Electronic Journal of Biotechnology 66 (2023) 38–51

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

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

Review

Alginate and γ-polyglutamic acid hydrogels: Microbial production strategies and biomedical applications. A review of recent literature

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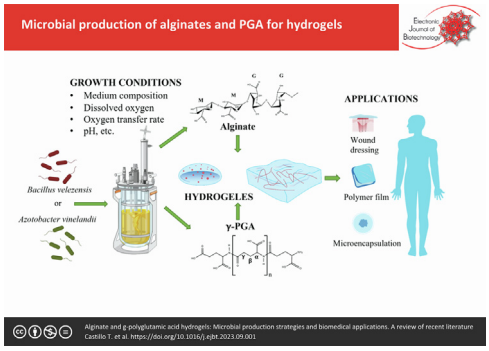
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 May 2023
Accepted 6 September 2023
Available online 14 September 2023

Keywords:
Alginates
Biomedical applications
Hydrogels
Microbial production
Polyglutamic acid

ABSTRACT

Hydrogels are three-dimensional networks of hydrophilic polymers. In general, these structures can be soft, elastic, porous and can absorb high quantities of water. Due to these characteristics, there is a growing interest in the use of hydrogels in diverse areas, from bioremediation to applications in the biomedical field. Although hydrogels can be elaborated with natural and synthetic polymers, natural polymers are attracting attention for their use in the biomedical and pharmaceutical fields. Alginate and γ-polyglutamic acid (γ-PGA) are microbial polymers, which show a great potential for hydrogel elaboration because of their biocompatibility that positioned them in emerging technologies, such as tissue engineering, microencapsulation, and soft robotics; these applications require specific characteristics of hydrogels in terms of their mechanical resistance, swelling capability, flexibility, softness, and stiffness. Thus, there is an emerging interest in the microbial production of alginates and γ-PGA, where it is possible to change their physicochemical and thermomechanical characteristics by the manipulation of the culture growth conditions of the microbial producers that can be oriented to specific applications. In this review, the chemical composition of biopolymers, hydrogel structure, the applications of hydrogels of alginates and γ-PGA, as well as their advantages and limitations are described; besides, the bacterial production of these polymers and the growth conditions that modify their chemical composition, are discussed.

How to cite: Castillo T, Flores C, Salgado-Lugo H, et al. Alginate and γ-polyglutamic acid hydrogels: Microbial production strategies and biomedical applications. A review of recent literature. Electron J Biotechnol 2023;66. https://doi.org/10.1016/j.ejbt.2023.09.001.

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso

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https://doi.org/10.1016/j.ejbt.2023.09.001
0717-3458/

Contents

1. Introduction

Hydrogels are formed by insoluble three-dimensional networks of hydrophilic polymers [1,2]. In these structures, both, weak interactions (i.e. hydrogen bonds) and strong chemical linkages (i.e. ionic and covalent bonds) are present [2].

There is a growing interest in these materials because of their potential in diverse fields including bioremediation [3], the food industry as a food packaging alternative, for immobilization of additives or as scaffolds for cell-cultured meat [4], as well as in the hygienical and cosmetical industry for the elaboration of personal-hygiene products (sanitary pads, nappies) [5] or cosmetics [6]. However, in the last decades, the relevance of hydrogels in the biomedical and pharmaceutical areas has been exponentially increased [7,8]. The biomedical applications of hydrogels include encapsulation and drug delivery, scaffolds for tissue engineering [5], biosensors, wound healing, and hemorrhage control agents [9,10,11].

It is important to point out that the applications of hydrogels are defined by their physicochemical and thermomechanical properties, mainly stiffness/softness [12], elasticity and mechanical resistance [13], porosity [14], and water absorption capability as well as their biodegradability and biocompatibility [15]. For instance, hydrogel scaffolds applied to the load-bearing tissue must provide sufficient strength to withstand the external stresses and load [16]. In turn, these properties are highly dependent on the chemical characteristics of those materials used in hydrogel's elaboration, such as their composition and molecular mass.

Hydrogels can be elaborated with a wide variety of polymers, such as homopolymers, copolymers, and their combinations [2,8]. In recent years, attention has been highlighted to hydrogels based on natural materials such as alginates and γ-polyglutamic acid because of their biocompatibility and biodegradability, which make them suitable for biomedical applications.

Alginates are polysaccharides formed by (1,4)-linked β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G). Alginates form gels in the presence of divalent metal cations by their interaction with the G residues in the structures known as "egg boxes" [17]. These polymers are naturally produced by the brown algae and bacteria of the genus *Azotobacter* and *Pseudomonas* [17,18]. From these sources, the bacterial ones show advantages like the possibility to manipulate the chemical composition of the alginates (i.e. mean molecular weight, G content, G/M ratio and acetylation degree) and therefore, their thermomechanical properties by manipulation of the growth conditions [19]. From those chemical characteristics, both the G/M ratio and the acetylation degree are the ones that mainly determine the characteristics of the designed hydrogels.

On the other hand, γ-polyglutamic acid (γ-PGA) is a polyaminoacid conformed by D/L-glutamic acid units linked by amide bonds [20]. The γ-PGA can form hydrogels by crosslinking between γ-carboxyl groups and the α-amino group [21]. This polymer is synthesized by several species of the *Bacillus* genus [20]. It is known that the molecular mass, which can be changed by manipulating the culture conditions, determines the mechanical properties of the hydrogels. Both, alginates and γ-PGA are water-soluble, non-toxic, biocompatible, biodegradable, and edible materials, and recently, important applications of these polymers as hydrogels have been highlighted.

Due to the relevance of hydrogels in the biomedical field, the research in this area has been growing exponentially in recent years. According to SCOPUS, the publications in this subject between 1980 and 2000 were 352. In contrast, from 2001 to present, 10,264 documents have been published. However, the information about hydrogels containing alginate and γ-PGA (that can be produced by microbial synthesis) represents less than 1 % of this literature.

In the present review, recent studies (from 2000 to present) related to the most representative biomedical applications of the hydrogels of alginates and γ-PGA (microencapsulation and tissue engineering, protein and drug deliver as well as wound healing) are reviewed; besides, the bacterial production of these materials, and the effect of the growing conditions as a strategy to obtain tailor-made polymers for specific applications, will be discussed. To our knowledge, there is not a comprehensive review of recent literature that integrates the importance of hydrogels elaborated with these biopolymers together with the relevant aspects of their production using bacterial models such as *Azotobacter vinelandii* and *Bacillus sp.*

2. Hydrogels

Hydrogels are polymeric matrices, water-containing, in which can coexist covalent-bond cross-linking, hydrogen bonds and electrostatic interactions [22]. Hydrogels conformed a three-dimensional structure, based on a crosslinked network, these structures are characterized by their flexibility, malleability, and

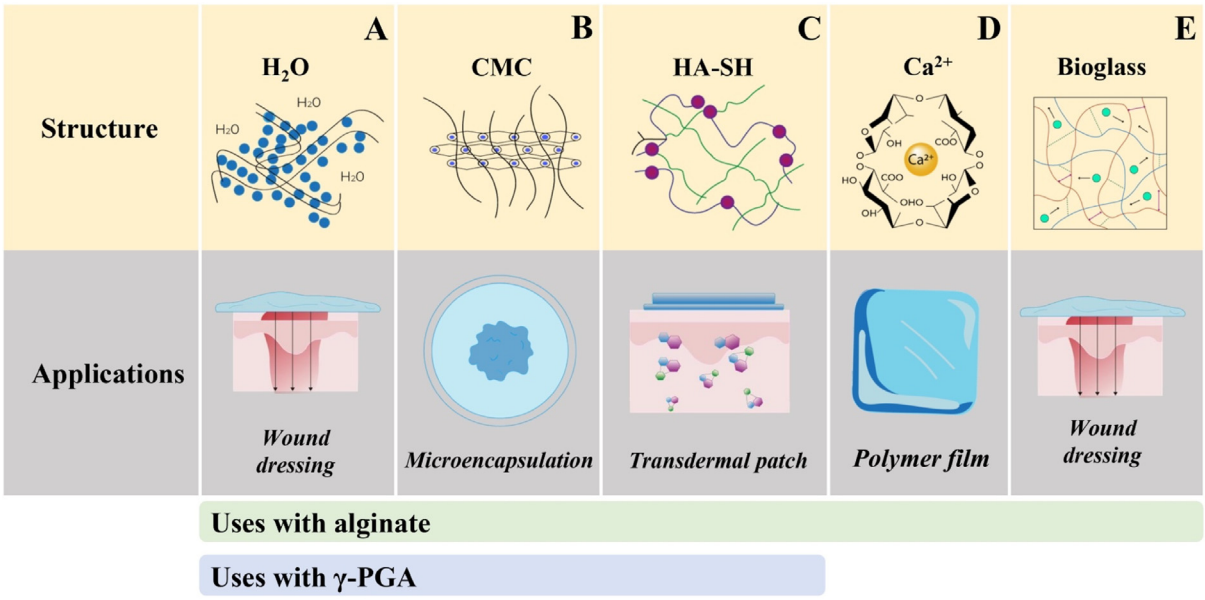

Fig. 1. Schematic representation of the interactions in alginate or γ-PGA hydrogels in the presence of Water (a), Carboxymethylcellulose (CMC) (b), Hyaluronic acid (HA) (c), Calcium ions (d), and Bioglass (e).

shape memory [22]. Hydrogels are suitable to be modeled by diverse techniques including electrospraying, electrospinning, and 3D printing [23].

