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Recent progress on gellan gum hydrogels provided by functionalization strategies

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Abstract

Gellan gum, a microbial exopolysaccharide fermentation product of *Pseudomonas elodea*, is a natural biomaterial that has shown promise for tissue engineering and regenerative medicine applications. Although this exopolysaccharide possesses many advantages, such interesting physicochemical properties and non-cytotoxicity, the mechanical properties and processability of gellan gum are not totally satisfactory in different tissue engineering contexts, i.e. gellan gum hydrogels are mechanically weak and the high gelling temperature is also unfavourable. An additional critical limitation is the lack of specific attachment sites for anchorage-dependent cells. However, the multiple hydroxyl groups and the free carboxyl per repeating unit of gellan gum can be used for chemical modification and functionalization in order to optimize its physicochemical and biological properties. A number of physical modification approaches have also been employed. This review outlines the recent progresses for gellan gum hydrogels and derivatives, and identifies the new challenges in tissue engineering, provided by blending and/or chemical modifications.

Keywords: Gellan gum, Hydrogels, Chemical modification, Blending, Tissue engineering, Regenerative Medicine

1. Introduction

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Gellan Gum (GG), a natural biocompatible polysaccharide and a food additive approved by the USFDA and European Union (E418), is a microbial fermentation product produced by the bacterium, *Sphingomonas (Pseudomonas) elodea* (ATCC 31461), which lives on the algae *Elodea Canadensis*. A culture of *S. elodea* is inoculated in a medium of glucose, nitrogen and inorganic salts and when the glucose is exhausted the gum is collected, after precipitation with alcohol, from the fermentation medium[1, 2] (Figure 1). GG is a linear, anionic and high molecular weight polysaccharide (~500 kDa)[3] comprising of a repeating tetrasaccharide unit consisting of two glucoses residues, one glucuronic acid and one rahmnose residue: 1,3-β-D-glucose (Glc), 1,4-β-D-glucuronic acid (GlcA), 1,4-β-D-glucose (Glc), 1,4-a-L-rhamnose (Rha)[4]. GG exists in two forms: in its acetylated (native) form, usually referred to as high acyl gellan gum (HAGG; Figure 1A), where two acyl groups, *O*-acetate and *L*-glycerate, are bound to the same glucose residue adjacent to glucuronic acid and in the deacetylated form (derived from alkaline hydrolysis of HAGG) usually known as low acyl gellan gum (LAGG; Figure 1B), the most common and most commercially available.

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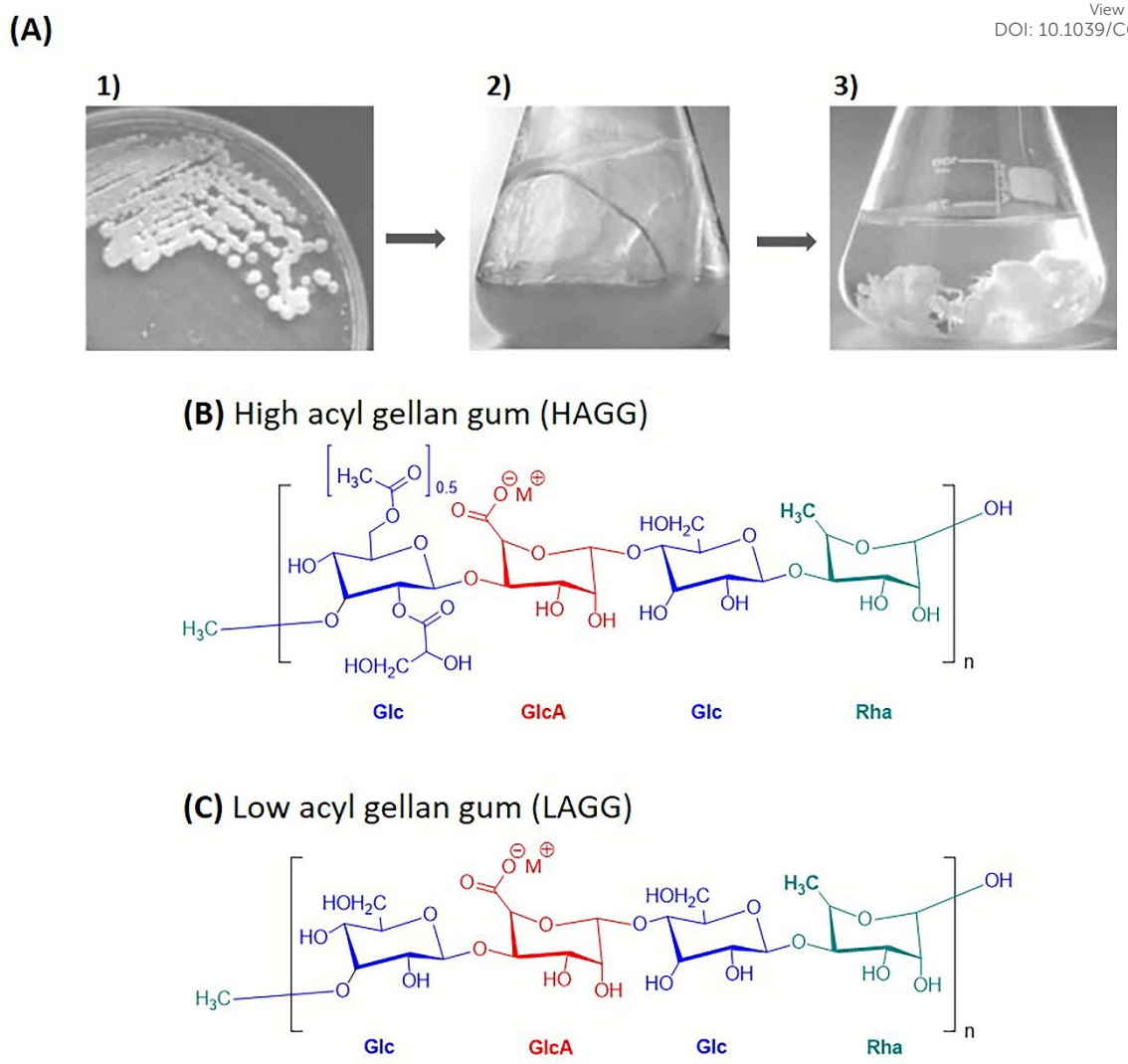

