antibacterial and antifungal agents. A GG hydrogel laced with silver nanoparticles showed preferential antibacterial activity *versus* Gram-negative bacteria, specifically *Escherichia coli and Aeromonashydrophila* (Sharanappa, Ambresh, & Gangadhar, 2019). A GG/chitosan nanocomplex entrapping ketoconazole optimized by experimental design also showed potent antifungal activity, with over 80 % inhibition against *Aspergillus niger* (Kumar, Kaur, Bernela, Rani, & Thakur, 2016). Further, GG has also been recently utilized in ocular *in situ* gelling delivery systems. Göttel and collaborators developed GG/pullulan nanofibers through electrospinning and applied curvature to the fibers into the shape of a lens. Curved fiber lenses spread more evenly throughout the cornea surface and prolonged *in vitro* residence time (Göttel, Souza e Silva, et al., 2020). Likewise, the flurbiprofen-cyclodextrin complex entrenched in a GG carrier exhibited higher permeation when compared to commercially available Ocufen® (Senjoti, Timmins, Conway, & Smith, 2020). Although, biomedical solutions incorporating gellan gum are commercially available, very little information is known regarding its potential side effects on humans as a drug delivery system. Nevertheless, gellan gum is recognized as a safe food additive by the FDA and EFSA and is biocompatible, and biodegradable with an extracellular matrix-like structure. So, it is expected that any adverse effects on humans as drug delivery vehicles would be minimal if existent at all. Additional advances in GG based materials for drug delivery applications can be found in Table 4.

4.4. Applications in immobilization, remediation and biosynthesis

The ongoing agricultural and industrial expansion often results in undesirable soil and water quality, leaving behind traces or significant amounts of pollutants and heavy metals (Perk, 2013). GG based materials have also found success in the contaminant removal field. Liang and co-workers reported carboxymethyl konjac glucomannan reinforced GG microspheres with mechanical strength 6 times superior to bare GG. The microspheres engaged in an ion exchange adsorption mechanism, wherein the crosslinker aluminium ions present in the microspheres structure were replaced with the uranyl groups present in the surrounding medium, as confirmed by the disappearance of aluminium peaks and emergence of uranium peaks in EDX. Furthermore, uranium also underwent coordination with hydroxyl and carboxyl groups in the GG structure resulting in a maximum adsorption of 98.10 mg/g, ensuring appropriate uranium removal (Liang et al., 2020). Indeed, GG could also improve the nickel adsorption of jumunjin sand upwards of 150 % through a similar cation exchange process, significantly clarifying

Table 4
Relevant developments in GG based materials for drug delivery applications.

Delivery system	Drug	Route of administration	Main results	Ref.
GG fluid gel	Diclofenac	Transdermal	GG gel exhibited similar mechanical and lubrication properties to Voltaren® while increasing skin permeation	(Mahdi, Conway, Mills, & Smith, 2016)
Acrylic ion exchange resin microparticles coated with GG	Cefuroxime Sodium (CFX)	Rectal Transdermal	GG coating increases adsorption of CFX onto microparticles and slightly decreases drug release rates	(Racovita, Lungan, Bunia, Popa, & Vasiliu, 2016)
Tamarind seed gum/ Polymethacrylamide-GG beads	Diclofenac	Oral	The formation of IPN post Polymethacrylamide grafting increased drug loading efficiency to 93.3 % and sustained release up to 10 h	(Nandi, Nandi, Khan, Pal, & Dey, 2018)
GG/PVA IPN microspheres	Aceclofenac	Oral	Dual crosslink production with Zinc and Glutaraldehyde proved useful in enhancing drug loading efficiency and controlled release	(Jana & Sen, 2019)
GG hydrogel loaded with Tween40/ S1570/Coconut oil/Glycerol microemulsions	Apigenin	Oral	The microemulsions stabilized the hydrophobic drug while the composite hydrogel exhibited distinct pH dependent release mechanisms	(Zhao & Wang, 2019)
Maleate-GG/Silk sericin/chitosan nanospheres	Rifampicin Pyrazinamide	Pulmonary	Nearly 80 % of both drugs was released after 120 h inducing a combinatory inhibition of *Mycobacterium tuberculosis* by damaging cell membranes	(Mehnath et al., 2019)
GG microspheres	Quercetin	Oral	GG microspheres enhanced the chemical stability of Quercetin in Simulated Gastric and Intestinal Fluid	(Dey et al., 2020)
GG/Pullulan nanofibers	Amphotericin B	Ocular	Nanofibers displayed a clear zone of inhibition of *Issatchenkia orientalis* and retained 95.5 % Amphotericin B after electron beam sterilization	(Göttel, Lucas, et al., 2020)

nickel contaminated water (Tran, Cho, Cho, Han, & Chang, 2021). In turn, itaconic acid modified with Ca—Al layered double hydroxide/GG nanocomposites loaded with silver nanoparticles produced through a green synthesis approach, could efficiently reduce organic pollutant 4-nitrophenol into 4-aminophenol (Shabani & Dinari, 2021). In addition, a GG/cuttlefish ink photothermal evaporator can effectively promote solar desalination of water bodies with an evaporation rate of 3.1 kg m^{-2} h^{-1}. This eco-friendly and sustainable tool could potentially yield high amounts of freshwater from polluted water or regular seawater (X. Liu et al., 2021).

Immobilization of cells and enzymes has been a focus of research for application in various industries. The immobilization facilitates enzyme recovery post operation, avoiding contamination of the final product (Wahba, 2018). It can protect cells or enzymes from harsh environments (*i.e.*, toxic compounds) and provide thermal and pH stability. Indeed, it can retain a high cell density, preventing washout and enabling reusability (Umar Mustapha, Halimoon, Wan Johari, & Abd Shukor, 2020). GG has been explored in this area since hydrogels as the main immobilizer are inexpensive, easy to produce and tend to be porous which makes the immobilized cells or enzymes accessible for the aqueous media containing nutrients or substrates (Fan, Yi, Hua, Zhang, & Yang, 2017). Immobilization of a cold-adapted β-Galactosidase in GG microspheres retained nearly full activity upwards of 70 °C, while free enzyme lost significant activity at 40 °C (Fan et al., 2017). Similarly, β-D-Galactosidase encapsulated in GG/chitosan and GG/polyethyleneimine beads was able to maintain appropriate activity outside of the normal pH and temperature range for free enzyme. Additionally, both systems were reusable for over 14 cycles and preserved approximately complete activity after 36 days in storage (Wahba, 2018). A performance comparison of free *vs* immobilized biocatalysts in GG materials is listed in Table 5.

4.5. Other emerging applications

As previously mentioned, GG found great success in several fields discussed above. Although this is the case, novel applications outside this scope emerged, and even though they are not yet as developed as the aforementioned fields, they present great interest and potential. For instance, methacrylated GG and hyaluronic acid hydrogel supplemented with paramagnetic manganese permitted the real time visualization of

Table 5
Developments in the field of GG materials for immobilization of cells and enzymes.

Application	Immobilization support	Cells/enzyme	Free	Immobilized	Stability	Ref.
Biodegradation of diesel contaminated water	GG/Alginate Microspheres	*Pseudomonas Aeruginosa*	Degradation of 32 % of diesel after 30 days	Degradation of 71.2 % diesel after 10 days and 90.8 % after 30 days	Immobilized cells retained 84.3 % viability while free cells dropped to 14.4 % after 30 days	(Park et al., 2021)
Biodegradation of carbofuran	GG Microspheres	Enterobacter sp. (BRC05)	Complete degradation of carbofuran at 50 mg/L after 12 h	Complete degradation of carbofuran at 50 mg/L after 9 h	Increase of carbofuran to 200 mg/L significantly impaired free cells while immobilized cells remained somewhat stable. Immobilized cells can be reutilized up to 9 times	(Umar Mustapha et al., 2020)
Biosynthesis of γ-aminobutyrate (GABA)	GG Beads	*Lactobacillus brevis*	Free cells were more susceptible to pH and temperature changes and had a final GABA yield of 11.97 g/L	Immobilized cells retained higher % activity with a final yield of 10.15 g/L	Although immobilized cells had slightly less yield, they could be reutilized 10 times	(Lyu et al., 2019)
Biosynthesis of ethanol	GG Beads	*Saccharomyces cerevisiae*	Free cells presented a yield in fermented glucose of around 40 %	The yield of immobilized cells varied between 50 and 85 % after 5 consecutive cycles	Immobilized cells could be reutilized for at least 40 fermentation cycles without losing bioactivity	(Iurciuc Tincu et al., 2016)

the hydrogel post intrathecal injection *via* magnetic resonance imaging (MRI). The hydrogel acted as a cell delivery system, while the manganese ions served as an imaging probe (Vieira et al., 2020). A similar MRI contrasting agent function was reported for GG microcapsules containing ferrofluids (Leopércio, Ribeiro, Gomes, Michelon, & Carvalho, 2021). Further, GG scaffolds grafted with fluorescein isothiocyanate emitted green fluorescence when examined by confocal microscopy. Consequently, after implantation of the scaffold in mice, fluorescence was detected using FOBI *in vivo* imaging, which confirmed the suitability of GG to be used for bioimaging endeavors (Cho et al., 2020). In addition, a sorafenib loaded GG hydrogel laden with bismuth sulfide nanodots was detectable *via* X-ray computed tomography, allowing real time tracking of pharmacokinetics and biodistribution of therapeutic composite. The application of combined NIR photothermal therapy and sustained release of sorafenib resulted in a 98.2 % tumor suppression rate (Y. Wu et al., 2021). In fact, in regard to cancer therapy, an oral patch of GG/glucosamine/clioquinol was found to suppress tumor development and could be used for the treatment of early stage cancer patients (Tsai et al., 2018). Moreover, the embedding of gold nanoparticles into GG hydrogels has made them suitable to be used as a surface-enhanced Raman spectroscopy substrate allowing the detection of lactate and thiocyanate, two cancer associated metabolites, acting as a biosensor (Abalde-Cela et al., 2020). Indeed, GG hydrogel sensors have also been reported in the area of flexible electronic skin, where the formulated self-healing GG-based hydrogel could be worn as a strain sensor, detecting large joint motions and minor physiological signals (S. Liu, Qiu, Yu, & Zhang, 2020).