It must be emphasized that, depending on the application of the hydrogels, the chemical composition of the former materials could be decisive for the design of hydrogels, because these characteristics can determine the thermomechanical properties such as swelling and absorption capability as well as the rigidity and elasticity. Based on this, both artificial and natural polymers can be used to elaborate hydrogels. Some of the most common polymers used for the elaboration of hydrogels are (among the artificial polymers) poly(methacrylic) acid [24] and polyacrylamide [25]. The biopolymers chitosan [26], alginates [10], gelatin and pectin [27] have also been used for making hydrogels. Fig. 1 represents some of the most common chemical interactions present in hydrogels.

In the biomedical area, due to the structural similarity of the hydrogels with the biological extracellular matrices, these materials offer an excellent platform for the proliferation, adhesion, and delivery of biologics and cells [28]. Because of these characteristics, the hydrogels have been used for 3D cell cultures, as scaffold materials for tissue regeneration and wound healing [7], as biomimetic soft robots [29], as well as shape memory supports [24]. Hydrogels can also be used for gene therapy [15], protein delivery and drug delivery [1,7]. The versatility of hydrogels is also reflected on their application which can be local, injectable, and oral. However, it is remarkable that for these applications, besides the thermomechanical properties, non-toxicity and biocompatibility are highly required.

In this context, the characteristics of the hydrogels of alginates and/or γ-polyglutamic acid including biocompatibility and biodegradability have generated a growing interest as will be discussed in the following sections.

2.1. Hydrogels of alginates and their biomedical applications

Alginates are highly recognized as viscosifying and gelling agents, and because of their good biocompatibility, alginate hydrogels have attracted attention for pharmaceutical and biomedical applications [10]. Alginates are polysaccharides formed by monomers of the mannuronic acid (M) and its epimer, the guluronic acid (G) (Fig. 2a). These monomers can be grouped as MMM, GGG, and MGM blocks, conforming chains of different mean molecular weights (MMWs), and those alginates from bacterial sources can be acetylated in the C-2 and C-3 positions of the mannuronic residues. Currently, the alginate is obtained from two different groups of organisms, brown algae such as *Ascophyllum nodosum* [30], *Lessonia nigrescens* [31], *Macrocystis pyrifera* [32], to mention a few, and from bacteria of the genus *Pseudomonas* and *Azotobacter* [18]; however, alginates from algal sources can be contaminated by heavy metals, and their chemical composition is highly variable. In contrast, the chemical composition (i. e. molecular weight (MW), M/G ratio, G content) of the bacterial alginates, mainly those produced by *A. vinelandii,* can be manipulated by the growth conditions [33] as will be further discussed.

Alginate hydrogels are soft functional materials that are currently used in the biomedical industry. These materials are used for tissue repair, cell therapy, and drug administration, to mention some applications (Table 1). Alginates can be used in their native form, partially oxidized, as well as in combination with other components both synthetic and natural, improving their chemical and physical characteristics, which allow advances in emerging areas with this biomaterial (Table 1; Fig. 2b).

2.1.1. Microencapsulation of cells and tissue engineering

Several studies have focused on the use of alginate hydrogels for microencapsulation, particularly in the medical field. Alginate microcapsules with some divalent metals such as Ca^{2+} and Ba^{2+} have been evaluated as models to transplant some types of cells because of their physicochemical characteristics and their biocompatibility [34,35].

King et al. [36] evaluated the biocompatibility and efficacy of pancreatic islets microencapsulated in alginate/poly-L-lysine (PLL)/alginate to potentially reverse hyperglycemia in mice. These authors observed that those islets with alginate containing a high % of G induced a transient reverse of hyperglycemia; in contrast, the islets encapsulated in alginate with high M content were able to reverse hyperglycemia and were functional for at least one month. However, the use of such capsules still cannot eliminate the detrimental effects of PLL on the biocompatibility of the capsules. Moreover, epimerized coating not containing a PLL showed good

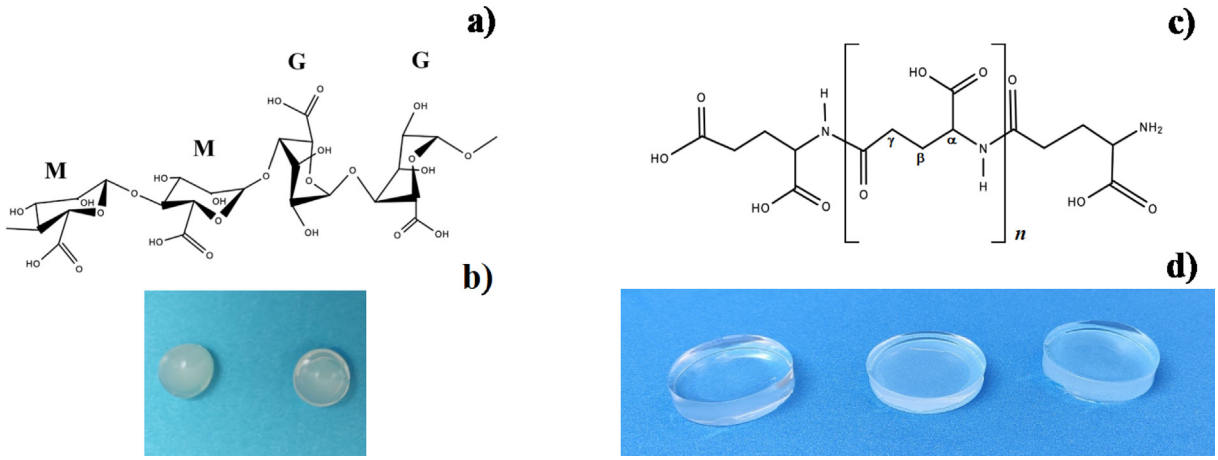

Fig. 2. Chemical structure of alginate (a). Photograph of alginate hydrogels (b). Chemical structure of γ-polyglutamic acid, *n* depends on the molecular weight of the polymer (c). Photograph of Polyacrylamide (PAAm)/ γ-PGA hydrogels prepared using different PAAm/ γ-PGA composition (d).

Table 1
Biomedical applications of alginate hydrogels.

Application	Composition	Observations	Reference
Microencapsulation of cells and tissue engineering	Alginate/poly-L-lysine (PLL)/alginate	The increase of the mannuronic content in alginates showed a higher and continuous activity for reverse hyperglycemia	[36]
	Alginate/ γ-PGA/Microcrystalline cellulose (MCC) for chondrocyte propagation	Hydrogels with a high storage modulus and capable of stimulating the chondrocyte propagation	[37]
Drug delivery	Alginate functionalized with β-cyclodextrin for delivering paclitaxel (anticarcinogenic agent)	β-cyclodextrin allows to modulate release of paclitaxel. It is a good alternative for other hydrophobic drugs.	[40]
	Alginate-Carboxymethyl cellulose Poly (N,N-dimethylacrilamide) for drug release	These hydrogels allowed the sustained drug release	[1]
	Alginate-graphene oxide for cephradine releasing	Adequate cephradine release	[41]
	Alginate-sterculia gum-carbopol for citicoline release	One gram of sterculia/alginate/Carbopol absorbed 19.6 g of synthetic artificial cerebrospinal fluids	[39]
Wound dressings	Alginate/Hyaluronic acid/Vancomycin/BMP-2	These hydrogels allowed *in vivo* the controlled release of vancomycin and BMP-2, suppressing bacteria proliferation, and increasing bone regeneration	[42]

biocompatibility; however, they were unstable. Further studies are required to determine the efficacy of the epimerized beads in the transplantation of islets of Langerhans to diabetic mice.