Figure 1 – (A) Typical fermentation process for gellan gum (GG) production: 1) Mucoid phenotype of the GG producing strain *S. elodea* (ATCC 31461), 2) culture broth after 72 h of growth and 3) precipitation of GG with isopropyl alcohol[5]. Chemical structure of GG repeating unit: (B) high acyl (HAGG) and (C) low acyl (LAGG).

Both acetylated and deacetylated gellan gum forms are capable of gelation after transition from a coiled form at high temperature (~90ºC for a 1% solution) to a double-helix structure when cooled (coil-helix transition; Figure 2) originating thermo-reversible gels with different mechanical properties and gelation behaviour according to the degree of deacetylation: the substituted form of the GG produces soft, elastic gels (Figure 2A),

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whereas the non-substituted form produces hard, rigid, transparent gels and higher thermal stability (Figure 2B)[6]. This gelation is an ionotropic process, which means that the presence of cations is necessary for the formation of a stable hydrogel structure[7, 8]. In this process, the quantity and chemical nature of cations present in solution greatly affects gellan gelation: divalent cations (e.g., Ca^{2+} and Mg^{2+}) promote a more efficient gelation than monovalent ones (e.g., Na^{+} and K^{+}) – process known as ionic crosslinking, as showed in the scheme in Figure 2[9, 10]. In monovalent cations, the gelation is mainly a result of the screening of the electrostatic repulsion between the ionized carboxylate groups on the gellan gum chains[11, 12]. In the case of divalent cations, the gelation and aggregation of gellan occurs via a chemical bonding between divalent cations and two carboxylate groups belonging to glucuronic acid molecules in the gellan chains, in addition to the screening effect. Additionally, it has been shown that GG can be crosslinked to form self-supporting hydrogel structures simply by the addition of standard cell culture media with no added ions[13]. Chemical crosslinkers such 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) are also reported to promote gellan gelation – process known as chemical/covalent crosslinking, illustrated in the scheme in Figure 2[14, 15].