GG gels have also been proven to be effective as tissue phantoms. Cortela and coworkers produced a GG based tissue mimicking material which exhibited mechanical, thermal and acoustic properties similar to biological soft tissues and could be utilized in efficacy tests for ultrasonic hyperthermia devices (Cortela, Negreira, & Pereira, 2020). Moreover, GG materials can also be used as MRI phantoms and the addition of radiation sensitive tetrazolium salts to a GG matrix with electrical conductivity can yield gel dosimeters for radiation therapy (Brzozowski, Penev, & Mequanint, 2021). In fact, development of solid polymer electrolytes achieved by doping GG with electrical conductivity has been a hot topic and early groundwork is being set in the optimization of electrical, thermal and mechanical properties of this models for the application of GG polyelectrolyte systems in batteries, capacitators, sensors and electrochromic devices, among others (Isfahani et al., 2021; Noor, 2020). Indeed, a flexible organic light-emitting diode (FOLED) with GG as the carbon biosubstrate displayed electrical properties similar to other FOLEDs found in literature (Faraco et al., 2021). Additionally, GG microgels have shown strong effects in the cleaning and preservation of artwork, being able to more efficiently wash and remove contaminants from *Breviarium* and *Xuan* papers over traditional wetting methods (Di Napoli et al., 2020; Li et al., 2021). In turn, water resistant GG and alginate blended films show promise of replacing polystyrenes in coating paper cups for hot drinks, tackling sustainability issues (N. Zhang, Xu, Gao, Fu, & Zheng, 2017). Lately, it has been determined that GG microspheres can also be employed in the capture of therapeutic biomolecules. GG microspheres produced through a simple W/O emulsion method and optimized by a design of experiments tool (Coelho et al., 2019), were applied in the capture of human soluble catechol-*O*-methyltransferase (hSCOMT). Indeed, GG microspheres reinforced with nickel and magnesium ions were applied in a batch method to selectively capture hSCOMT from complex *K. pastoris* lysates utilizing different strategies. Therein, nickel reinforced microspheres presented the best results utilizing an ionic strategy where target protein was mostly recovered in a single fraction by manipulating pH and salt levels, achieving bioactivity levels for the recovered fraction of 200 % and purification of 77.37 fold. In turn, magnesium reinforced GG microspheres, presented a bioactivity recovery of 19 % and purification of 0.73 fold operating on an affinity strategy, taking advantage of the fact that magnesium is a hSCOMT cofactor (Gomes, Gonçalves, et al., 2021). On the other hand, copper-crosslinked GG microspheres produced through the same optimized W/O emulsion method, were applied in the direct capture of a pDNA vaccine, encoding HPV E7 oncoprotein, from a *E. coli* lysate. At first, the copper microspheres were able to capture 15.61 % of pDNA with 2.42 % of purity. However, the functionalization of copper reinforced GG microspheres with polyethylenimine increased pDNA binding sites and improved total capture to 88.09 % with 3.18 % purity (Gomes, Costa, et al., 2021). Overall, the application of GG microspheres in a simple batch method can efficiently and selectively capture therapeutic proteins and nucleic acids, largely clarifying initial lysates in a viable, fast and cost-effective manner, excluding the need for harsh or toxic reagents.

5. Conclusions

The review provides a thorough overview of current therapeutic uses for gellan gum-based systems, which present themselves as innovative and different approaches in a number of domains. The noteworthy characteristics of GG, including its capacity to gel, sensitivity to heat and ions, mucoadhesion, and tunable physical and mechanical properties, encourage research interest in this biomaterial. Several gellan-based materials have been created within the past years using a variety of fabrication techniques, which indicates that GG is one of the most promising biomaterials for use in a variety of fields as drug delivery, tissue engineering, wound healing, capture of biomolecules, enzyme and cells immobilization and in the remediation field. Therefore, with deeper investigation, this cheap material can undoubtedly produce stunning results in several sectors of economic activity.

Declaration of competing interest

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

Data availability

The authors do not have permission to share data.

Acknowledgments

The authors acknowledge the support from FEDER funds through the POCI-COMPETE 2020–Operational Programme Competitiveness and Internationalisation in Axis I–Strengthening Research, Technological Development and Innovation (Project POCI-01-0145-FEDER-007491), from FCT–Fundação para a Ciência e Tecnologia. This work was also supported by the Health Sciences Research Centre CICS-UBI (UIDB/00709/2020 and UIDP/00709/2020), the Applied Molecular Biosciences Unit UCIBIO (UIDB/04378/2020 and UIDP/04378/2020) and the Associate Laboratory Institute for Health and Bioeconomy–i4HB (project LA/P/0140/2020) which are financed by National Funds from FCT/MCTES. D. Gomes also acknowledges the doctoral fellowship from FCT (ref: 2020.06792.BD).

References

Aadil, K. R., Nathani, A., Sharma, C. S., Lenka, N., & Gupta, P. (2019). Investigation of poly(vinyl) alcohol-gellan gum based nanofiber as scaffolds for tissue engineering applications. *Journal of Drug Delivery Science and Technology, 54*, Article 101276. https://doi.org/10.1016/j.jddst.2019.101276

Abalde-Cela, S., Rebelo, R., Wu, L., Barbosa, A. I., Rodríguez-Lorenzo, L., Kant, K., Reis, R. L., Correlo, V. M., & Diéguez, L. (2020). A SERS-based 3D nanobiosensor: Towards cell metabolite monitoring. *Materials Advances, 1*(6), 1613–1621. https://doi.org/10.1039/D0MA00121J

Adrover, A., Paolicelli, P., Petralito, S., Di Muzio, L., Trilli, J., Cesa, S., Tho, I., & Casadei, M. A. (2019). Gellan Gum/Laponite beads for the modified release of drugs: Experimental and modeling study of gastrointestinal release. *Pharmaceutics, 11*(4). https://doi.org/10.3390/pharmaceutics11040187

Agarwal, S., Saha, S., Balla, V. K., Pal, A., Barui, A., & Bodhak, S. (2020). Current developments in 3D bioprinting for tissue and organ regeneration–A review. *Frontiers in Mechanical Engineering, 6*. https://doi.org/10.3389/fmech.2020.589171

Albert, S., & Mittal, G. S. (2002). Comparative evaluation of edible coatings to reduce fat uptake in a deep-fried cereal product. *Food Research International, 35*(5), 445–458. https://doi.org/10.1016/S0963-9969(01)00139-9

Amiri, F., Babaei, M., Jamshidi, N., Agheb, M., Rafienia, M., & Kazemi, M. (2022). Fabrication and assessment of a novel hybrid scaffold consisted of polyurethane-gellan gum-hyaluronic acid-glucosamine for meniscus tissue engineering. *International Journal of Biological Macromolecules, 203*, 610–622. https://doi.org/10.1016/j.ijbiomac.2022.01.091

Babu, R. J., Sathigari, S., Kumar, M. T., & Pandit, J. K. (2010). Formulation of controlled release gellan gum macro beads of amoxicillin. *Current Drug Delivery, 7*(1), 36–43. https://doi.org/10.2174/156720110790396445

Bajaj, I. B., Saudagar, P. S., Singhal, R. S., & Pandey, A. (2006). Statistical approach to optimization of fermentative production of gellan gum from sphingomonas paucimobilis ATCC 31461. *Journal of Bioscience and Bioengineering, 102*(3), 150–156. https://doi.org/10.1263/jbb.102.150

Bajaj, I. B., Survase, S. A., Saudagar, P. S., & Singhal, R. S. (2007). Gellan gum: Fermentative production, downstream processing and applications. *Food Technology and Biotechnology, 45*(4), 341–354.

Balasubramanian, R., Kim, S. S., Lee, J., & Lee, J. (2019). Effect of TiO2 on highly elastic, stretchable UV protective nanocomposite films formed by using a combination of k-carrageenan, xanthan gum and gellan gum. *International Journal of Biological Macromolecules, 123*, 1020–1027. https://doi.org/10.1016/j.ijbiomac.2018.11.151

Banik, R. M., Kanari, B., & Upadhyay, S. N. (2000). Exopolysaccharide of the gellan family: Prospects and potential. *World Journal of Microbiology and Biotechnology, 16*(5), 407–414. https://doi.org/10.1023/A:1008951706621

Banik, R. M., Santhiagu, A., & Upadhyay, S. N. (2007). Optimization of nutrients for gellan gum production by sphingomonas paucimobilis ATCC-31461 in molasses based medium using response surface methodology. *Bioresource Technology Reports, 98*, 792–797. https://doi.org/10.1016/j.biortech.2006.03.012

BeMiller, J. N. (2019). Gellans, curdlan, dextrans, levans, and pullulan. In J. N. BeMiller (Ed.), *Carbohydrate chemistry for food scientists* (pp. 271–278). Elsevier Inc.. https://doi.org/10.1016/B978-0-12-812069-9.00012-1

Bhardwaj, N., & Kundu, S. C. (2010). Electrospinning: A fascinating fiber fabrication technique. *Biotechnology Advances, 28*(3), 325–347. https://doi.org/10.1016/j.biotechadv.2010.01.004

Boni, F. I., Prezotti, F. G., & Cury, B. S. (2016). Gellan gum microspheres crosslinked with trivalent ion: Effect of polymer and crosslinker concentrations on drug release and mucoadhesive properties. *Drug Development and Industrial Pharmacy, 42*(8), 1283–1290. https://doi.org/10.3109/03639045.2015.1125915

Bonifacio, M. A., Cochis, A., Cometa, S., Gentile, P., Scalzone, A., Scalia, A. C., Rimondini, L., & De Giglio, E. (2020). From the sea to the bee: Gellan gum-honey-diatom composite to deliver resveratrol for cartilage regeneration under oxidative stress conditions. *Carbohydrate Polymers, 245*, Article 116410. https://doi.org/10.1016/j.carbpol.2020.116410

Bonifacio, M. A., Cochis, A., Cometa, S., Scalzone, A., Gentile, P., Procino, G., Milano, S., Scalia, A. C., Rimondini, L., & De Giglio, E. (2020). Advances in cartilage repair: The influence of inorganic clays to improve mechanical and healing properties of antibacterial gellan gum-manuka honey hydrogels. *Materials Science and Engineering: C, 108*, Article 110444. https://doi.org/10.1016/j.msec.2019.110444