Another example of the capability of alginate hydrogels for cell microencapsulation was described by Wang et al. [37]; these authors evaluated biomimetic hydrogels of alginates blended with γ-PGA and microcrystalline cellulose as a scaffold for chondrocyte cells for cartilage regeneration. In these hydrogels, the cartilage matrix deposition was promoted, and the expression of cartilage genes was upregulated, making these hydrogels adequate for cartilage regeneration [37].

In another study, alginate hydrogels were successfully evaluated for neuronal network cultivations with a microelectrode array [38]. In that study, the micro-pattern of alginate hydrogel was adequate to form subpopulations of neuronal networks at the electrodes. The authors observed that alginate hydrogel showed a methodological advantage by allowing the segregation and integration of sub-populations of the neuronal networks. Based on their results, the authors suggested that the functional and structural properties of neuronal networks could be studied by using network models of alginate hydrogels [38].

Despite the great potential of alginate hydrogels for cell microencapsulation, cell cultivation and tissue engineering, more studies are needed in order to identify those chemical properties of alginates that could be relevant for these applications.

2.1.2. Drug delivery

Hydrogels as local drug delivery systems can increase drug concentration for its delivery *in situ* and this mechanism could be of great importance for the treatment of certain cancers, i. e. brain cancer, in which the blood–brain barrier can limit the bioavailability of the drug [39]. The challenges of alginate hydrogels as drug delivery systems include low retention of the small water-soluble molecules and, on the other hand, the poor loading and release of the hydrophobic molecules [40]. Diverse strategies have been suggested to improve hydrogels of alginates as drug delivery systems such as alginate combination with other materials like β-cyclodextrins [40], graphene oxide [41], carboxymethyl-cellulose [1], sterculia gum – Carbopol [39] (Table 1).

In this context, Omtvedt et al. [40] evaluated alginate hydrogels grafted with moieties of β-cyclodextrins. These authors observed that the introduction of β-cyclodextrins into the delivery system generally decreased the mechanical properties of the gels (compared to non-modified alginate hydrogels) but did not influence gelation kinetics [40]. The authors claim that in these hydrogels, the presence of β-cyclodextrins can retain and modulate the release of hydrophobic drugs such as paclitaxel [40].

In another approach, Hu et al. [1] designed a double-layer hydrogel based on a pH-sensitive inner core of alginate-carboxymethyl cellulose and an outer layer of polyacrylamide. In these hydrogels, the alginate and carboxymethylcellulose interact

by chemical and physical crosslinking, in these supports, the synthetic polymer brought stability to the hydrogel avoiding the expansion of the inner core. These hydrogel systems were adequate for sustained drug release [1].

Another successful application effort was reported by Singh et al. [39]; these authors evaluated hydrogels of alginate (1.33% w/v), sterculia gum (1.33% w/v), and carbopol (2.66% w/v). This hydrogel was able to absorb up to 19.35 g of artificial cerebrospinal fluids per gram. In this hydrogel, citicoline (a neuroprotector) was released in a first-order fashion. In addition, this hydrogel showed antioxidant activity and it was permeable to O_2 and H_2O, but impermeable to microorganisms. This approach could be an option for drug-delivery devices in the brain [39].

Similarly, alginate hydrogels loaded with graphene oxide prepared with tetraethyl orthosilicate as cross-linker were evaluated for cephradine release [41]. The hydrogel that contains 10 mg of graphene oxide was non-toxic and showed good swelling thermal stability and antimicrobial properties [41].

2.1.3. Wound dressings

Alginates could be used as self-healing hydrogels for wound-healing and cell-delivering. This strategy could be relevant in surgical procedures for *in situ* sustained release of antibiotics and proteins such as growth factors. This strategy was suggested for the prevention or treatment of osteomyelitis by orthopedic surgeons [42]. Jung et al. [42] evaluated alginate and hyaluronic acid hydrogels containing vancomycin and bone morphogenetic protein-2 (BMP-2). The gelling time was 4 min, and both vancomycin and BMP-2 were released for 6 weeks. These hydrogels were tested *in vivo* in rat models, in which, hydrogels suppressed bacteria proliferation and enhanced bone regeneration [42].

Hoefer et al. [43] evaluated wound dressings elaborated with bacterial and marine alginates. The bacterial alginates had a M/G ratio of 40/60, a MMW of 3420 kDa, and were acetylated. In contrast, the M/G ratio and the MMW in the algal alginates were 65/35 and 1100 kDa, respectively. The fibers obtained from the bacterial alginates were thinner than those fibers from algal alginates. However, the absorbency of the needle webs of bacterial alginates was twice higher than those of algal alginates. It would be interesting to study the effect of the MMW of bacterial alginates on the strength and stability of gelled dressing and its advantages in the management of patient wounds.

2.2. Hydrogels of γ-polyglutamic acid and their biomedical applications

Polyglutamic acid (PGA) is a natural polyaminoacid of repeating units of glutamic acid. There are two isoforms of PGA: α-PGA and γ-PGA (the classification is based on the carboxyl group attached through α or γ-linkage, respectively) [44] (Fig. 2c). There are important differences between these isomers: the α-PGA is chemically synthesized [45] and has a low molecular weight (<10 kDa) which limits its application; its biosynthesis has been reported only by using recombinant technology [46]. The γ-PGA is produced by GRAS (Generally Regarded As Safe) microorganisms [47], mainly by *Bacillus* sp. The molecular weight of γ-PGA varies between 10 and 8000 kDa [48] and depends on the producing strain and the culture conditions. γ-PGA is water-soluble, biodegradable, edible, and nontoxic to humans and the environment; these characteristics make γ-PGA suitable for diverse applications in various industrial sectors.

The use of γ-PGA for hydrogels is a viable option due to its availability, amenability to modifications, biodegradability, biocompatibility, and high water-retention capability. γ-PGA can promote cell migration and proliferation by enhancing cell adhesion, essential characteristics in applications in the biomedical area. In addition, the Food and Drug Administration (FDA) has approved γ-PGA for use in drug delivery systems, wound dressings, and tissue engineering [49]. Table 2 shows some of the most significant biomedical and pharmaceutical applications of the γ-PGA hydro-

Table 2
Biomedical applications of γ-polyglutamic acid hydrogels.

Application	Composition	Observations	Reference
Microencapsulation of cells and tissue engineering	γ-PGA/Keratin	Hydrogels showed maximum swelling ratio of about 2500%, maximum elastic modulus (stiffness) of about 4.5 kPa with controllable pore size, high porosity and soft enough for cell differentiation.	[50]
	Chitosan/γ-PGA with surfaces modified with elastin, human serum albumin (HSA) and poly-L-lysine and using genipin as a cross-linker	Chitosan/γ-PGA scaffolds showed higher porosity, suitable for cartilage cell growth; the compressive strength (4.5 MPa) and elongation strength (10.7%) provided the survival of chondrocytes *in vitro*.	[51]
Drugs and protein release	Hyaluronic acid (HA)/γ-PGA hydrogels by photopolymerization	Hydrogels showed outstanding anti-compression ability and shape recovery capability. Also showed good biocompatibility and weak and sustained protein release.	[16]
	γ-PGA)/bacterial cellulose (BC) hydrogels obtained by the covalent cross-linking of γ-PGA in the BC nanofibers suspensions	Hydrogels composite exhibited excellent strength and toughness (tensile strength was improved 8.16 times compared with γ-PGA single network hydrogels) and excellent cytocompatibility.	[56]
	γ-PGA/3-glycidoxypropyl trimethoxysilane(GPTMS)/ tetraethylorthosilicate (TEOS)	Hydrogels showed good conductivity as well as high sensitivity to external strain. By adjusting the silica content, hydrogels showed different microporous structure, compressive strength, swelling and drug (doxorubicin and tetracycline) delivery.	[53]
	N-isopropylacrylamide (NIPAAm)/γ-PGA loaded with naproxen, and using allyl glycidyl ether	Hydrogels showed homogeneous porous structure. Biocompatibility and cell proliferation were significantly improved in composite hydrogels with respect to NIPAAm-based hydrogels.	[52]
	γ-PGA hydrogels were prepared from bulk and electrospun nanofibers	Hydrogel-based electrospun nanofibers were an appropriate medium to keep the activity of bacteriophages and chemical bactericides.	[54]
Wound healing	Hydrogel of chitosan/heparin/γ-PGA with superoxide dismutase	Wound healing was faster. γ-PGA provided good hydrophilicity and biocompatibility to keep a moist environment favorable for cell growth.	[55]
	γ-PGA hydrogel loaded with cell-free fat extract (Ceffe)	Hydrogel had a sustained release of Ceffe, promoted neovascularization, cell proliferation and collagen production in healing of diabetic wounds.	[49]
	γ-PGA/silk sericin (SS) hydrogel	The tensile modulus increased with the increase of SS content; granulation and capillary formation were significantly stimulated, in addition to biodegradability and cell proliferation.	[57]

gels and Fig. 2d shows hybrid hydrogels from γ-PGA (produced by *Bacillus velezensis* 83 cultures) and polyacrylamide.