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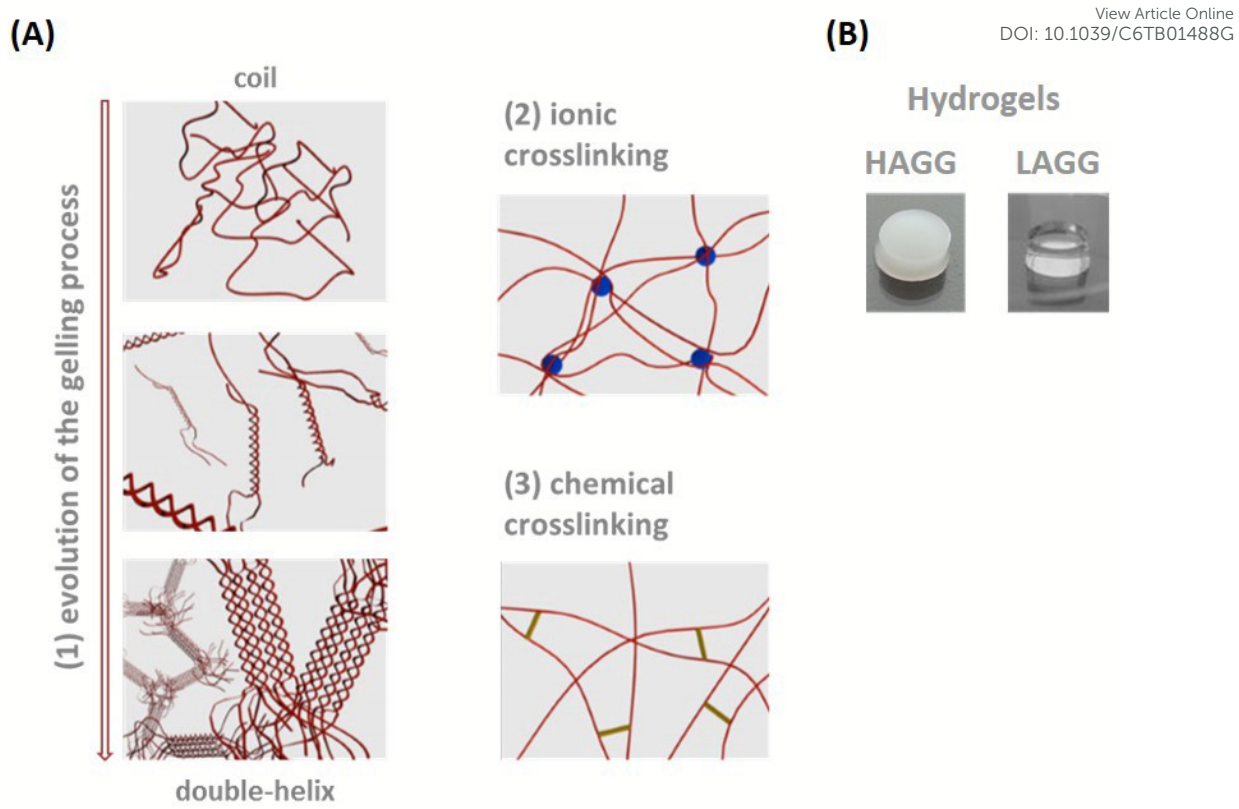

Figure 2 – (A) Gradual transformation of gellan gum in aqueous solutions: (1) due to a change in temperature, the transition of a thermally reversible coil to double-helix chain occurs (coil-helix transition). Then, the helices self-assemble in clusters joined together by the untwined polysaccharide regions; (2) ionic-crosslinking: polymer backbone carrying the charge bind with cations (blue circles); (3) chemical crosslinking: covalent bond between polymer chains. (B) Photographs of 2% (w/v) HAGG and LAGG hydrogels.

The described properties have been supporting the exploitation of gellan gum for different tissue engineering (TE) and regenerative medicine applications[13, 16]. Both acellular and cellular strategies using GG were successfully proposed for drug delivery[17-20], cartilage[21, 22] and intervertebral disc repair[23, 24]. Further attractive characteristics of gellan for TE are its biocompatibility, non-cytotoxicity, mild conditions of processing, structural similarity with native glycosaminoglycans (due to the presence of glucuronic

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acid residues in the repeat unit) and also the mechanical similarity to the elastic moduli of common tissue. Recent investigations have demonstrated that various cell types immobilized into and onto gellan gum hydrogels could maintain high viability and appropriate functionalities, implying the potential use of gellan gum for TE purposes[13]. The GG hydrogel has been shown to efficiently sustain the deliver and growth of human articular chondrocytes and support the deposition of a hyaline-like ECM, leading to the formation of a functional cartilage[21]. Additionally, GG has been found to have low induction of inflammation *in vivo*, so GG-based materials have been evaluated for potential therapeutic applications that include films to reduce post-surgical adhesions and discs for nucleus pulposus regeneration[25, 26].

However, it must be taken into account that simple gellan gum hydrogels gradually dissolve in physiological fluids. Major limitations of hydrogels include their insufficient mechanical performance and relative harsh gelation conditions for cell encapsulation. Although GG produces stable hydrogels, the mechanical functionality and long-term stability needs to be tuned, in order to fully comply the TE requirements. The reduced temperature window for viable cell encapsulation (high gelling temperature) and the lack of specific attachment sites for anchorage dependent cells are additional critical limitations attributed to gellan gum hydrogels. Chemical modifications permit to overcome some limitations of the traditional GG hydrogels, such as reduced physical stability and flexibility, limited handling possibility and reduced temperature spectrum for viable cell encapsulation and homogeneous cell dispersion. Gellan gum can be easily modified into new derivatives with enhanced properties, due to several hydroxyl groups and one free carboxyl group in the glucuronic acid monomer. Additionally, a number of physical modification approaches have also been employed in order to improve physicochemical and biological properties. A large variety of other potential applications

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of gellan and its derivatives, in pharmacy and medicine as example, are reported in several reviews[27-29]. Recent reports describe gellan sulfate for anticoagulant activity[30].