Bonifacio, M. A., Cometa, S., Cochis, A., Gentile, P., Ferreira, A. M., Azzimonti, B., Procino, G., Ceci, E., Rimondini, L., & De Giglio, E. (2018). Antibacterial effectiveness meets improved mechanical properties: Manuka honey/gellan gum composite hydrogels for cartilage repair. *Carbohydrate Polymers, 198*, 462–472. https://doi.org/10.1016/j.carbpol.2018.06.115

Brzozowski, P., Penev, K. I., & Mequanint, K. (2021). Gellan gum gel tissue phantoms and gel dosimeters with tunable electrical, mechanical and dosimetric properties. *International Journal of Biological Macromolecules, 180*, 332–338. https://doi.org/10.1016/j.ijbiomac.2021.03.047

Cardoso, V. O., Kiraly, V. R., Boni, F. I., Ferreira, N. N., Ferreira, L. B., Pereira, F. V., Borges, J. C., Cury, B. F., & Gremião, M. D. (2021). Rational design of nanocarriers based on gellan gum/retrograded starch exploiting polyelectrolyte complexation and ionic cross-linking processes: A potential technological platform for oral delivery of bevacizumab. *Journal of Drug Delivery Science and Technology, 66*, Article 102765. https://doi.org/10.1016/j.jddst.2021.102765

Chen, B., Cai, Y., Liu, T., Huang, L., Zhao, X., Zhao, M., Deng, X., & Zhao, Q. (2020). Formation and performance of high acyl gellan hydrogel affected by the addition of physical-chemical treated insoluble soybean fiber. *Food Hydrocolloids, 101*, Article 105526. https://doi.org/10.1016/j.foodhyd.2019.105526

Chen, Y., Liu, Y., Xie, J., Zheng, Q., Yue, P., Chen, L., Hu, P., & Yang, M. (2020). Nose-to-brain delivery by nanosuspensions-based in situ gel for breviscapine. *International Journal of Nanomedicine, 15*, 10435–10451. https://doi.org/10.2147/ijn.s265659

Cho, H. H., Been, S. Y., Kim, W. Y., Choi, J. M., Choi, J. H., Song, C. U., Song, J. E., Bucciarelli, A., & Khang, G. (2021). Comparative study on the effect of the different harvesting sources of demineralized bone particles on the bone regeneration of a composite gellan gum scaffold for bone tissue engineering applications. *ACS Applied Bio Materials, 4*(2), 1900–1911. https://doi.org/10.1021/acsabm.0c01549

Cho, H. H., Choi, J. H., Been, S. Y., Kim, N., Choi, J. M., Kim, W., Kim, D., Jung, J. J., Song, J. E., & Khang, G. (2020). Development of fluorescein isothiocyanate conjugated gellan gum for application of bioimaging for biomedical application. *International Journal of Biological Macromolecules, 164*, 2804–2812. https://doi.org/10.1016/j.ijbiomac.2020.08.146

Choi, J. H., Kim, N., Rim, M. A., Lee, W., Song, J. E., & Khang, G. (2020). Characterization and potential of a bilayered hydrogel of gellan gum and demineralized bone particles for osteochondral tissue engineering. *ACS Applied Materials & Interfaces, 12*(31), 34703–34715. https://doi.org/10.1021/acsami.0c10415

Choi, J. H., Park, A., Lee, W., Youn, J., Rim, M. A., Kim, W., Kim, N., Song, J. E., & Khang, G. (2020). Preparation and characterization of an injectable dexamethasone-cyclodextrin complexes-loaded gellan gum hydrogel for cartilage tissue engineering. *Journal of Controlled Release, 327*, 747–765. https://doi.org/10.1016/j.jconrel.2020.08.049

Coelho, J., Eusebio, D., Gomes, D., Frias, F., Passarinha, L. A., & Sousa, A. (2019). Biosynthesis and isolation of gellan polysaccharide to formulate microspheres for protein capture. *Carbohydrate Polymers, 220*, 236–246. https://doi.org/10.1016/j.carbpol.2019.05.011

Cortela, G. A., Negreira, C. A., & Pereira, W. C. A. (2020). Durability study of a gellan gum-based tissue-mimicking phantom for ultrasonic thermal therapy. *The Journal of the Acoustical Society of America, 147*(3), 1531. https://doi.org/10.1121/10.0000813

Costa, A. L. R., Gomes, A., Ushikubo, F. Y., & Cunha, R. L. (2017). Gellan microgels produced in planar microfluidic devices. *Journal of Food Engineering, 209*, 18–25. https://doi.org/10.1016/j.jfoodeng.2017.04.007

Costa, J. B., Park, J., Jorgensen, A. M., Silva-Correia, J., Reis, R. L., Oliveira, J. M., Atala, A., Yoo, J. J., & Lee, S. J. (2020). 3D bioprinted highly elastic hybrid constructs for advanced fibrocartilaginous tissue regeneration. *Chemistry of Materials, 32*(19), 8733–8746. https://doi.org/10.1021/acs.chemmater.0c03556

Costa, J. N., Leal, A. R., Nascimento, L. G. L., Rodrigues, D. C., Muniz, C. R., Figueiredo, R. W., Mata, P., Noronha, J. P., & Sousa, P. H. M. (2020). Texture, microstructure and volatile profile of structured guava using agar and gellan gum. *International Journal of Gastronomy and Food Science, 20*, Article 100207. https://doi.org/10.1016/j.ijgfs.2020.100207

Criado, P., Fraschini, C., Becher, D., Mendonça Pereira, F. G., Salmieri, S., & Lacroix, M. (2020). Modified cellulose nanocrystals (CNCs) loaded in gellan gum matrix enhance the preservation of agaricus bisporus mushrooms. *Journal of Food Processing and Preservation, 44*(11), Article e14846. https://doi.org/10.1111/jfpp.14846

Criado, P., Fraschini, C., Shankar, S., Salmieri, S., & Lacroix, M. (2021). Influence of cellulose nanocrystals gellan gum-based coating on color and respiration rate of agaricus bisporus mushrooms. *Journal of Food Science, 86*(2), 420–425. https://doi.org/10.1111/1750-3841.15580

Danalache, F., Carvalho, C. Y., Alves, V. D., Moldão-Martins, M., & Mata, P. (2016). Optimisation of gellan gum edible coating for ready-to-eat mango (Mangifera indica L.) bars. *International Journal of Biological Macromolecules, 84*, 43–53. https://doi.org/10.1016/j.ijbiomac.2015.11.079

de Souza, F. S., de Mello Ferreira, I. L., da Silva Costa, M. A., de Lima, A. L., da Costa, M. P., & da Silva, G. M. (2016). Evaluation of different methods to prepare superabsorbent hydrogels based on deacetylated gellan. *Carbohydrate Polymers, 148*, 309–317. https://doi.org/10.1016/j.carbpol.2016.04.019

Dey, M., Ghosh, B., & Giri, T. K. (2020). Enhanced intestinal stability and pH sensitive release of quercetin in GIT through gellan gum hydrogels. *Colloids Surf B Biointerfaces, 196*, Article 111341. https://doi.org/10.1016/j.colsurfb.2020.111341

Dhanka, M., Shetty, C., & Srivastava, R. (2018). Methotrexate loaded gellan gum microparticles for drug delivery. *International Journal of Biological Macromolecules, 110*, 346–356. https://doi.org/10.1016/j.ijbiomac.2017.12.026

Di Napoli, B., Franco, S., Severini, L., Tumiati, M., Buratti, E., Titubante, M., Nigro, V., Gnan, N., Micheli, L., Ruzicka, B., Mazzuca, C., Angelini, R., Missori, M., & Zaccarelli, E. (2020). Gellan gum microgels as effective agents for a rapid cleaning of paper. *ACS Applied Polymer Materials, 2*(7), 2791–2801. https://doi.org/10.1021/acsapm.0c00342

Ding, X., Shi, J., Wei, J., Li, Y., Wu, X., Zhang, Y., Jiang, X., Zhang, X., & Lai, H. (2021). A biopolymer hydrogel electrostatically reinforced by amino-functionalized bioactive glass for accelerated bone regeneration. *Science Advances, 7*(50), Article eabj7857. https://doi.org/10.1126/sciadv.abj7857

Dohle, E., Scherrieble, A., Doser, M., Al-Maawi, S., Hoss, M., Dauner, M., Sader, R., Kirkpatrick, C. J., & Ghanaati, S. (2020). Co-culture model for cutaneous wound healing to assess a porous fiber-based drug delivery system. *Tissue Engineering. Part C, Methods, 26*(9), 475–484. https://doi.org/10.1089/ten.TEC.2020.0145

Duffy, G. L., Liang, H., Williams, R. L., Wellings, D. A., & Black, K. (2021). 3D reactive inkjet printing of poly-ε-lysine/gellan gum hydrogels for potential corneal constructs. *Materials Science and Engineering: C, 131*, Article 112476. https://doi.org/10.1016/j.msec.2021.112476

Ebnesajjad, S. (2014). *Chapter 4 – Surface and material characterization techniques*.