2.2.1. Microencapsulation of cells and tissue engineering

Cartilage has a limited ability to self-repair due to the absence of blood vessels and nerves; it is mainly composed of chondrocytes, glycosaminoglycans, and type II collagen. The application of biological material as scaffolds, using a biomimetic extracellular matrix (ECM), through tissue engineering strategies has been effective for functional cartilage regeneration by combining scaffolds and chondrocytes in *in vitro* or *in vivo* cultures. Scaffolds must present the mechanical strength and the required quantity of cells to stimulate and mimic native tissue architecture. In this regard, Bajestani et al. [50] studied the material properties and cell compatibility of γ-PGA/keratin hydrogels. These hydrogels showed important features, highlighting a controllable matrix pore size, and the improvement of mechanical properties as a maximum swelling ratio of about 2500% and maximum elastic modulus (stiffness) of about 4.5 kPa, (for the swollen sample), suggesting its potential for cartilage repair. Likewise, the compressive modulus of swollen samples was enhanced 10-fold, as the concentration of the chemical activator (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and hydroxy benzotriazole) increased. The biocompatibility of γ-PGA/keratin hydrogels was shown using mesenchymal stem cells [50]. Bajestani et al. [50] showed the outstanding features of γ-PGA/keratin hydrogels in studies conducted *in vitro;* however, it is necessary to carry out studies towards their *in vivo* evaluation.

In another investigation, Kuo et al. [51] prepared Chitosan/γ-PGA scaffolds, with surfaces modified with elastin, human serum albumin (HSA) and poly-L-lysine using genipin as cross-linker, for its application in the production of engineered cartilage. Using a 3:1 ratio of Chitosan/γ-PGA, scaffolds showed a porosity of 86 %, and pores size of about 120–150 μm, which can provide enough space for the growth of cartilage cells, transfer of enough nutrients and better cell-matrix interactions. In addition, a high-water absorption capacity and an increase in cell viability were obtained. The mechanical properties of the hydrogels were modified as a function of the concentration of the components: the compressive strength and Young's modulus values decreased when the γ-PGA concentration was increased; however, the elongation value increased from 7.0% to 22.1%. A concentration of 3% of chitosan/γ-PGA scaffolds at a 3:1 ratio, showed a compressive strength of 4.5 MPa and elongation of 10.7%, suggesting a suitable application for the human body (a knee weight-bearing requires a strength of 3–7 MPa). It is noteworthy that these hydrogels showed a low degradation rate after four weeks in cultures of chondrocytes *in vitro* [51].

2.2.2. Drug release

In the biomedical field, the synthesis of temperature and pH-sensitive hydrogels is required. The N-isopropylacrylamide (NIPAAm) and its derivatives are the most investigated as thermal-responsive polymers for hydrogels. These hydrogels are not biodegradable; however, the addition of γ-PGA provides them biodegradable and biocompatible characteristics [52]. Yang et al. [52] synthesized hydrogels based on NIPAAm with γ-PGA and allyl glycidyl ether (AGE, a crosslinking agent, pH-responsive). The NIPAAm/γ-PGA/AGE hydrogels had a good thermal response and pH sensitivity [52]. Under simulated physiological conditions of intestinal (pH = 6.8) and gastric fluid (pH = 2.4) at 37°C, the drug naproxen was efficiently loaded and released in a controlled manner from hydrogels [52]. The investigation of Yang et al. [52] shows the advantages provided by the different components of composites hydrogels, where the biocompatibility and biodegradability provided by γ-PGA stand out.

Another example of pH-responsive hybrid hydrogels was reported by Gao et al. [53]. It was shown that the physicochemical properties of the compounds constituting the hydrogel improved its mechanical strength, conductivity and cytocompatibility. Gao et al. [53] prepared hydrogels composited of γ-PGA and 3-glycidoxypropyl trimethoxysilane (GPTMS) with different contents of silica which was derived from hydrolyzed tetraethylorthosilicate (TEOS). The microporous structure, compressive strength, swelling as well as drug delivery behavior, and cytocompatibility (evaluated *in vitro*) of hybrid hydrogels were effectively tailored by adjusting the silica content. In addition, the hydrogel showed high sensitivity to external stress as well as good conductivity. These characteristics are relevant if the hydrogel is used as a sensor to detect or monitor diverse human motions in real time. For example, one composition of the hydrogel safely and accurately detected subtle physiological signals such as the bending of various joints and wrist pulse before and after exercises. Also, doxorubicin (DOX) and tetracycline (TET) were used as models for drug release, showing that the hybrid hydrogels loaded more TET than DOX, due to the different hydrophilic property of TET and DOX. This investigation showed the great potential of γ-PGA-GPTMS)/TEO hydrogels for biomedical applications.

Ma et al. [16] prepared hyaluronic acid (HA)/γ-PGA hydrogels for applications as load-bearing tissue. Pure HA hydrogels are stiff, brittle, and with rapid degradation. HA/γ-PGA hydrogels showed better toughness (could withstand larger compressive stress) and excellent shape recovery capability and highly porous (required for the delivery of drugs), highlighting their great potential for load-bearing tissue application. In addition, bovine serum albumin-loaded HA/γ-PGA hydrogels showed a weak burst release and sustained release of the protein. Another advantage of these hydrogels is that the mechanical properties, swelling, and enzymatic degradation behavior could be modulated by adjusting the molar ratio of HA to γ-PGA.

Kasbiyan et al. [54] evaluated hydrogels derived from γ-PGA nanofibers for antibacterial drug delivery used for disinfection in both home and hospital settings; bacteriophages were also evaluated as alternative for bacterial infections with antibiotic resistance. The hydrogels derived from γ-PGA nanofibers were compared with bulk hydrogels. Hydrogels obtained from electrospinning showed enough mechanical consistency, maintained the chemical bactericide activity against *Staphylococcus aureus,* and were compatible with bacteriophages [54].

2.2.3. Wound dressings

For the application of γ-PGA hydrogels as wound dressings, some important characteristics to take into account are: should be porous (for homogeneous cell distribution, nutrient, and oxygen diffusion), biocompatible, and could promote cell adhesion, proliferation, and differentiation. It is important to mention that physical properties, such as swelling, permeation, mechanical strength, and surface characteristics of γ-PGA hydrogels can be modulated through structural modifications [50]. Wound healing is especially important in the case of chronic wounds, like diabetic foot ulcers, where severe oxidative stress hampers the healing of wounds. In this line, and to avoid oxidative damage, Zhang et al. [55] elaborated composite hydrogels of chitosan/heparin/γ-PGA) (CS/Hep/γ-PGA-H) loaded with the antioxidant superoxide dismutase (SOD). The authors highlighted the advantages that each component contributed to the composite hydrogel: CS could accelerate the regeneration of normal tissue by promoting cell proliferation; Hep could relieve pain, inhibit clotting and inflammation, restore blood flow, and enhance healing, while γ-PGA provided good hydrophilicity and biocompatibility to keep a moist environment at the wound, favorable for cell growth, while SOD can decrease reactive oxygen species generation [55].