The present review provides a comprehensive overview about the reported strategies for i) tuning the physicochemical properties (mechanical performance/degradation behaviour), and ii) improving the biological performance of gellan (cell adhesion and spreading).

2. Strategies for tuning the physicochemical properties

2.1. Mechanical performance and degradation behaviour

In gellan gum hydrogel, as for other ionic-crosslinked polymerized hydrogels (e.g. alginate), an important loss of stability may occur *in vivo*, with possible misplace of structural integrity[16, 21] leading to exchange of divalent cations for monovalent ones which are present in higher concentrations in physiological environment. A number of strategies have been adopted to address the mechanical weakness, degradation rate and permeability of gellan gum hydrogels, namely by (i) the introduction of reactive double bonds (possibility of photo-initiated crosslinking); (ii) combination with inorganic materials; (iii) blending with biomolecules, using an interpenetrating polymer network (IPN) and (iv) tuning the processing methodologies.

2.1.1. Functionalization of gellan with double bonds: (meth)acrylation

The introduction of the (meth)acrylates groups in gellan gum backbone allows the possibility of hydrogels formation by light irradiation, an important family of chemically crosslinked hydrogels for cell encapsulation. The photo-crosslinking reaction involves the presence of a photo-initiator compound and irradiation by light, typically ultraviolet (UV), to initiate a free radical polymerization reaction that propagate through carbon–

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carbon double bonds to form high-molecular-weight kinetic chains and form covalent crosslinks between the polymer chain (Figure 3A). Several authors performed gellan gum methacrylation (Table 1, entry 1), through chemical modification of LAGG, using different reactions conditions, tuning the crosslinking degree of the polymer and combining physical and chemical crosslinking methods[23, 31, 32]. The studies of photo-crosslinked methacrylated gellan (phGG-MA; Figure 3B) indicate that the crosslinking density remarkably increased after the modification. In fact, methacrylated gellan gum (GG-MA) hydrogel has been demonstrated to possess improved mechanical properties, with Young's modulus values between 0.15 and 148 kPa, (according to the crosslinking mechanism used) as compared to gellan gum hydrogel (56 kPa)[33, 34]. The *in vitro* swelling kinetics and the degradation rate (in 0.1 mM, NaOH, 37ºC, 24 h) is influenced by the crosslinking mechanism used to form the hydrogels[32]. The non-cytotoxicity of methacrylated gellan gum hydrogels has already been demonstrated both *in vitro* and *in vivo*[23, 33, 34], and the main advantage of these modified hydrogels is that they can be applied by injection using a minimally invasive system, decreasing the complexity and invasiveness of a spine surgery as reported by Silva-Correia et al.[21, 35]. The ionic-crosslinked methacrylated GG (iGG-MA) and phGG-MA hydrogels have been proposed as potential alternative implants for nucleus pulposus regeneration[23, 33]. To achieve a similar goal, a series of gellan gum unsaturated esters were synthesized by Hamcerencu et al.[36] by esterification of GG with acrylic acid, acryloyl chloride and maleic anhydride, under various conditions (Table 1, entry 2) in order to obtain derivatives carrying reactive double bonds, easily photo-polymerizable. The hydrogels obtained present higher stability. Moreover, hydrogels prepared by further co-polymerization of the esters with *N*-isopropylacrylamide showed pH- and temperature-sensitive properties[37].

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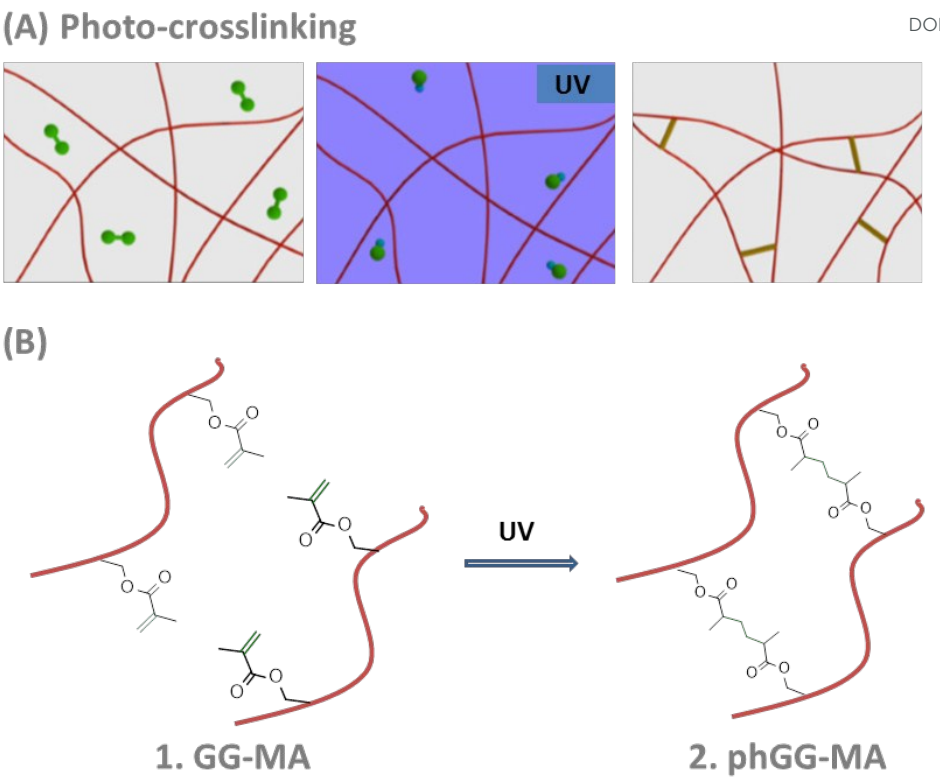