Fan, Y., Yi, J., Hua, X., Zhang, Y., & Yang, R. (2017). Preparation and characterization of gellan gum microspheres containing a cold-adapted beta-galactosidase from rahnella sp. R3. *Carbohydrate Polymers, 162*, 10–15. https://doi.org/10.1016/j.carbpol.2017.01.033

Faraco, T. A., Silva, H. X., Barud, H. S., Ribeiro, T. C., Maciel, I. O., Quirino, W. G., Fragneaud, B., Cremona, M., Pandoli, O., & Legnani, C. (2021). Biosubstrates obtained from gellan gum for organic light-emitting diodes. *ACS Applied Electronic Materials, 3*(5), 2333–2340. https://doi.org/10.1021/acsaelm.1c00217

Federico, S., Pitarresi, G., Palumbo, F. S., Fiorica, C., Catania, V., Schillaci, D., & Giammona, G. (2021). An asymmetric electrospun membrane for the controlled release of ciprofloxacin and FGF-2: Evaluation of antimicrobial and chemoattractant properties. *Materials Science and Engineering: C, 123*, Article 112001. https://doi.org/10.1016/j.msec.2021.112001

Fialho, A. M., Martins, L. O., Donval, M. L., Leitao, J. H., Ridout, M. J., Jay, A. J., Morris, V. J., & Sa-Correia, I. I. (1999). Structures and properties of gellan polymers produced by sphingomonas paucimobilis ATCC 31461 from lactose compared with those produced from glucose and from cheese whey. *Applied and Environmental Microbiology, 65*(6), 2485–2491. https://doi.org/10.1128/aem.65.6.2485-2491.1999

Fialho, A. M., Moreira, L. M., Granja, A. T., Popescu, A. O., Hoffmann, K., & Sa-Correia, I. (2008). Occurrence, production, and applications of gellan: Current state and perspectives. *Applied Microbiology and Biotechnology, 79*(6), 889–900. https://doi.org/10.1007/s00253-008-1496-0

Forecast, M. D. (2021). In *Gellan gum market by product type (high acyl content, low acyl content), by application (food(confectionary, jams and jellies, fabricated foods, water-based gels, pie fillings and puddings, dairy products, processed meats, beverages), personal care and cosmetics, industrial and household cleaners, pharmaceuticals, and others), and by region (North America, Europe, Asia Pacific, Latin America, and Middle East & Africa) – Global industry analysis, size, share, growth, trends, and competitive analysis forecasts report 2021-2026* (pp. 1–175). Market Data Forecast.

Gadziński, P., Froelich, A., Kowalska, U., Soból, M., Szybowicz, M., & Osmałek, T. (2020). Encapsulation of roxithromycin into gellan gum matrices and the impact of other natural polymers on drug release. *Acta Poloniae Pharmaceutica - Drug Research, 77*(2), 319–330. https://doi.org/10.32383/appdr/118532

Gangane, P. S., & Kawtikwar, P. S. (2020). Development of donepezil hydrochloride loaded gellan gum based nasal mucoadhesive microspheres by spray drying method. *Indian Journal of Pharmaceutical Education and Research, 54*(4), 935–945. https://doi.org/10.5530/ijper.54.4.187

Gehrcke, M., de Bastos Brum, T., da Rosa, L. S., Ilha, B. D., Soares, F. Z. M., & Cruz, L. (2021). Incorporation of nanocapsules into gellan gum films: A strategy to improve the stability and prolong the cutaneous release of silibinin. *Materials Science and Engineering: C, 119*, Article 111624. https://doi.org/10.1016/j.msec.2020.111624

Ghezzi, M., Pescina, S., Padula, C., Santi, P., Del Favero, E., Cantù, L., & Nicoli, S. (2021). Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. *Journal of Controlled Release, 332*, 312–336. https://doi.org/10.1016/j.jconrel.2021.02.031

Giannakourou, M. C., & Tsironi, T. N. (2021). Application of processing and packaging hurdles for fresh-cut fruits and vegetables preservation. *Foods, 10*(4). https://doi.org/10.3390/foods10040830

Giavasis, I., Harvey, L. M., & McNeil, B. (2000). Gellan gum. *Critical Reviews in Biotechnology, 20*(3), 177–211. https://doi.org/10.1080/07388550008984169

Giri, T. K. (2016). 20 - alginate containing nanoarchitectonics for improved cancer therapy. In A. M. Holban, & A. M. Grumezescu (Eds.), *Nanoarchitectonics for smart delivery and drug targeting* (pp. 565–588). William Andrew Publishing.

Gomes, D., Costa, D., Queiroz, J. A., Passarinha, L. A., & Sousa, A. (2021). A new insight in gellan microspheres application to capture a plasmid DNA vaccine from an Escherichia coli lysate. *Separation and Purification Technology, 274*, Article 119013. https://doi.org/10.1016/j.seppur.2021.119013

Gomes, D., Gonçalves, C., Gonçalves, A. M., Queiroz, J. A., Sousa, A., & Passarinha, L. A. (2021). Applications of gellan natural polymer microspheres in recombinant catechol-O-methyltransferase direct capture from a komagataella pastoris lysate. *International Journal of Biological Macromolecules, 172*, 186–196. https://doi.org/10.1016/j.ijbiomac.2020.12.225

Göttel, B., Lucas, H., Syrowatka, F., Knolle, W., Kuntsche, J., Heinzelmann, J., Viestenz, A., & Mäder, K. (2020). In situ gelling amphotericin B nanofibers: A new option for the treatment of keratomycosis. *Frontiers in Bioengineering and Biotechnology, 8*. https://doi.org/10.3389/fbioe.2020.600384

Göttel, B., Souza e Silva, J., Oliveira, C., Syrowatka, F., Fiorentzis, M., Viestenz, A., Viestenz, A., & Mäder, K. (2020). Electrospun nanofibers – A promising solid in-situ gelling alternative for ocular drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics, 146*, 125–132. https://doi.org/10.1016/j.ejpb.2019.11.012

Groll, J., Burdick, J. A., Cho, D. W., Derby, B., Gelinsky, M., Heilshorn, S. C., Jüngst, T., Malda, J., Mironov, V. A., Nakayama, K., Ovsianikov, A., Sun, W., Takeuchi, S., Yoo, J. J., & Woodfield, T. B. F. (2018). A definition of bioinks and their distinction from biomaterial inks. *Biofabrication, 11*(1), Article 013001. https://doi.org/10.1088/1758-5090/aaec52

Gupta, V., & Trivedi, P. (2018). Chapter 15 - in vitro and in vivo characterization of pharmaceutical topical nanocarriers containing anticancer drugs for skin cancer treatment. In A. M. Grumezescu (Ed.), *Lipid nanocarriers for drug targeting* (pp. 563–627). William Andrew Publishing.

Huang, J., Zhu, S., Li, C., Zhang, C., & Ji, Y. (2020). Cost-effective optimization of gellan gum production by sphingomonas paucimobilis using corn steep liquor. *Preparative Biochemistry & Biotechnology, 50*(2), 191–197. https://doi.org/10.1080/10826068.2019.1692215

Isfahani, V. B., Pereira, R. P., Fernandes, M., Sabadini, R. C., Pereira, S., Dizaji, H. R., Arab, A., Fortunato, E., Pawlicka, A., Rego, R., Bermudez, V., & Silva, M. M. (2021). Gellan-gum and LiTFSI-based solid polymer electrolytes for electrochromic devices. *ChemistrySelect, 6*(20), 5110–5119. https://doi.org/10.1002/slct.202004614

Ismail, N. A., Amin, K. A. M., Majid, F. A. A., & Razali, M. H. (2019). Gellan gum incorporating titanium dioxide nanoparticles biofilm as wound dressing: Physicochemical, mechanical, antibacterial properties and wound healing studies. *Materials Science and Engineering: C, 103*, Article 109770. https://doi.org/10.1016/j.msec.2019.109770

Iurciuc, C.-E., Savin, A., Atanase, L. I., Danu, M., Martin, P., & Popa, M. (2018). Encapsulation of Saccharomyces cerevisiae in hydrogel particles based gellan ionically cross-linked with zinc acetate. *Powder Technology, 325*, 476–489. https://doi.org/10.1016/j.powtec.2017.11.017

Iurciuc Tincu, C. E., Alupei, L., Savin, A., Ibanescu, C., Martin, P., & Popa, M. (2016). Yeast cells immobilized in spherical gellan particles cross-linked with magnesium acetate. *Journal of Biotechnology, 236*, 45–56. https://doi.org/10.1016/j.jbiotec.2016.08.002

Jain, R., Shetty, S., & Yadav, K. S. (2020). Unfolding the electrospinning potential of biopolymers for preparation of nanofibers. *Journal of Drug Delivery Science and Technology, 57*, Article 101604. https://doi.org/10.1016/j.jddst.2020.101604

Jana, S., Pramanik, R., Nayak, A. K., & Sen, K. K. (2022). Gellan gum (GG)-based IPN microbeads for sustained drug release. *Journal of Drug Delivery Science and Technology, 69*, Article 103034. https://doi.org/10.1016/j.jddst.2021.103034

Jana, S., & Sen, K. K. (2019). Gellan gum/PVA interpenetrating network micro-beads for sustained drug delivery. *Materials Today: Proceedings, 11*, 614–619. https://doi.org/10.1016/j.matpr.2019.03.018

Jiang, J., Tang, Y., Zhu, H., Wei, D., Sun, J., & Fan, H. (2020). Dual functional modification of gellan gum hydrogel by introduction of methyl methacrylate and RGD contained polypeptide. *Materials Letters, 264*, Article 127341. https://doi.org/10.1016/j.matlet.2020.127341

Jin, H., Lee, N.-K., Shin, M.-K., Kim, S.-K., Kaplan, D. L., & Lee, J.-W. (2003). Production of gellan gum by sphingomonas paucimobilis NK2000 with soybean pomace. *Biochemical Engineering Journal, 16*(3), 357–360. https://doi.org/10.1016/S1369-703X(03)00076-7

Jongprasitkul, H., Turunen, S., Parihar, V. S., & Kellomäki, M. (2022). Two-step crosslinking to enhance the printability of methacrylated gellan gum biomaterial ink for extrusion-based 3D bioprinting. *Bioprinting, 25*, Article e00185. https://doi.org/10.1016/j.bprint.2021.e00185

Jung, S., Oh, H. K., Kim, M. S., Lee, K. Y., Park, H., & Kook, M. S. (2020). Effect of gellan Gum/Tuna skin film in guided bone regeneration in artificial bone defect in rabbit calvaria. *Materials (Basel), 13*(6). https://doi.org/10.3390/ma13061318

Kačarević, Ž. P., Rider, P. M., Alkildani, S., Retnasingh, S., Smeets, R., Jung, O., Ivanišević, Z., & Barbeck, M. (2018). An introduction to 3D bioprinting: Possibilities, challenges and future aspects. Retrieved from *Materials, 11*(11), 2199 https://www.mdpi.com/1996-1944/11/11/2199.