Another remarkable application of the γ-PGA hydrogel on wound healing in diabetic patients was reported by Yin et al. [49]. These authors prepared a wound dressing by loading a Cell-free fat extract (Ceffe obtained from human fresh fat tissue) with the γ-PGA hydrogel (Ceffe/γ-PGA) and evaluated them in wounds of diabetic mice. Ceffe was enriched with growth factors because these compounds are rapidly degraded in the body. γ-PGA acted as a drug delivery system compatible with wound healing and protecting the growth factors from damage in the protease-rich environments in the wound and extends the biological activity of the growth factors [49]

Shi et al. [57] developed γ-PGA/silk sericin (SS) hydrogels used as a wound dressing. The hydrogels maintained a moist healing environment, protected the wound from bacterial infection, and exudate absorption, as well as promoted cell proliferation (being biodegradable and non-cytotoxic) to reconstruct damaged tissue. In addition, they allowed a controlled release of naproxen. With the application of this hydrogel, the wound healed more rapidly than the control test (where only gauzes were used).

3. Microbial production of alginates and polyglutamic acid for hydrogels

For their applications in the biomedical field, such as scaffolds for tissue engineering, the porosity, stiffness, swelling, and biocompatibility of natural hydrogels, like alginates and γ-PGA, are highly desirable [10]. However, these hydrogels of natural origin are characterized by low mechanical properties and composition variability [8]. As was previously described, the thermomechanical properties of natural hydrogels can be improved by blending them with other materials (Fig. 1). Another alternative is the bacterial production of alginates or γ-PGA, in which the strict control of the growth conditions allows the manipulation of the chemical composition of these polymers and therefore their physicochemical and thermomechanical properties (Fig. 3).

3.1. Bacterial production of alginates for the elaboration of hydrogels

Although many works on alginate hydrogels have focused on algal alginate, the current source of alginate [58,59], bacterial

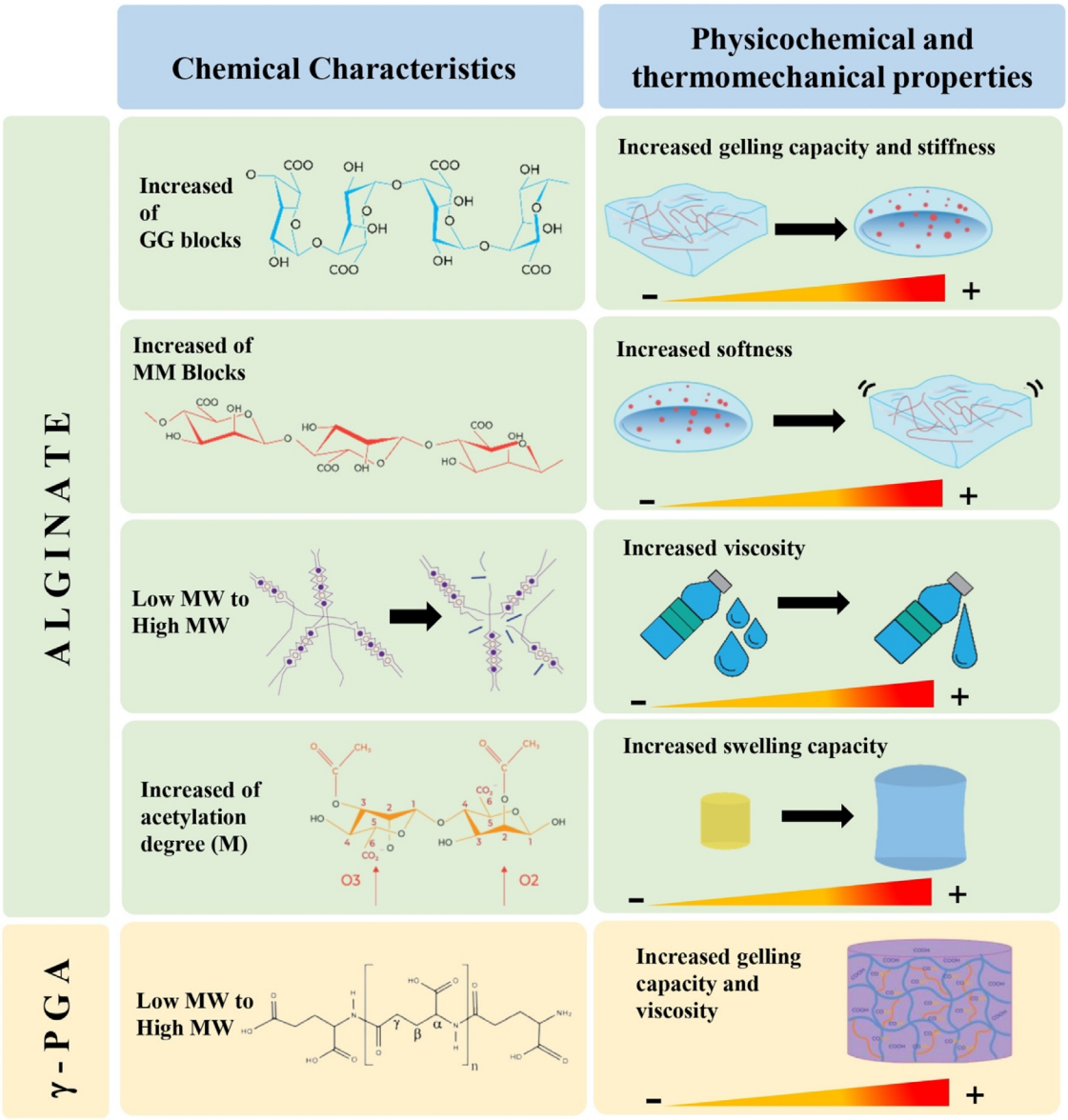

Fig. 3. Schematic representation of the chemical characteristics of alginates and γ-PGA and their relationship with hydrogel's mechanical and rheological characteristics.

alginate, displays properties that cannot be obtained and controlled in the algal ones [60], i.e. can be acetylated, and show higher molecular weights, lower M/G ratios, as well as large and homogeneous G blocks.

Alginate hydrogels exhibit different physical and biocompatibility characteristics depending on the production source, as well as the extraction method and composition [61]. A characteristic that determines the mechanical resistance and stiffness of the hydrogel of alginates is indicated by their affinity to multivalent cations such as Ca^{2+}, Mg^{2+}, Fe^{3+}, and Zn^{2+}, and this property is determined by the presence of G blocks in the alginate chain. The interaction of the G blocks with divalent cations such as calcium gives rise to the formation of structures called "egg-box"; thus, the alginates with high G content form rigid hydrogels (Fig. 3) [33,62]. In addition, elasticity and swelling characteristics of the alginate hydrogels are related to the acetylation degree, the presence of acetyl groups increases the viscosity of alginate solutions [63], as well as the water absorption and retention in hydrogels (Fig. 3) [43]. Hoefer et al. [43], observed that bacterial acetylated alginates increased up to 2-fold their gel formation capability as compared to commercial algal alginates (non-acetylated) with a similar G/M ratio, besides, the o-acetyl groups can interfere in the activity of the mannuronan epimerases and lyases [64]. In addition, Dudun et al. [64] observed that bacterial alginates with a MW of 574 kDa formed hydrogels with a higher density and lower cytotoxicity for mesenchymal stem cells in contrast to bacterial alginate with a low MW (212 kDa).

Based on these characteristics, there is an increasing interest in the alginates from microbial sources, mainly from *A. vinelandii,* that can be manipulated by the growth conditions [65,66]. In this regard, Hoefer et al. [43] processed alginates isolated from *A. vinelandii* into fibers and into needle webs showing an enhanced gelling capability as compared to the fibers from algal alginates.

In *A. vinelandii,* alginate is synthesized from fructose-6P, which is transformed into mannuronic acid, and it is transported through the periplasmic space where the mannuronic acid residues are polymerized into the poly-mannuronic acid and some residues can be epimerized into the guluronic acid by eight C-5 epimerases, the periplasmic AlgG, and the extracellular AlgE1-AlgE7. Some mannuronic residues can be o-acetylated in the C-2 and C-3 positions, by the proteins AlgI, AlgF, and AlgV (the latter also known as AlgJ in *Pseudomonas*), it must be noticed that acetylated residues cannot be further epimerized (Fig. 4a) [33,65].

The growth conditions that affect the guluronic acid content in the alginates produced by *A. vinelandii* involved the dissolved oxygen tension (DOT), oxygen uptake rate (OUR), agitation rate, dilution rate, and carbon source [67,68,69,70] Table 3.