Figure 3 - (A) Simplified scheme of photo-crosslinking: the photo-initiator molecules (green circles) form free radicals when irradiated by light and crosslink the polymer chains; (B) schematic representation of the photo-crosslinking reaction between methacrylates groups on methacrylated gellan gum (GG-MA) chain.

Molecules and cell nutrients should be able to diffuse through the matrix of hydrogels without being blocked. Thus, the microstructure and permeability of hydrogels are crucial factors for enabling cell nutrition, penetration, and proliferation. Transmission mode optical projection tomography (OPT) was used by Soto et al.[38] to analyse image microtextures of GG hydrogels obtained by different crosslinking methods, and emission mode OPT to study mass transport within these hydrogels. Acellular ionic-crosslinked unmodified GG, iGG-MA and phGG-MA have shown different image textures (caused by the different microstructure of the hydrogels) that are associated to differences in crosslinking density and distribution. Moreover, the index of homogeneity calculated

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from the diffusion of differently sized FITC-dextran molecules have shown that molecular mobility is different between the three types of GG-based hydrogels.

2.1.2. Blending with inorganic materials, polysaccharides and enzymes

The combination of inorganic materials, with their flexibility, and biopolymers with their poor rigidity, becomes a logical and attractive solution to improve the mechanical properties of gellan gum. The reinforcement of GG was recently achieved by introduction of fine bioactive glass (BAG) particles[39, 40], hydroxyapatite (HAp)[41-43] and calcium phosphate (CaP)[44]. GG-based hydrogels blended with HAp particles have been proposed for bone and osteochondral applications showing greater mechanical and biological properties in comparison to the polymeric hydrogel[41]. Bilayered GG/GG-HAp hydrogels were also produced for osteochondral tissue engineering applications, by joining both solutions of GG 2% (w/v) with and without HAp (20 wt.%) for bone and cartilage parts, respectively[43]. Other approaches such as enzymatic mineralization of GG with CaP[44] and alkaline phosphatase (ALP)[45] through incubation in a mineralization solution containing calcium glycerophosphate (CaGP) led to improved mechanical properties (in terms of both amount of mineral formed and stiffness, which increased with increasing ALP concentration), and enhanced adhesion and proliferation of osteoblastic cells[44]. Additionally, it was recently shown by Oliveira et al.[46] that 1.5% (w/v) GG-MA hydrogel presents a self-inducing osteogenic ability. In that work, it was demonstrated for the first time the GG-MA capability to induce autonomous osteogenic differentiation of human adipose-derived stem cells in basal medium without addition of any osteoconductive or osteogenic stimuli and in absence of any cell-ligand peptide/protein.

Lee and co-workers[47] also attempted to optimize the physical parameters of GG hydrogels for cartilage applications by blending LAGG and HAGG. In that work,

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dynamic mechanical analysis showed that increased concentrations of low acyl gellan gum resulted in increased stiffness and the addition of high acyl gellan gum greatly resulted in decreased stiffness. The degradation behaviour of LAGG, HAGG and LAGG/HAGG blended hydrogels were investigated recently by Silva and co-authors[48] and it was observed that all the three types of gels degraded for 28 days. More recently, different formulations of GG hydrogels were produced by mixing varying amounts of GG-MA and HAGG for application as acellular or cellular NP substitutes[25]. Results showed that as HAGG content increased, the modulus of the hydrogels decreased. Moreover, increases in HAGG content induced greater weight loss in the GG-MA/HAGG formulation compared to GG-MA hydrogel.