Kang, K. S., Veeder, G. T., Mirrasoul, P. J., Kaneko, T., & Cottrell, I. W. (1982). Agar-like polysaccharide produced by a pseudomonas species: Production and basic properties. *Applied and Environmental Microbiology, 43*(5), 1086–1091. https://doi.org/10.1128/aem.43.5.1086-1091.1982

Kanyuck, K. M., Norton-Welch, A. B., Mills, T. B., & Norton, I. T. (2021). Structural characterization of interpenetrating network formation of high acyl gellan and maltodextrin gels. *Food Hydrocolloids, 112*, Article 106295. https://doi.org/10.1016/j.foodhyd.2020.106295

Kaur, J., & Kaur, G. (2018). Optimization of pH conditions and characterization of polyelectrolyte complexes between gellan gum and cationic guar gum. *Polymers for Advanced Technologies, 29*(12), 3035–3048. https://doi.org/10.1002/pat.4424

Kedzierewicz, F., Lombry, C., Rios, R., Hoffman, M., & Maincent, P. (1999). Effect of the formulation on the in-vitro release of propranolol from gellan beads. *International Journal of Pharmaceutics, 178*(1), 129–136. https://doi.org/10.1016/s0378-5173(98)00351-2

Kim, D., Cho, H. H., Thangavelu, M., Song, C., Kim, H. S., Choi, M. J., Song, J. E., & Khang, G. (2020). Osteochondral and bone tissue engineering scaffold prepared from gallus var domesticus derived demineralized bone powder combined with gellan gum for medical application. *International Journal of Biological Macromolecules, 149*, 381–394. https://doi.org/10.1016/j.ijbiomac.2020.01.191

Kim, D., Thangavelu, M., Cheolui, S., Kim, H. S., Choi, M. J., Song, J. E., & Khang, G. (2019). Effect of different concentration of demineralized bone powder with gellan gum porous scaffold for the application of bone tissue regeneration. *International Journal of Biological Macromolecules, 134*, 749–758. https://doi.org/10.1016/j.ijbiomac.2019.04.184

Kim, H. S., Kim, D., Jeong, Y. W., Choi, M. J., Lee, G. W., Thangavelu, M., Song, J. E., & Khang, G. (2019). Engineering retinal pigment epithelial cells regeneration for transplantation in regenerative medicine using PEG/Gellan gum hydrogels. *International Journal of Biological Macromolecules, 130*, 220–228. https://doi.org/10.1016/j.ijbiomac.2019.01.078

Kim, N., Choi, J. H., Choi, M. J., Kim, J. S., Kim, W., Song, J. E., & Khang, G. (2020). Characterization of platelet-rich Plasma/Gellan gum hydrogel composite for biological performance to induce chondrogenesis from adipose-derived stem cells. *Macromolecular Research, 28*(12), 1098–1103. https://doi.org/10.1007/s13233-020-8155-4

Kouhi, M., Varshosaz, J., Hashemibeni, B., & Sarmadi, A. (2020). Injectable gellan gum/lignocellulose nanofibrils hydrogels enriched with melatonin loaded forsterite nanoparticles for cartilage tissue engineering: Fabrication, characterization and cell culture studies. *Materials Science and Engineering: C, 115*, Article 111114. https://doi.org/10.1016/j.msec.2020.111114

Kubo, W., Miyazaki, S., & Attwood, D. (2003). Oral sustained delivery of paracetamol from in situ-gelling gellan and sodium alginate formulations. *International Journal of Pharmaceutics, 258*(1–2), 55–64. https://doi.org/10.1016/s0378-5173(03)00163-7

Kuhn, K. R., e Silva, F. G. D., Netto, F. M., & da Cunha, R. L. (2019). Production of whey protein isolate – gellan microbeads for encapsulation and release of flaxseed bioactive compounds. *Journal of Food Engineering, 247*, 104–114. https://doi.org/10.1016/j.jfoodeng.2018.12.002

Kumar, S., Kaur, P., Bernela, M., Rani, R., & Thakur, R. (2016). Ketoconazole encapsulated in chitosan-gellan gum nanocomplexes exhibits prolonged antifungal activity. *International Journal of Biological Macromolecules, 93*(Pt A), 988–994. https://doi.org/10.1016/j.ijbiomac.2016.09.042

Kundu, B., Brancato, V., Oliveira, J. M., Correlo, V. M., Reis, R. L., & Kundu, S. C. (2020). Silk fibroin promotes mineralization of gellan gum hydrogels. *International Journal of Biological Macromolecules, 153*, 1328–1334. https://doi.org/10.1016/j.ijbiomac.2019.10.269

Kundu, P., Datta, R., & Maiti, S. (2015). Hexadecyl gellan amphiphilic nanoparticles: Physicochemical properties and in vivo lipid-lowering potential. *Journal of Drug Delivery Science and Technology, 27*, 9–17. https://doi.org/10.1016/j.jddst.2015.03.004

Lankalapalli, S., & Kolapalli, V. R. (2009). Polyelectrolyte complexes: A review of their applicability in drug delivery technology. *Indian Journal of Pharmaceutical Sciences, 71*(5), 481–487. https://doi.org/10.4103/0250-474x.58165

Lázaro, N., Sevilla, A. L., Morales, S., & Marqués, A. M. (2003). Heavy metal biosorption by gellan gum gel beads. *Water Research, 37*(9), 2118–2126. https://doi.org/10.1016/S0043-1354(02)00575-4

Leal, A. R., Oliveira, L. S., Costa, J. N., Alves, C. A., Mata, P., & Sousa, P. H. (2022). In vitro bioaccessibility of antioxidant compounds from structured fruits developed with gellan gum and agar. *Rev Ciência Agronômica, 53*, 1–10. https://doi.org/10.5935/1806-6690.20220005

Lee, S., Choi, J. H., Park, A., Rim, M., Youn, J., Lee, W., Song, J. E., & Khang, G. (2020). Advanced gellan gum-based glycol chitosan hydrogel for cartilage tissue engineering biomaterial. *International Journal of Biological Macromolecules, 158*, 452–460. https://doi.org/10.1016/j.ijbiomac.2020.04.135

Lee, W., Choi, J. H., Lee, J., Youn, J., Kim, W., Jeon, G., Lee, S. W., Song, J. E., & Khang, G. (2021). Dopamine-functionalized gellan gum hydrogel as a candidate biomaterial for a retinal pigment epithelium cell delivery system. *ACS Applied Bio Materials, 4*(2), 1771–1782. https://doi.org/10.1021/acsabm.0c01516

Leopércio, B. C., Ribeiro, S., Gomes, F., Michelon, M., & Carvalho, M. S. (2021). Microcapsules with controlled magnetic response. *Microfluidics and Nanofluidics, 25*(12), 98. https://doi.org/10.1007/s10404-021-02498-9

Li, A., Xu, H., Yu, P., Xing, J., Ding, C., Yan, X., Xie, J., & Li, J. (2020). Injectable hydrogels based on gellan gum promotes in situ mineralization and potential osteogenesis. *European Polymer Journal, 141*, Article 110091. https://doi.org/10.1016/j.eurpolymj.2020.110091

Li, H., Severini, L., Titubante, M., Gong, D., Micheli, L., Mazzuca, C., & Gong, Y. (2021). Gellan gum hydrogel as an aqueous treatment method for Xuan paper. Restaurator. *International Journal for the Preservation of Library and Archival Material, 42*(1), 37–54. https://doi.org/10.1515/res-2020-0010

Li, W., Wu, D., Hu, D., Zhu, S., Pan, C., Jiao, Y., Li, L., Luo, B., Zhou, C., & Lu, L. (2020). Stress-relaxing double-network hydrogel for chondrogenic differentiation of stem cells. *Materials Science and Engineering: C, 107*, Article 110333. https://doi.org/10.1016/j.msec.2019.110333

Liang, L., Lin, X., Liu, Y., Sun, S., Chu, H., Chen, Y., Liu, D., Luo, X., Zhang, J., & Shang, R. (2020). Carboxymethyl konjac glucomannan mechanically reinforcing gellan gum microspheres for uranium removal. *International Journal of Biological Macromolecules, 145*, 535–546. https://doi.org/10.1016/j.ijbiomac.2019.12.188

Lin, C.-C., & Chiu, J.-Y. (2021). Glycerol-modified γ-PGA and gellan composite hydrogel materials with tunable physicochemical and thermal properties for soft tissue engineering application. *Polymer, 230*, Article 124049. https://doi.org/10.1016/j.polymer.2021.124049

Liu, S., Qiu, Y., Yu, W., & Zhang, H. (2020). Highly stretchable and self-healing strain sensor based on gellan gum hybrid hydrogel for human motion monitoring. *ACS Applied Polymer Materials, 2*(3), 1325–1334. https://doi.org/10.1021/acsapm.9b01200

Liu, X., Tian, Y., Wu, Y., Chen, F., Mu, Y., Minus, M. L., & Zheng, Y. (2021). Fully biomass-based hybrid hydrogel for efficient solar desalination with salt self-cleaning property. *ACS Applied Materials & Interfaces, 13*(36), 42832–42842. https://doi.org/10.1021/acsami.1c11636

Lozano, R., Stevens, L., Thompson, B. C., Gilmore, K. J., Gorkin, R., Stewart, E. M., Panhuis, M., Romero-Ortega, M., & Wallace, G. G. (2015). 3D printing of layered brain-like structures using peptide modified gellan gum substrates. *Biomaterials, 67*, 264–273. https://doi.org/10.1016/j.biomaterials.2015.07.022

Lyu, C.-J., Liu, L., Huang, J., Zhao, W.-R., Hu, S., Mei, L.-H., & Yao, S.-J. (2019). Biosynthesis of γ-aminobutyrate by engineered lactobacillus brevis cells immobilized in gellan gum gel beads. *Journal of Bioscience and Bioengineering, 128*(2), 123–128. https://doi.org/10.1016/j.jbiosc.2019.01.010

Ma, P. X. (2004). Scaffolds for tissue fabrication. *Materials Today, 7*(5), 30–40. https://doi.org/10.1016/S1369-7021(04)00233-0

Mahajan, H. S., & Patil, P. P. (2017). In situ cross linked chitosan-gellan gum polyelectrolyte complex based nanogels containing curcumin for delivery to cancer cells. *Indian Journal of Pharmaceutical Education and Research, 51*, s40–s45. https://doi.org/10.5530/ijper.51.2s.48