Sabra et al. [67] in chemostat cultivations of *A. vinelandii* DSMZ93-541B observed that both DOT and dilution rate affected guluronic content (G). In that study, the highest G content (50%) was achieved in cultivations at a DOT of 10% and dilution rates between 0.15 and 0.22 h^{-1}. In another approach, using *A. vinelandii* ATCC9046 strain, Moral and Sanin [68] evaluated the monomeric composition of the alginate produced in bioreactors at 1, 3, 5, and 10% of DOT, similarly to that reported by Sabra et al. [67], the highest G content was observed at the 10% of DOT reaching up to 100% of G monomers; however, at this condition, the alginate concentration was only 0.76 g L^{-1}.

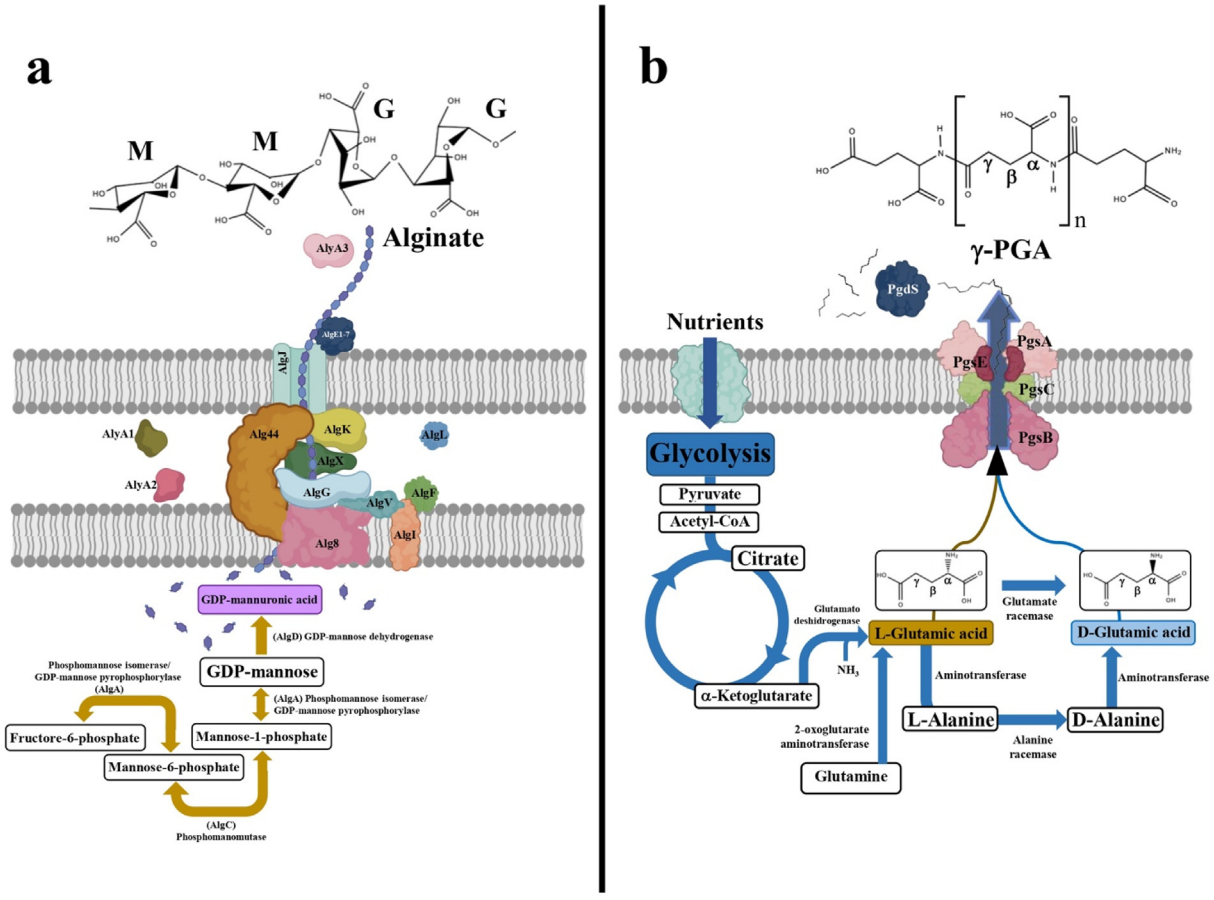

Fig. 4. Graphical representation of the biosynthesis pathway of alginates in *A. vinelandii* (a). Graphical representation of the biosynthesis pathway of γ -polyglutamic acid in *Bacillus* sp. (b).

Table 3
Growth conditions that influence the chemical characteristics (M/G ratio and acetylation degree) of alginates synthesized by *A. vinelandii*.

Chemical characteristics	Strain	Growth conditions	Chemical properties of alginates	Reference
G/M ratio and G content	*A. vinelandii* DSMZ93-541B	Chemostat cultivations. Dilution rates of (0.08–0.26 h^{-1}) and DOT of (1–10%)	G content and MMW were higher at high DOT	[67]
	A. vinelandii ATCC9046	Bioreactor batch cultivations (5% DOT) Agitation rates (200, 400 and 700 rpm)	The highest G content (80%) was achieved at 200 and 700 rpm	[69]
		Bioreactor batch cultivations at 1–10% DOT Sucrose initial concentration of 20 g L^{-1}	High G content (80 and 100%) was achieved in the cultivations at 1 and 10%, respectively	[68]
		Bioreactor batch cultivations (5% DOT) Sucrose initial concentration of 10, 20 and 40 g L^{-1}	The highest G content (80%) was achieved with a sucrose initial concentration of 10 g L^{-1}	[71]
		Bioreactor batch cultivations (5% DOT) Sucrose initial concentration of 20 g L^{-1} and calcium concentrations (25, 50 and 100 mg L^{-1})	The highest G content (60%) was obtained for calcium concentrations of 50 and 100 mg L^{-1}	[68]
		Bioreactor batch cultivations at 3 and 30 L and three OTRmax (5, 10 and 19 mmol $L^{-1}h^{-1}$)	The highest G/M ratio (0.86) was achieved at 10 and 19 mmol $L^{-1}h^{-1}$. The G/M ratio decreased from 0.86 to 0.67 when the cultivations were scaled from 3 to 30 L bioreactor	[70]
Acetylation degree	*A. vinelandii* ATCC9046	Shake flask cultivations, different MOPS concentrations were evaluated.	The highest acetylation degree (1.4%) was obtained at the highest MOPS concentration evaluated (13.5 mM)	[63]
		Bioreactor chemostat cultivations at 1 and 9% of DOT and different D (0.04 – 0.1 h^{-1})	The highest acetylation degree was 7% and it was achieved in cultivations conducted at 0.04 h^{-1} and 9% of DOT	[72]

In another study, Moral et al. [69] described the effect of the agitation rate on the monomer composition at a constant DOT (5%). Those authors demonstrated that the highest percentage of G was achieved in cultures developed at 200 and 700 rpm, and this percentage dropped to 60% in the cultivations at 400 rpm. These authors proposed that maximum GG block content obtained at 700 rpm would be a response to the high shear rate in the cultivation. However, since at 200 rpm, a high content of residues G was reached, this argument does not seem to have good support.

The effect of the agitation rate on the guluronic content could be related to the oxygen transfer rate (OTR_{max}) as recently suggested by Díaz-Barrera et al. [70]. These authors observed that in 3 L bioreactor cultivations at three different agitation rates of 300, 500, and 700 rpm, under which the OTR_{max} was of 5, 10 and 19 mmol $L^{-1}h^{-1}$, respectively, the highest G/M ratio (0.86) was achieved at 10 and 19 mmol $L^{-1}h^{-1}$. Interestingly, in this study, the G/M ratio was affected by the bioreactor scale, decreasing from 0.86 achieved in the cultivations in 3 L bioreactors to 0.67 when the cultivations were conducted in a 30 L bioreactor and using as the scaling criterion a OTR_{max} of 10 mmol $L^{-1}h^{-1}$ [70].

The guluronic content of the alginates synthesized by *A. vinelandii* can also be affected by the components of the culture media, mainly the type and concentration of the carbon source and the calcium concentration [68,71]. In this regard, Moral and Yildiz [71] observed that the G content of the alginates reached up to 80% in the cultivations of *A. vinelandii* with an initial sucrose concentration of 10 g L^{-1} and decreased to 60% when the initial sucrose concentration increased to 20 g L^{-1}. In the case of the carbon source, Moral and Yildiz [71] observed that in the alginate synthesized by *A. vinelandii* using maltose as a carbon source, the guluronic content was 60% and it dropped to 40% in the cultivations of *A. vinelandii* grown with molasses as carbon source. Regarding the Ca^{2+} concentration, the activity of the extracellular epimerases depends on the concentration of this cofactor; in this context, Moral et al. [69] evaluated the percentage of G residues in the alginate produced in cultivations of *A. vinelandii* with 25, 50, and 100 mg L^{-1} of calcium, and found that the alginate with the lowest G content was achieved employing 25 mg L^{-1} of Ca^{2+}.