Blending GG with other types of polysaccharides for TE applications has also been considered. Cencetti et al.[49] prepared an hydrogel composed of low acyl gellan gum (2%) and sulphated hyaluronic acid (1%) to be evaluated in postsurgical epidural scar prevention. The presence of the sulphated polymer adds hydrophilic properties to gellan structure and allows obtaining a hydrogel with improved mechanical and rheological properties. The produced material showed a high elastic modulus value and good stability at room temperature up to 12 months. More recently, Bellini et al.[50] reported the preparation and characterization of a novel *in situ* ionically gelling hydrogel composed of gellan gum, hyaluronic acid and calcium chloride, for osteochondral defects repair. The hydrogel composition is crucial in the *in situ* gelation and adhesion to the bone surface. The best formulation was found to be HA/$CaCl_2$ (2%:1%) and 2% GG: good hydrogels with good/excellent resistance to shaking and to water flow with best performance of adhesion. A photo-crosslinking of modified GG-MA and GelMA, photo-reactive forms of gellan and gelatine, produced mechanically strong double network (DN) hydrogels that can encapsulate cells for application as scaffolds for load-bearing tissues.

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DN hydrogels exhibited higher strength, which approaches closer to the strength of cartilage[51]. Gellan gum was also applied to improve the mechanical properties of other materials applied in bone reconstruction. As example, gellan was used to increase the mechanical strength of scaffolds containing hydroxyapatite and gelatin[52] and a new biohydrogel of gelatin and gellan gum interpenetrating network (IPN) structure was prepared using a combination of enzymatic (mTG enzyme) and ionic-crosslinking approaches. The resulting IPN exhibited tunable and significantly increased mechanical strength, decreased swelling ratios and lower degradation rate[53]. The degradation rate can also be tuned by means of incorporating hydrolytically or enzymatically labile segments into the hydrogel. Enzymatic degradation studies of gellan hydrogels with lysozyme and trypsin[54], and in the presence of galactomannase[55] were reported. A significant decrease in the viscosity of gellan was noted in the presence of galactomannanase at a concentration of 15 mg/mL, thus indicating that the polysaccharide degrades in an enzymatic reaction.

2.1.3. Processing methodologies

The ability of gellan gum in the context of tissue engineering arises from the processing versatility of the precursor hydrogels, giving rise to networks with improvement physicochemical properties. Silva et al.[56] reported the development of GG spongy-like hydrogels with enriched mechanical performance and flexibility in relation to traditional hydrogels. During the subsequent freezing, freeze-drying and rehydration steps of the processing method, specific parameters, such as crosslinking solution, stabilization time, freezing temperature and time, along with the consecutive stages of preparation affected the physical/mechanical properties of GG spongy-like hydrogels.

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Another strategy to reinforce gellan gum hydrogels was proposed by Pereira et al.[24]. In that work, formulations of low acyl and high acyl hydrogels have been processed as microparticles/matrix systems and the results have shown an improved system with better control over the mechanical properties and degradation rate of the hydrogel matrices. Promising anti-adhesive gellan gum-based films grafted with a photoactive chromophore moiety (cinnamate) were proposed by Lee and co-authors[57] (Table 1, entry 3). By this way, photosensitivity was achieved and the reaction does not need the addition of a photo-initiator. The anti-adhesion films prepared from gellan gum-cinnamate polymers exhibited high gels content and suitable mechanical properties. D'Arrigo et al.[58] developed self-assembling nanohydrogels based on sonicated gellan gum conjugating prednisolone with carboxylic moieties (Table 1, entry 4) using a short spacer between gellan and prednisolone. The material revealed a tendency to self-aggregate in the aqueous media and to form biocompatible nanohydrogels with the average size of 300 nm.

2.2. Gelation temperature

In a tissue engineering context, a substantial problem remains for gellan gum hydrogels in order to meet the requirement of encapsulating living cells while maintaining their injectability. In physiological cationic conditions, the $T_{gelation}$ of commercial available and unmodified gellan gum is too high (> 42 ºC) to suspend cells for injectable purposes encapsulation. Because cells are present during the gelation process, the number of suitable chemistries and formulations are limited. Although, several reports on gellan gum hydrogels demonstrate that the gelation temperature can be tailored to be physiologically relevant by means of: (i) adjusting the molecular weight of gellan (*via*

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oxidative cleavage), and ii) functionalization of gellan with carboxymethyl and thiol groups.

2.2.1. Chemically scissoring (oxidation)

Gong et al.[59] successfully modified the gelation point of gellan gum by cleavage of the chains into smaller fragments with an oxidative agent – sodium periodate (Table 1, entry 5), leading to a decrease in polymer molecular weight and an increase of crosslinking aldehyde groups. The gelation temperature was optimised to 37.5ºC by controlling the GG molecular weight. In this *in vitro* study, gellan hydrogels showed a great potential for being used as injectable vehicles for chondrocyte delivery. However, later Tang et al.[60] observed that the oxidation cleavage damages the crosslinking points in gellan network and contributes to increase water absorption and degradation. The authors proposed an additional crosslinking of gellan with carboxymethyl chitosan (CHC-GG) to stabilize the gel structure through a Schiff-base reaction (Table 1, entry 6). By this way, the number of free aldehyde groups decreases and consequently chondrocytes proliferation and viability were enhanced.