Mahdi, M. H., Conway, B. R., Mills, T., & Smith, A. M. (2016). Gellan gum fluid gels for topical administration of diclofenac. *International Journal of Pharmaceutics, 515*(1–2), 535–542. https://doi.org/10.1016/j.ijpharm.2016.10.048

Malda, J., Visser, J., Melchels, F. P., Jüngst, T., Hennink, W. E., Dhert, W. J. A., Groll, J., & Hutmacher, D. W. (2013). 25th anniversary article: Engineering hydrogels for biofabrication. *Advanced Materials, 25*(36), 5011–5028. https://doi.org/10.1002/adma.201302042

Manna, B., Gambhir, A., & Ghosh, P. (1996). Production and rheological characteristics of the microbial polysaccharide gellan. *Letters in Applied Microbiology, 23*(3), 141–145. https://doi.org/10.1111/j.1472-765X.1996.tb00050.x

Martins, L. O., & Sá-Correia, I. (1994). Temperature profiles of gellan gum synthesis and activities of biosynthetic enzymes1. *Biotechnology and Applied Biochemistry, 20*(3), 385–395. https://doi.org/10.1111/j.1470-8744.1994.tb00324.x

McClements, D. J. (2007). Critical review of techniques and methodologies for characterization of emulsion stability. *Critical Reviews in Food Science and Nutrition, 47*(7), 611–649. https://doi.org/10.1080/10408390701289292

Mehnath, S., Ayisha Sithika, M. A., Arjama, M., Rajan, M., Amarnath Praphakar, R., & Jeyaraj, M. (2019). Sericin-chitosan doped maleate gellan gum nanocomposites for effective cell damage in mycobacterium tuberculosis. *International Journal of Biological Macromolecules, 122*, 174–184. https://doi.org/10.1016/j.ijbiomac.2018.10.167

Meneguin, A. B., Beyssac, E., Garrait, G., Hsein, H., & Cury, B. S. F. (2018). Retrograded starch/pectin coated gellan gum-microparticles for oral administration of insulin: A technological platform for protection against enzymatic degradation and improvement of intestinal permeability. *European Journal of Pharmaceutics and Biopharmaceutics, 123*, 84–94. https://doi.org/10.1016/j.ejpb.2017.11.012

Michelon, M., Leopércio, B. C., & Carvalho, M. S. (2020). Microfluidic production of aqueous suspensions of gellan-based microcapsules containing hydrophobic compounds. *Chemical Engineering Science, 211*, Article 115314. https://doi.org/10.1016/j.ces.2019.115314

Mirón-Mérida, V. A., Yáñez-Fernández, J., Montañez-Barragán, B., & Barragán Huerta, B. E. (2019). Valorization of coffee parchment waste (Coffea arabica) as a source of caffeine and phenolic compounds in antifungal gellan gum films. *LWT, 101*, 167–174. https://doi.org/10.1016/j.lwt.2018.11.013

Miyazaki, S., Aoyama, H., Kawasaki, N., Kubo, W., & Attwood, D. (1999). In situ-gelling gellan formulations as vehicles for oral drug delivery. *Journal of Controlled Release, 60*(2–3), 287–295. https://doi.org/10.1016/s0168-3659(99)00084-x

Miyoshi, E., & Nishinari, K. (1999). Rheological and thermal properties near the sol-gel transition of gellan gum aqueous solutions. In K. Nishinari (Ed.), *Physical chemistry and industrial application of gellan gum* (pp. 68–82). Berlin HeidelbergBerlin, Heidelberg: Springer.

Modi, D., Nirmal, J., Warsi, M. H., Bhatia, M., Hasan, N., Kesharwani, P., & Jain, G. K. (2022). Formulation and development of tacrolimus-gellan gum nanoformulation for treatment of dry eye disease. *Colloids and Surfaces B: Biointerfaces, 211*, Article 112255. https://doi.org/10.1016/j.colsurfb.2021.112255

Morris, E. R., Nishinari, K., & Rinaudo, M. (2012). Gelation of gellan – a review. *Food Hydrocolloids, 28*(2), 373–411. https://doi.org/10.1016/j.foodhyd.2012.01.004

Moslemy, P., Guiot, S. R., & Neufeld, R. J. (2004). Activated sludge encapsulation in gellan gum microbeads for gasoline biodegradation. *Bioprocess and Biosystems Engineering, 26*(4), 197–204. https://doi.org/10.1007/s00449-004-0360-6

Moslemy, P., Neufeld, R. J., & Guiot, S. R. (2002). Biodegradation of gasoline by gellan gum-encapsulated bacterial cells. *Biotechnology and Bioengineering, 80*(2), 175–184. https://doi.org/10.1002/bit.10358

Mouser, V. H. M., Melchels, F. P. W., Visser, J., Dhert, W. J. A., Gawlitta, D., & Malda, J. (2016). Yield stress determines bioprintability of hydrogels based on gelatin-methacryloyl and gellan gum for cartilage bioprinting. *Biofabrication, 8*(3), Article 035003. https://doi.org/10.1088/1758-5090/8/3/035003

Muktar, M. Z., Bakar, M. A. A., Amin, K. A. M., Che Rose, L., Wan Ismail, W. I., Razali, M. H., Razak, S. I., & Panhuis, M. (2021). Gellan gum hydrogels filled edible oil microemulsion for biomedical materials: Phase diagram, mechanical behavior, and in vivo studies. *Polymers (Basel), 13*(19). https://doi.org/10.3390/polym13193281

Müller, M., Fisch, P., Molnar, M., Eggert, S., Binelli, M., Maniura-Weber, K., & Zenobi-Wong, M. (2020). Development and thorough characterization of the processing steps of an ink for 3D printing for bone tissue engineering. *Materials Science and Engineering: C, 108*, Article 110510. https://doi.org/10.1016/j.msec.2019.110510

Murphy, S. V., & Atala, A. (2014). 3D bioprinting of tissues and organs. *Nature Biotechnology, 32*(8), 773–785. https://doi.org/10.1038/nbt.2958

Muthukumar, C., Jusuarockiam, N., Krishnasamy, S. K., T, S. M. K., Jawaid, M., Ridwan, I. M., & Britto, J. J. (2020). 2 - Role of characterization techniques in evaluating the material properties of nanoparticle-based polymer materials. In R. Bouhfid, A. E. K. Qaiss, & M. Jawaid (Eds.), *Polymer nanocomposite-based smart materials* (pp. 21–39). Woodhead Publishing.

Nair, L. S., & Laurencin, C. T. (2007). Biodegradable polymers as biomaterials. *Progress in Polymer Science, 32*(8), 762–798. https://doi.org/10.1016/j.progpolymsci.2007.05.017

Nampoothiri, K. M., Singhania, R. R., Sabarinath, C., & Pandey, A. (2003). Fermentative production of gellan using sphingomonas paucimobilis. *Process Biochemistry, 38*(11), 1513–1519. https://doi.org/10.1016/S0032-9592(02)00321-7

Nandi, G., Nandi, A. K., Khan, N. S., Pal, S., & Dey, S. (2018). Tamarind seed gum-hydrolyzed polymethacrylamide-g-gellan beads for extended release of diclofenac sodium using 3(2) full factorial design. *International Journal of Biological Macromolecules, 114*, 214–225. https://doi.org/10.1016/j.ijbiomac.2018.03.064

Nascimento da Costa, J., Lima Nascimento, L. G., Leal, A. R., Danalache, F., Moreira Leite, B. S., Figueiredo, R. W., Mata, P., Alves, V. D., & Machado de Sousa, P. H. (2021). Effect of agar and gellan gum on structured guava (Psidium guajava L.): Rheological behavior and gastrointestinal digestion in vitro. *Food Bioscience, 42*, Article 101165. https://doi.org/10.1016/j.fbio.2021.101165

Ng, J. Y., Zhu, X., Mukherjee, D., Zhang, C., Hong, S., Kumar, Y., Gokhale, R., & Ee, P. L. R. (2021). Pristine gellan gum-collagen interpenetrating network hydrogels as mechanically enhanced anti-inflammatory biologic wound dressings for burn wound therapy. *ACS Applied Bio Materials, 4*(2), 1470–1482. https://doi.org/10.1021/acsabm.0c01363

Nimesh, S. (2013). 3 - tools and techniques for physico-chemical characterization of nanoparticles. In S. Nimesh (Ed.), *Gene therapy* (pp. 43–63). Woodhead Publishing.

Noor, I. M. (2020). Determination of charge carrier transport properties of gellan gum–lithium triflate solid polymer electrolyte from vibrational spectroscopy. *High Performance Polymers, 32*(2), 168–174. https://doi.org/10.1177/0954008319890016

Oliveira, A. C., Lima, G. F., Klein, R. S., Souza, P. R., Vilsinski, B. H., Garcia, F. P., Nakamura, C. V., & Martins, A. F. (2021). Thermo-and pH-responsive chitosan/gellan gum hydrogels incorporated with the β-cyclodextrin/curcumin inclusion complex for efficient curcumin delivery. *Reactive and Functional Polymers, 165*, Article 104955. https://doi.org/10.1016/j.reactfunctpolym.2021.104955

Oliveira, A. C., Sabino, R. M., Souza, P. R., Muniz, E. C., Popat, K. C., Kipper, M. J., Zola, R. S., & Martins, A. F. (2020). Chitosan/gellan gum ratio content into blends modulates the scaffolding capacity of hydrogels on bone mesenchymal stem cells. *Materials Science and Engineering: C, 106*, Article 110258. https://doi.org/10.1016/j.msec.2019.110258

Osmalek, T., Froelich, A., & Tasarek, S. (2014). Application of gellan gum in pharmacy and medicine. *International Journal of Pharmaceutics, 466*(1–2), 328–340. https://doi.org/10.1016/j.ijpharm.2014.03.038

Pacelli, S., Paolicelli, P., Petralito, S., Subham, S., Gilmore, D., Varani, G., Yang, G., Lin, D., Casadei, M. A., & Paul, A. (2020). Investigating the role of polydopamine to modulate stem cell adhesion and proliferation on gellan gum-based hydrogels. *ACS Applied Bio Materials, 3*(2), 945–951. https://doi.org/10.1021/acsabm.9b00989

Pakzad, Y., Fathi, M., & Mozafari, M. (2019). Chapter 17 - Characterization methodologies of functional polymers. In M. Mozafari, & N. P. Singh Chauhan (Eds.), *Advanced functional polymers for biomedical applications* (pp. 359–381). Elsevier.