The acetylation degree of bacterial alginates can be also manipulated by the growth conditions [63,72,73,74] Table 3. Some of the growth conditions that affect the acetylation degree of alginates are the media composition, dissolved oxygen tension (DOT), oxygen transfer rate (OTR), as well as the specific growth rate (μ). Peña et al. [63] reported, in shake flask cultivations, an increase in the acetylation degree of alginates as a response to the increase in the concentration of the 3-(N-morpholino)-propane-sulfonic acid (MOPS) in the medium of cultivation, achieving the highest acetylation degree (1.4%) in the polymer produced in the cultivations with a MOPS concentration of 13.6 mM. The authors proposed that the MOPS could contribute to a better control of the pH in the periplasmic space, and therefore, the acetylase activity would be higher than that likely obtained when no MOPS was supplemented to the medium.

As a function of the OTR_{max}, Peña et al. [19] and Castillo et al. [74], in shake flask cultivations of *A. vinelandii* observed that in cultures conducted at low OTR_{maz} (≈2–5 mmol $L^{-1}h^{-1}$), the acetylation degree of alginates was higher than those synthesized at higher values of OTR. On the other hand, Castillo et al. [72] and Díaz-Barrera et al. [73] described the influence of the dissolved oxygen tension (DOT) and dilution rate (D) on the acetylation degree of alginates in chemostat cultivations of *A. vinelandii* ATCC9046. In those studies, at different values of D, the acetylation degree of alginates was higher in the cultivations conducted at high DOT (8 and 9%) than the acetylation observed at 1% of DOT.

Regarding the effect of the D in the chemostat cultures, Castillo et al. [72] in diazotrophic conditions, observed the highest acetylation degree (7%) in the cultivations at 9% of DOT and D = 0.04 h^{-1}, and it dropped as the D increased. In contrast, Díaz-Barrera et al. [73] in no-diazotrophic cultivations observed the highest acetylation degree (6%) at D = 0.08 h^{-1} and 9% of DOT.

In the case of the molecular weight of alginates, the effect of the growth conditions, mainly the DOT, OTR, and the specific growth rate has been widely documented [19,75,76,77,78,79,80,81,82,83], and summarized in comprehensive reviews [33,65,84,85].

3.2. Bacterial production of γ-PGA for the elaboration of hydrogels

The γ-polyglutamic acid (γ-PGA) is an extracellular biopolymer produced by Gram-positive bacteria [86], mainly by bacteria belonging to *Bacillus* genus [87,88], and some Gram-negative bacteria such as *Fusobacterium nucleatum,* archea and eukaryotes

[89,90]. The γ-PGA-producing *Bacillus* species include *B. licheniformis*, *B. subtilis*, *B. megaterium*, *B. pumilus*, *B. mojavensis*, and *B. amyloliquefaciens* [88]. *Bacillus subtilis* has the 'generally regarded as safe' status of the US Food and Drug Administration and has the 'qualified presumption of safety' status of the European Food Safety Authority [87,91]. These characteristics make *B. subtilis* a promising model to produce γ-PGA [92].

γ-PGA is synthesized in a ribosome-independent manner. The γ-PGA-producing bacteria are classified into two groups according to the requirements of glutamic acid, precursor monomer of the polymer [93]: glutamic acid-dependent strains, which cannot produce γ-PGA without glutamic acid added to the culture medium; and the non-glutamic acid-dependent strains, that can synthesize glutamate from *de novo* synthesis, via the tricarboxylic acid (TCA) cycle.

The biosynthetic pathway of polyglutamic acid is shown in Fig. 4b. The endogenous production of L-glutamic acid starts with the carbon source uptake which is metabolized via glycolysis or pentose phosphate pathway to acetyl-CoA and TCA cycle intermediates. α-ketoglutaric acid (α-KG) from the TCA cycle is the direct precursor of glutamic acid. The polymerization process starts from D- and/or L-glutamate. To incorporate D glutamate into the growing chain, L-glutamate is converted into D-glutamate by a racemization reaction. The identification and characterization of racemases in different *Bacillus* species have been reported [94,95,96].

γ-PGA can be released into the extracellular environment or membrane-associated; in both cases, the synthase complex has at least four genes: *pgs*B,C,A, and E or *cap*B,C,A, and E, respectively. Thus, if γ-PGA is extracellular, the enzyme complex glutamate synthase PgsB,C,A and E is responsible for the polymerization [97].

During PGA bacterial production, the decrease of γ-PGA concentration and the molecular weight of the polymer have been observed during the stationary phase [98,99] and this behavior is a function of both the strain and the culture conditions. In *Bacillus* sp. two extracellular enzymes capable of degrading γ-PGA have been identified: endo-γ-glutamyl peptidase and exo-γ-glutamyl peptidase [100].

The γ-PGA presents two physicochemical characteristics that determine its physical properties and consequently, its application: enantiomeric composition and its molecular weight. γ-PGA can be composed of only D, L, or both glutamate enantiomers. γ-PGA with a high content of L-glutamate is used in cosmetics due to its skin-compatible feature; in some other fields, γ-PGA with a high content of D-glutamate is used, because it degrades slowly [94]. Regarding the molecular weight, in general, high-MW γ-PGA (>1000 kDa) is used for hydrogels (for personal hygiene items such as baby diapers, sanitary pads, or adult-incontinence products), thickeners, cryoprotectants, and flocculants; whereas, low-MW γ-PGA is used for humectants and drug delivery agents [101]. In contrast, the molecular mass of γ-PGA required for biomedical applications such as cartilage repair, wound healing, and wound dressing is < 450 kDa [50,55,56]. In addition, the enantiomeric composition and the molecular weight of γ-PGA have an important relationship since it has been reported that the enantiomeric composition of the polymer influences the degradation of γ-PGA, because of the specificity of the hydrolases [102]. As it was mentioned previously, both the enantiomeric composition and the molecular weight can be modified by the culture conditions used during the bacterial production process of the polymer.

In consequence, the focus of several investigations has been the optimization of culture media composition and culture conditions to obtain a polymer of MMW required for specific applications and/or to increase the polymer concentration, as well as to study the influence of culture conditions on the enantiomeric composition of the polymer (Table 4). In the evaluation of the culture medium, Feng et al. [103] found that high Fe^{3+} concentrations in the medium decrease the molecular weight of γ-PGA but also increase the γ-PGA yield in *B. licheniformis* ATCC 9945A cultures. Cromwick and Gross [104] reported that the increase in $MnSO_4$ concentration (from 0 to 33.8 μmol L^{-1}) in the medium increased the polymer concentration from 5 to 17 g L^{-1}.

The oxygen concentration in the fermentation process is one of the factors that determines the MMW of the γ-PGA; the influence of this parameter has been evaluated through changes in the aeration and agitation rate [98,99,105,106]. Cromwick et al. [98] evaluated the influence of pH (5.5, 6.5, 7.4, 8.2), the flow rate (0.5 to 2.0 L min^{-1}), and agitation rate (from 250 to 800 rpm) on the MMW of γ-PGA, showing that an increase in the oxygen availability (through high aeration and high agitation rate in the culture) at pH 6.5 allowed to obtain a polymer with a higher MMW (2000 kDa) and increased the concentration from 6.3 to 23 g L^{-1} in *B. licheniformis* ATCC 9945A cultures.

Bajaj and Singhal [105] evaluated different aeration conditions (1.0 to 3.0 vvm) and agitation rates (250 to 1,000 rpm) simultaneously in *B. licheniformis* NCIM 2324 cultures. The authors reported that the MMW of γ-PGA was higher (1100–2300 kDa) in cultures developed between 1 and 2 vvm and 750 rpm and 1000 rpm, with respect to the other conditions evaluated. In addition, high aeration and agitation rates stimulated the growth of the bacteria. Subsequently, Zhao et al. [106] developed fed-batch cultivations, where glucose was added and the agitation rate was increased from 200 to 700 rpm, the MMW of the polymer increased at least 2-fold with the increase of the agitation rate. In addition, the flocculation activity of γ-PGA increased with the increase of the MMW.