2.2.2. Carboxymethylation and thiolation

Carboxymethylation[61] and thiolation[62, 63] of gellan can improve the *in situ* gelling properties of gellan gum by the immobilization of carboxymethyl and thiol groups, respectively. Carboxymethylation was achieved by reaction with chloroacetic acid (Table 1, entry 7), improving its aqueous solubility (soluble at room temperature up to 10%). Concerning thiolation, the thiol group containing the amino acid L-cysteine has been introduced to the gellan gum backbone via carbodiimide chemistry using EDC as activator (Table 1, entry 8)[62]. The injectability of the thiolated GG hydrogel was

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demonstrated *in vitro* to be near body temperature[63]. Thiolated gellan gum hydrogels also exhibit quick gelation, lower gelling temperature and stable structure which make them promising as injectable hydrogels. Thiol-functionalization of gellan was also carried out by esterification with thioglycolic acid (Table 1, entry 9)[64]. It was observed that gellan-thioglycolic acid revealed a decrease in sensitivity to cation-induced gelation but an improvement in mucoadhesive properties.

3. Strategies to improve biological performance

3.1. Cell adhesion and spreading

3.1.1. Functionalization of gellan with bioactive peptides/proteins conjugation

Cell adhesion and migration, crucial to attain successful results in a tissue engineering and regenerative medicine approach, are features that are not naturally demonstrated by gellan gum hydrogels. The lack of anchorage dependent cells (ADC) affinity has been attributed to the extreme hydrophilic nature of the hydrogels, which *per se* allows water molecules to bind to the polymer backbone. This behaviour is also attributed to the negative charge of the polymer that repulse cells, limiting the adsorption of cell-adhesive proteins prior to cell attachment. Strategies to overcome cell adhesion features to GG hydrogels have been provided by the combination with proteins and peptides sequences (Figure 4). Wang et al.[65] prepared gellan gum microspheres covalently functionalised with gelatin, a partially denatured derivative of collagen, through redox-mediated crosslinking to enable ADC binding (Table 1, entry 10). In fact, both human dermal fibroblasts and human foetal osteoblasts used in this study successfully attached to the surface of the spheres. Moreover, good cell viability, morphology and proliferation were observed in both cases.

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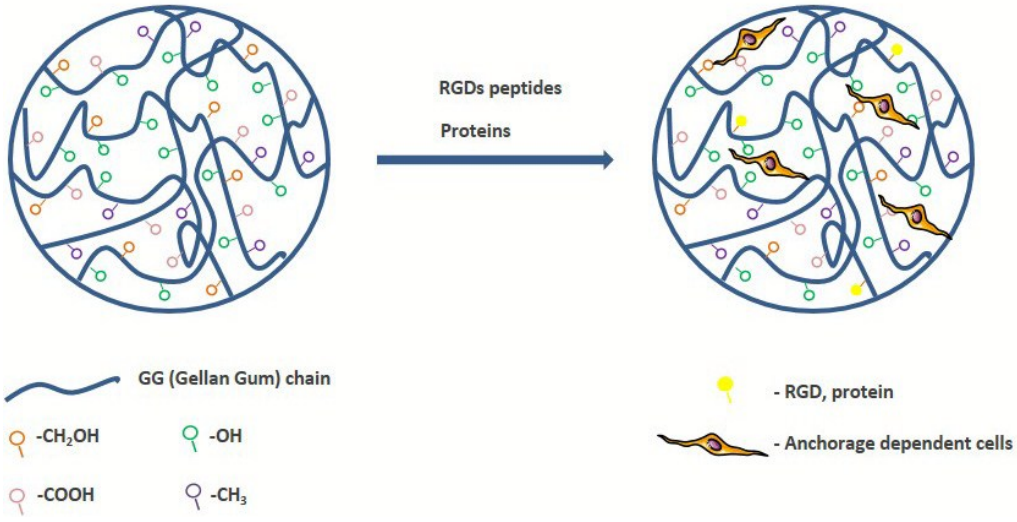

Figure 4 - Schematic representation of the biological functionalization of gellan gum hydrogels by the combination with proteins and peptides sequences.