Palumbo, F. S., Federico, S., Pitarresi, G., Fiorica, C., & Giammona, G. (2020). Gellan gum-based delivery systems of therapeutic agents and cells. *Carbohydrate Polymers, 229*, Article 115430. https://doi.org/10.1016/j.carbpol.2019.115430

Palumbo, F. S., Federico, S., Pitarresi, G., Fiorica, C., Scaffaro, R., Maio, A., Gulino, E. F., & Giammona, G. (2021). Effect of alkyl derivatization of gellan gum during the fabrication of electrospun membranes. *Journal of Industrial Textiles, 51*(2_suppl), 2467S–2482S. https://doi.org/10.1177/15280837211007508

Paolicelli, P., Petralito, S., Varani, G., Nardoni, M., Pacelli, S., Di Muzio, L., Tirillò, J., Bartuli, C., Cesa, S., Casadei, M. A., & Adrover, A. (2018). Effect of glycerol on the physical and mechanical properties of thin gellan gum films for oral drug delivery. *International Journal of Pharmaceutics, 547*(1), 226–234. https://doi.org/10.1016/j.ijpharm.2018.05.046

Park, H., Kim, H., Kim, G.-Y., Lee, M.-Y., Kim, Y., & Kang, S. (2021). Enhanced biodegradation of hydrocarbons by Pseudomonas aeruginosa-encapsulated alginate/gellan gum microbeads. *Journal of Hazardous Materials, 406*, Article 124752. https://doi.org/10.1016/j.jhazmat.2020.124752

Patel, A., Sant, V., Velankar, S., Dutta, M., Balasubramanian, V., Sane, P., Agrawal, V., Wilson, J., Rohan, L. C., & Sant, S. (2020). Self-assembly of multiscale anisotropic hydrogels through interfacial polyionic complexation. *Journal of Biomedical Materials Research Part A, 108*(12), 2504–2518. https://doi.org/10.1002/jbm.a.37001

Patil, P., Chavanke, D., & Wagh, M. P. (2012). *A review on ionotropic gelation method: Novel approach for controlled gastroretentive gelispheres*.

Peng, X., Liu, G., Zhu, L., Yu, K., Qian, K., & Zhan, X. (2020). In vitro and in vivo study of novel antimicrobial gellan–polylysine polyion complex fibers as suture materials. *Carbohydrate Research, 496*, Article 108115. https://doi.org/10.1016/j.carres.2020.108115

Perk, M. (2013). *Soil and water contamination : From molecular to catchment scale* (2nd ed.). London: Taylor & Francis.

Pitarresi, G., Martorana, A., Palumbo, F. S., Fiorica, C., & Giammona, G. (2020). New gellan gum-graft-poly(d, l-lactide-co-glycolide) copolymers as promising bioinks: Synthesis and characterization. *International Journal of Biological Macromolecules, 162*, 1653–1667. https://doi.org/10.1016/j.ijbiomac.2020.07.254

Prajapati, V. D., Jani, G. K., Zala, B. S., & Khutliwala, T. A. (2013). An insight into the emerging exopolysaccharide gellan gum as a novel polymer. *Carbohydrate Polymers, 93*(2), 670–678. https://doi.org/10.1016/j.carbpol.2013.01.030

Prezotti, F. G., Cury, B. S. F., & Evangelista, R. C. (2014). Mucoadhesive beads of gellan gum/pectin intended to controlled delivery of drugs. *Carbohydrate Polymers, 113*, 286–295. https://doi.org/10.1016/j.carbpol.2014.07.021

Pugliese, R., Beltrami, B., Regondi, S., & Lunetta, C. (2021). Polymeric biomaterials for 3D printing in medicine: An overview. *Annals of 3D Printed Medicine, 2*, Article 100011. https://doi.org/10.1016/j.stlm.2021.100011

Racovita, S., Lungan, M. A., Bunia, I., Popa, M., & Vasiliu, S. (2016). Adsorption and release studies of cefuroxime sodium from acrylic ion exchange resin microparticles coated with gellan. *Reactive and Functional Polymers, 105*, 103–113. https://doi.org/10.1016/j.reactfunctpolym.2016.06.003

Raghunandan, K., Kumar, A., Kumar, S., Permaul, K., & Singh, S. (2018). Production of gellan gum, an exopolysaccharide, from biodiesel-derived waste glycerol by sphingomonas spp. *3Biotech, 8*(1), 71. https://doi.org/10.1007/s13205-018-1096-3

Ramos, M., Mellinas, C., Solaberrieta, I., Garrigós, M. C., & Jiménez, A. (2021). Emulsions incorporated in polysaccharide-based active coatings for fresh and minimally processed vegetables. *Foods, 10*(3). https://doi.org/10.3390/foods10030665

Raval, N., Maheshwari, R., Kalyane, D., Youngren-Ortiz, S. R., Chougule, M. B., & Tekade, R. K. (2019). Chapter 10 - importance of physicochemical characterization of nanoparticles in pharmaceutical product development. In R. K. Tekade (Ed.), *Basic fundamentals of drug delivery* (pp. 369–400). Academic Press.

Razali, M. H., Ismail, N. A., & Mat Amin, K. A. (2020). Titanium dioxide nanotubes incorporated gellan gum bio-nanocomposite film for wound healing: Effect of TiO2 nanotubes concentration. *International Journal of Biological Macromolecules, 153*, 1117–1135. https://doi.org/10.1016/j.ijbiomac.2019.10.242

Richa, R. C. A. (2019). Synthesis and rheological characterization of a novel thermostable quick setting composite hydrogel of gellan and pullulan. *International Journal of Biological Macromolecules, 125*, 979–988. https://doi.org/10.1016/j.ijbiomac.2018.12.147

Rider, P., Kačarević, Ž. P., Alkildani, S., Retnasingh, S., & Barbeck, M. (2018). Bioprinting of tissue engineering scaffolds. *Journal of Tissue Engineering, 9*. https://doi.org/10.1177/2041731418802090, 2041731418802090.

Rim, M. A., Choi, J. H., Park, A., Youn, J., Lee, S., Kim, N. E., Song, J. E., & Khang, G. (2020). Characterization of Gelatin/Gellan Gum/Glycol chitosan ternary hydrogel for retinal pigment epithelial tissue reconstruction materials. *ACS Applied Bio Materials, 3*(9), 6079–6087. https://doi.org/10.1021/acsabm.0c00672

Rostami, M., Ghorbani, M., Aman Mohammadi, M., Delavar, M., Tabibiazar, M., & Ramezani, S. (2019). Development of resveratrol loaded chitosan-gellan nanofiber as a novel gastrointestinal delivery system. *International Journal of Biological Macromolecules, 135*, 698–705. https://doi.org/10.1016/j.ijbiomac.2019.05.187

Rydz, J., Šišková, A., & Andicsová Eckstein, A. (2019). Scanning electron microscopy and atomic force microscopy: Topographic and dynamical surface studies of blends, composites, and hybrid functional materials for sustainable future. *Advances in Materials Science and Engineering, 2019*, Article 6871785. https://doi.org/10.1155/2019/6871785

Sajna, K. V., Gottumukkala, L. D., Sukumaran, R. K., & Pandey, A. (2015). Chapter 18 - White biotechnology in cosmetics. In A. Pandey, R. Höfer, M. Taherzadeh, K. M. Nampoothiri, & C. Larroche (Eds.), *Industrial biorefineries & white biotechnology* (pp. 607–652). Amsterdam: Elsevier.

Santos, K. L., Machado de Sousa, P. H., Rangel Moreira Cavalcanti-Mata, M. E., & Barros de Vasconcelos, L. (2021). Mixed leather of açaí, banana, peanut, and guarana syrup: The effect of agar and gellan gum use on quality attributes. Retrieved from *International Journal of Gastronomy and Food Science, 26*, Article 100407. https://doi.org/10.1016/j.ijgfs.2021.100407.

Santos, T. P., Costa, A. L. R., Michelon, M., Costa, L. P., & Cunha, R. L. (2020). Development of a microfluidic route for the formation of gellan-based microgels incorporating jabuticaba (Myrciaria cauliflora) extract. *Journal of Food Engineering, 276*, Article 109884. https://doi.org/10.1016/j.jfoodeng.2019.109884

Sapper, M., Bonet, M., & Chiralt, A. (2019). Wettability of starch-gellan coatings on fruits, as affected by the incorporation of essential oil and/or surfactants. *LWT, 116*, Article 108574. https://doi.org/10.1016/j.lwt.2019.108574

Senjoti, F. G., Timmins, P., Conway, B. R., & Smith, A. M. (2020). Optimizing ophthalmic delivery of a poorly water soluble drug from an aqueous in situ gelling system. *European Journal of Pharmaceutics and Biopharmaceutics, 154*, 1–7. https://doi.org/10.1016/j.ejpb.2020.06.016

Shabani, S., & Dinari, M. (2021). Ag/LDH-itaconic acid-gellan gam nanocomposites: Facile and green synthesis, characterization, and excellent catalytic reduction of 4-nitrophenol. *International Journal of Biological Macromolecules, 193*(Pt B), 1645–1652. https://doi.org/10.1016/j.ijbiomac.2021.11.001

Sharanappa, C., Ambresh, P. A., & Gangadhar, B. (2019). Gellan gum based silver nanocomposite hydrogel: Preparation, characterisation and anti-bacterial study. *Materials Today: Proceedings, 18*, 3937–3945. https://doi.org/10.1016/j.matpr.2019.07.334

Shirsath, N. R., & Goswami, A. K. (2021). Vildagliptin-loaded gellan gum mucoadhesive beads for sustained drug delivery: Design, optimisation and evaluation. *Materials Technology, 36*(11), 647–659. https://doi.org/10.1080/10667857.2020.1786783

Sinha Ray, S. (2013). 4 - techniques for characterizing the structure and properties of polymer nanocomposites. In S. Sinha Ray (Ed.), *Environmentally friendly polymer nanocomposites* (pp. 74–88). Woodhead Publishing.