Considering that in the γ-PGA biosynthesis, oxygen is essential since it intervenes in the formation of ATP, used for the biosynthesis of glutamate precursor (through glycolysis and the TCA cycle) and for its polymerization by PGA synthetase that requires ATP to perform the glutamic acid polymerization process [93]. Therefore, the influence of dissolved oxygen concentration on biosynthesis and on MMW of the polymer has been studied. Different strategies that allow to increase the oxygen solubility in the culture medium have been reported; one of them is the use of oxygen vectors (compounds that enhance oxygen transfer rate) in the culture medium. Zhang et al. [101] assessed n-hexane, n-heptane, and n-dodecane on the production and molecular weight of γ-PGA. The highest concentration (39.4 g L^{-1}) and highest MMW of γ-PGA were reached (1900 kDa) with n-heptane.

Recently, it was reported a study regarding the influence of the oxygen transfer rate (OTR) on γ-PGA production and its MMW [99] showed that the MMW of γ-PGA is closely linked to the OTR_{max}. Changes in the OTR_{max} significantly affected the MMW and its evolution during the production process, showing that non-limiting OTR conditions, above 5.7 mmol $L^{-1}h^{-1}$, increased the MMW of the polymer; however, it also stimulated the depolymerization of γ-PGA. Although some studies have suggested that *Bacillus* spp metabolizes γ-PGA, to utilize glutamate as a source of carbon and nitrogen when these nutrients are depleted [20,98], Flores et al. [99] showed that γ-PGA depolymerization takes place even in the presence of glucose, and this depolymerization is promoted by the increase in oxygen availability in the culture.

The enantiomeric composition, another characteristic that determines the application of the γ-PGA hydrogels, is dependent on the strain and cultivation conditions. In general, for most *Bacilli* including *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens,* and *B. megaterium*, the γ-PGA produced contained both D- and L-glutamates [107]. For example, γ-PGA produced by *B. subtilis* is constituted by a 60% ± 15% of D-isomer while *B. anthracis* produces the γ-PGA with a content of D-isomer of nearly 100% [108]. *B. subtilis* (natto) strain could produce γ-PGA with 50–80% D-glutamate content [109] and *B. licheniformis* could produce γ-

Table 4
Influence of culture conditions on production, MMW and enantiomeric composition of γ-PGA.

Strain	Culture conditions	Chemical properties and applications of γ-PGA	Reference
B. licheniformis ATCC 9945A	Bioreactor batch cultivations, pH: 5.5, 6.5, 7.4, 8.2 Flow rate from 0.5-2.0 L min^{-1}; agitation rate from 200-800 rpm.	MMW was higher (2000 kDa) at pH 6.5 and at high aeration and agitation rate.	[98]
B. licheniformis P-104	Bioreactor fed-batch cultivations, glucose was added and agitation rate was increased from 200-700 rpm	MMW up to 1500 a 3500 kDa. Flocculation activity increased with the increase in the MMW	[106]
B. licheniformis ATCC 9945a	Shake flask cultures $FeCl_3$ concentration was evaluated	MMW decreased from 1310 to 318 kDa when the $FeCl_3$ concentration increased from 0 to 7.4 mmol L^{-1}.	[103]
B. licheniformis NCIM 2324	Bioreactor cultivations: aeration (from 1.0 to 3.0 vvm) and agitation rate (from 250 to 1,000 rpm) were simultaneously evaluated.	MMW was higher (1100 kDa) in the fermentation runs with low aeration and agitation rate.	[105]
B. subtilis NX-2	Bioreactor cultivations: n-hexane, n-heptane and n-dodecane were used as vectors to increase oxygen concentration in the culture.	MMW was higher (1900 kDa) with the use of n-heptane.	[101]
B. velezensis 83	Shake flask cultures at different oxygen transfer rates (modifying agitation rate and filling volume).	Higher MMW (2850 kDa) were obtained at higher oxygen transfer rate	[99]
B. subtilis NX-2	Bioreactor cultivations	L-glutamate content decreased from 59 to 10% with the increased of $MnSO_4$ from 0 to 615 μM	[104]
B. subtilis NX-2	Shake flask cultures	D-glutamate content increased from 18% to 77% by increasing the Mn^{2+} concentration from 0 to 0.09 g L^{-1}.	[94]
B. megaterium WH320	Shake flask cultures	L-glutamate content reached up to 95% using 5% of NaCl in the culture medium; γ-PGA increased from < 1 to 8.6 g L^{-1} and the higher molecular size was obtained (>1000 kDa).	[108]

vvm.- volume of air per volume of medium.

PGA with 10–100% D-glutamate content. In the culture medium, the concentration of nutrients as $MnSO_4$ modifies the enantiomeric composition of γ-PGA [94,104].

In cultures of *B. subtilis* NX-2, the D-glutamate content of γ-PGA increased from 18 to 77%, when the Mn^{2+} concentration, in the culture medium, increased from 0 to 0.09 g L^{-1} [94]. Cromwick and Gross [87] found an inverse relationship between the L-glutamate content in the polymer and the $MnSO_4$ concentration: L-glutamate content decreased from 59% to 10% when $MnSO_4$ concentration was increased from 0 to 615 μM.

NaCl concentration in the culture medium has shown to have a significant effect on D/L content in γ-PGA [108]; when a concentration of 5% of NaCl was used, L-glutamate content reached a maximum of 95 %, while at a low NaCl concentration, the content of the L isomer decreased [108].

In this section, it has been shown that culture conditions determine the concentration and physicochemical characteristics of γ-PGA, such as its molecular weight and stereochemical composition. However, it stands out that the oxygen concentration in the culture influences both the concentration and the molecular weight; in addition, it is possible to consider that it also influences the stereochemistry of the polymer. It has recently been suggested that PgsA, a component of the polymerization complex, presents epimerase activity [110,111]. Therefore, it would be of interest to study the MMW, concentration and stereochemistry of the polymer as a function of the oxygen concentration in the culture. It must be emphasized that controlling the oxygen supply in bioreactors may be a suitable strategy for producing tailor-made γ-PGA, increasing its potential to be used for hydrogel elaboration for biomedical applications.

4. Conclusions and future prospects

Hydrogels of γ-polyglutamic acid or alginate have attracted considerable attention in important industrial sectors, especially in the biomedical area, due to their strong water retention and physical properties which can resemble the native extracellular matrix. In addition, the properties, such as biocompatibility, non-immunogenicity, biodegradability, hydrophilicity, and no toxicity that both polymers show, have expanded and diversified their applications, making them excellent substitutes for synthetic polymers. On the other hand, the possibility to produce these biopolymers by microbial cultivations with the advantage of their production with tailor-made chemical characteristics and thermomechanical properties has highlighted the importance of extending the experimental research that increases the biotechnological production of both polymers for specific applications.

It is important to point out that both, alginate, and γ-PGA hydrogels will continue to be useful tools in biomedical research, not only in their native form but also as chemically modified polymers and in combination with other materials as the need for tissue replacement and regeneration will increase over the next decades. On the other hand, for tissue engineering, new design of functional hydrogels based on alginates and γ-PGA for their application through 3D technology or modular tissue engineering should be developed in the near future.

Similarly, in the case of alginate or γ-PGA hydrogels for drug release, more efforts will be required in the proper design of supports with a high load capacity and controlled released of molecules of pharmaceutic interest.

Regarding the use of hydrogels for wound healing, there are still several challenges that must be overcome in the future. For example, the development of new hydrogels for the management of chronic wounds is important, such as those that occur in patients with diabetes.

In addition, it is important to test alginates and γ-PGA acid hydrogels with different chemical characteristics with clinical applications through *in vitro* and *in vivo* biological models. Likewise, the development of hydrogels with these biopolymers at the laboratory level is a priority, with potential for the development of products that can be tested at the clinical level.

In this regard, the knowledge of the mechanisms involved in the synthesis of alginate and γ-PGA will allow to establish strategies

for the synthesis of tailor-made hydrogels with suitable physical and mechanical properties for applications within the biomedical field. On the other hand, in the case of bacterial alginates, more studies related to the stability, biocompatibility, and cytotoxicity of hydrogels with different degrees of purity, focused mainly for applications in the microencapsulation of cells of medical interest, would be necessary.

Conflict of 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.

Acknowledgments

The authors acknowledge Karla Batista Celestino for her support in the graphical design of figures and Shirley Ainsworth for her support in the bibliographic search. The financial support of DGAPA-UNAM projects IG201021 and IG200222 is acknowledged with thanks.

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