Šlouf, M., Vacková, T., Lednický, F., & Wandrol, P. (2014). 6. Polymer surface morphology: Characterization by electron microscopies. In S. Luigia (Ed.), *Polymer surface characterization* (pp. 169–206). Berlin, Boston: De Gruyter.

Stephen, A. M., & Phillips, G. O. (2006). *Food polysaccharides and their applications* (2nd ed.). CRC Press. https://doi.org/10.1201/9781420015164

Stijnman, A. C., Bodnar, I., & Hans Tromp, R. (2011). Electrospinning of food-grade polysaccharides. *Food Hydrocolloids, 25*(5), 1393–1398. https://doi.org/10.1016/j.foodhyd.2011.01.005

Tako, M., Sakae, A., & Nakamura, S. (1989). Rheological properties of gellan gum in aqueous media. *Agricultural and Biological Chemistry, 53*(3), 771–776. https://doi.org/10.1080/00021369.1989.10869354

ter Horst, B., Moakes, R. J. A., Chouhan, G., Williams, R. L., Moiemen, N. S., & Grover, L. M. (2019). A gellan-based fluid gel carrier to enhance topical spray delivery. *Acta Biomaterialia, 89*, 166–179. https://doi.org/10.1016/j.actbio.2019.03.036

Tomadoni, B., Moreira, M. R., Pereda, M., & Ponce, A. G. (2018). Gellan-based coatings incorporated with natural antimicrobials in fresh-cut strawberries: Microbiological and sensory evaluation through refrigerated storage. *LWT, 97*, 384–389. https://doi.org/10.1016/j.lwt.2018.07.029

Tomovska, R., Agirre, A., Veloso, A., & Leiza, J. R. (2014). Characterization techniques for polymeric materials. In *Reference module in chemistry, molecular sciences and chemical engineering*. Elsevier.

Tran, T. P. A., Cho, H., Cho, G.-C., Han, J.-I., & Chang, I. (2021). Nickel (Ni2+) removal from water using gellan gum-sand mixture as a filter material. *Applied Sciences, 11*(17), 7884. Retrieved from https://www.mdpi.com/2076-3417/11/17/7884.

Trucco, D., Vannozzi, L., Teblum, E., Telkhozhayeva, M., Nessim, G. D., Affatato, S., Al-Haddad, H., Lisignoli, G., & Ricotti, L. (2021). Graphene oxide-doped gellan Gum–PEGDA bilayered hydrogel mimicking the mechanical and lubrication properties of articular cartilage. *Advanced Healthcare Materials, 10*(12), Article 2100873. https://doi.org/10.1002/adhm.202100873

Tsai, W., Tsai, H., Wong, Y., Hong, J., Chang, S., & Lee, M. (2018). Preparation and characterization of gellan gum/glucosamine/clioquinol film as oral cancer treatment patch. *Materials Science & Engineering. C, Materials for Biological Applications, 82*, 317–322. https://doi.org/10.1016/j.msec.2017.05.040

Umar Mustapha, M., Halimoon, N., Wan Johari, W. L., & Abd Shukor, M. Y. (2020). Enhanced carbofuran degradation using immobilized and free cells of enterobacter sp. Isolated from soil. *Molecules, 25*(12). https://doi.org/10.3390/molecules25122771

Vashisth, P., Nikhil, K., Roy, P., Pruthi, P. A., Singh, R. P., & Pruthi, V. (2016). A novel gellan–PVA nanofibrous scaffold for skin tissue regeneration: Fabrication and characterization. *Carbohydrate Polymers, 136*, 851–859. https://doi.org/10.1016/j.carbpol.2015.09.113

Vashisth, P., & Pruthi, V. (2016). Synthesis and characterization of crosslinked gellan/PVA nanofibers for tissue engineering application. *Materials Science and Engineering: C, 67*, 304–312. https://doi.org/10.1016/j.msec.2016.05.049

Vashisth, P., Raghuwanshi, N., Srivastava, A. K., Singh, H., Nagar, H., & Pruthi, V. (2017). Ofloxacin loaded gellan/PVA nanofibers - synthesis, characterization and

evaluation of their gastroretentive/mucoadhesive drug delivery potential. *Materials Science and Engineering: C, 71*, 611–619. https://doi.org/10.1016/j.msec.2016.10.051

Vieira, S., da Silva Morais, A., Garet, E., Silva-Correia, J., Reis, R. L., González-Fernández, Á., & Miguel Oliveira, J. (2019). Self-mineralizing ca-enriched methacrylated gellan gum beads for bone tissue engineering. *Acta Biomaterialia, 93*, 74–85. https://doi.org/10.1016/j.actbio.2019.01.053

Vieira, S., da Silva Morais, A., Garet, E., Silva-Correia, J., Reis, R. L., González-Fernández, Á., & Oliveira, J. M. (2021). Methacrylated gellan Gum/Poly-l-lysine polyelectrolyte complex beads for cell-based therapies. *ACS Biomaterials Science & Engineering, 7*(10), 4898–4913. https://doi.org/10.1021/acsbiomaterials.1c00486

Vieira, S., Strymecka, P., Stanaszek, L., Silva-Correia, J., Drela, K., Fiedorowicz, M., Malysz-Cymborska, I., Rogujski, P., Janowski, M., Reis, R. L., Lukomska, B., Walczak, P., & Oliveira, J. M. (2020). Methacrylated gellan gum and hyaluronic acid hydrogel blends for image-guided neurointerventions. *Journal of Materials Chemistry B, 8*(27), 5928–5937. https://doi.org/10.1039/D0TB00877J

Wahba, M. I. (2018). Processed gellan gum beads as covalent immobilization carriers. *Biocatalysis and Agricultural Biotechnology, 14*, 270–278. https://doi.org/10.1016/j.bcab.2018.03.019

Wang, L., Li, M., Li, X., Liu, J., Mao, Y., & Tang, K. (2020). A biomimetic hybrid hydrogel based on the interactions between amino hydroxyapatite and Gelatin/Gellan gum. *Macromolecular Materials and Engineering, 305*(9), Article 2000188. https://doi.org/10.1002/mame.202000188

Wei, Y.-C., Cheng, C.-H., Ho, Y.-C., Tsai, M.-L., & Mi, F.-L. (2017). Active gellan gum/purple sweet potato composite films capable of monitoring pH variations. *Food Hydrocolloids, 69*, 491–502. https://doi.org/10.1016/j.foodhyd.2017.03.010

Williams, R., & Mittal, G. S. (1999). Low-fat fried foods with edible coatings: Modeling and simulation. *Journal of Food Science, 64*(2), 317–322. https://doi.org/10.1111/j.1365-2621.1999.tb15891.x

Wu, D., Yu, Y., Tan, J., Huang, L., Luo, B., Lu, L., & Zhou, C. (2018). 3D bioprinting of gellan gum and poly (ethylene glycol) diacrylate based hydrogels to produce human-scale constructs with high-fidelity. *Materials & Design, 160*, 486–495. https://doi.org/10.1016/j.matdes.2018.09.040

Wu, Y., Liang, Y., Liu, Y., Hao, Y., Tao, N., Li, J., Sun, X., Zhou, M., & Liu, Y.-N. (2021). A Bi2S3-embedded gellan gum hydrogel for localized tumor photothermal/antiangiogenic therapy. *Journal of Materials Chemistry B, 9*(14), 3224–3234. https://doi.org/10.1039/D1TB00257K

Xu, L., Bai, X., Yang, J., Li, J., Xing, J., Yuan, H., Xie, J., & Li, J. (2020). Preparation and characterisation of a gellan gum-based hydrogel enabling osteogenesis and inhibiting enterococcus faecalis. *International Journal of Biological Macromolecules, 165*, 2964–2973. https://doi.org/10.1016/j.ijbiomac.2020.10.083

Xue, J., Wu, T., Dai, Y., & Xia, Y. (2019). Electrospinning and electrospun nanofibers: Methods, materials, and applications. *Chemical Reviews, 119*(8), 5298–5415. https://doi.org/10.1021/acs.chemrev.8b00593

Yu, I., Kaonis, S., & Chen, R. (2017). A study on degradation behavior of 3D printed gellan gum scaffolds. *Procedia CIRP, 65*, 78–83. https://doi.org/10.1016/j.procir.2017.04.020

Zhang, N., Xu, J., Gao, X., Fu, X., & Zheng, D. (2017). Factors affecting water resistance of alginate/gellan blend films on paper cups for hot drinks. *Carbohydrate Polymers, 156*, 435–442. https://doi.org/10.1016/j.carbpol.2016.08.101

Zhang, X., Liu, D., Jin, T. Z., Chen, W., He, Q., Zou, Z., & Guo, M. (2021). Preparation and characterization of gellan gum-chitosan polyelectrolyte complex films with the incorporation of thyme essential oil nanoemulsion. *Food Hydrocolloids, 114*, Article 106570. https://doi.org/10.1016/j.foodhyd.2020.106570

Zhao, X., & Wang, Z. (2019). A pH-sensitive microemulsion-filled gellan gum hydrogel encapsulated apigenin: Characterization and in vitro release kinetics. *Colloids and Surfaces B: Biointerfaces, 178*, 245–252. https://doi.org/10.1016/j.colsurfb.2019.03.015

Zhu, M., Tan, J., Liu, L., Tian, J., Li, L., Luo, B., Zhou, C., & Lu, L. (2021). Construction of biomimetic artificial intervertebral disc scaffold via 3D printing and electrospinning. *Materials Science and Engineering: C, 128*, Article 112310. https://doi.org/10.1016/j.msec.2021.112310

Zhu, S., Yao, L., Pan, C., Tian, J., Li, L., Luo, B., Zhou, C., & Lu, L. (2021). 3D printed gellan gum/graphene oxide scaffold for tumor therapy and bone reconstruction. *Composites Science and Technology, 208*, Article 108763. https://doi.org/10.1016/j.compscitech.2021.108763

Zia, K. M., Tabasum, S., Khan, M. F., Akram, N., Akhter, N., Noreen, A., & Zuber, M. (2018). Recent trends on gellan gum blends with natural and synthetic polymers: A review. *International Journal of Biological Macromolecules, 109*, 1068–1087. https://doi.org/10.1016/j.ijbiomac.2017.11.099