Another strategy to reinforce cell adhesion into gellan gum hydrogel was proposed by Silva et al.[66, 67]. In that work, gellan gum was modified using Diels-Alder click chemistry with a fibronectin derived synthetic peptide (GRGDS). The immobilization of the synthetic peptide was achieved in two synthetic steps (Table 1, entry 11). The authors reported the impact of the GG-GRGDS hydrogel on the behaviour of Neural Stem/Progenitor Cells (NSPCs)[66] (Figure 5A-B) and bone marrow Mesenchymal Stem Cells (BM-MSCs)[67]. NSPCs and BM-MSCs were found to adhere and proliferate within the modified gels, when compared to unmodified ones. The results showed that the presence of a peptide in gellan gum hydrogel, BM-MSCs presented higher proliferation and metabolic activity than in the unmodified material. Moreover, cell morphology and secretome of BM-MSCs were drastically positively influenced by the modification, as proven by the enhancement of survival and differentiation of primary cultures of hippocampal neurons *in vitro*. More recently, Ferris et al.[68] covalently linked a short peptide containing the RGD sequence G_4GRGDSY, to gellan gum, using the popular

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carbodiimide chemistry (Table 1, entry 11). Both murine skeletal muscle C2C12 and the rat pheochromocytoma PC12 cell lines proliferated when surface seeded or encapsulated with the GG-RGD gels (Figure 5C).

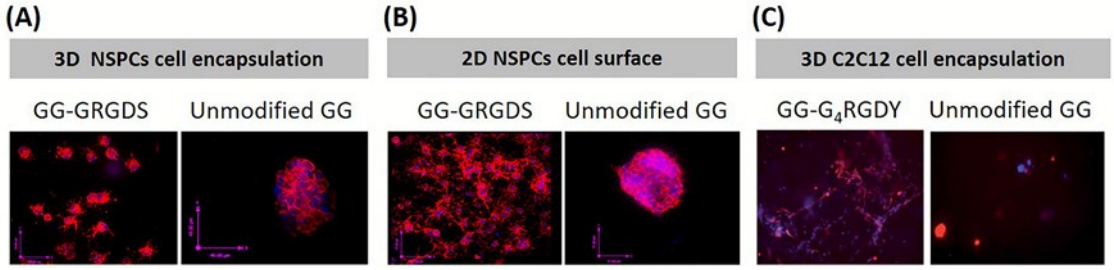

Figure 5 – Morphology and dispersion of NSPCs (encapsulated (A) or surface-seeded (B)) and C2C12 (encapsulated (C)) on the GG-GRGDS hydrogel, after 7 days (A-B) or 5 days (C) in culture. Cell spreading and visible cytoplasmatic extensions were only observed in the GG-GRGDS. In the unmodified GG the cells proliferate as neurospheres. Adapted from (A-B) Silva et al.[66] with permission from Elsevier and (C) Ferris et al.[68] with permission from The Royal Society of Chemistry.

Various works have previously shown the influence of hydrogel microarchitecture, such as the degree of porosity and pore architecture[69], as well as of matrix stiffness[70], hydrophilicity[71] and charge[72] over cell attachment. The spongy-like hydrogels developed by da Silva et al.[56] and described in the section 2.1.3, also demonstrated cell-adhesive character: when rehydrated with a cell suspension, cells became entrapped and able to adhere and proliferate. Depending on the cell line, a pre-incubating cell-adhesion step with a cell-adhesive protein, such as serum or fibronectin, is required. Another interesting and newly development is the modification of GG with surfactants to function as a bio-ink for cell printing applications (3D-bioprinting)[73]. More recently, the development of this new bio-ink make it possible to Lozano and co-workers[74] to produce 3D brain-like

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bioprinted structures that consist of discrete layers of primary cortical neurons encapsulated in GG-RGD hydrogels. These gellan gum-based brain-like structures provide an opportunity to mimic more accurately in *in vitro* studies the 3D microstructure of neuronal tissues.

The permeability of cells from GG hydrogels is crucial for cell culture and for cell survival. GG-based hydrogels present different permeability to cells. The chick embryo chorioallantoic membrane (CAM) assay performed by Silva-Correia et al.[26] in GG, iGG-MA and phGG-MA hydrogels showed that the hydrogels present different permeability to cells. iGG-MA and phGG-MA hydrogels are non-permissive to endothelial cells ingrowth, behaviour not observed in the unmodified GG hydrogels. The results showed that the methacrylation of GG allowed tuning the hydrogel permeability to cells.

4. Conclusions and final remarks

Gellan gum is a versatile polysaccharide and its advantageous properties, such as biodegradability, compatibility, rapid gelation in the presence of cations, high water content and non-toxicity make a particular and fundamental role for their application in the field of tissue engineering and regenerative medicine applications. In this review, we described the importance of appropriate blending/chemical modifications to overcome biofunctional limitations of traditional gellan gum hydrogels such as poor mechanical strength, reduced temperature window for viable cell encapsulation/homogenous cell dispersion and lack of cell adhesion. It is now clear that gellan gum, which is able to be functionalised and processed in so many ways, can be regarded as one of the most promising biomaterials for application in tissue engineering and regenerative medicine, namely for the encapsulation of cells and drug delivery in the treatment of metabolic and musculoskeletal diseases.

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Table 1 – Reaction scheme and general conditions used for gellan gum functionalization.

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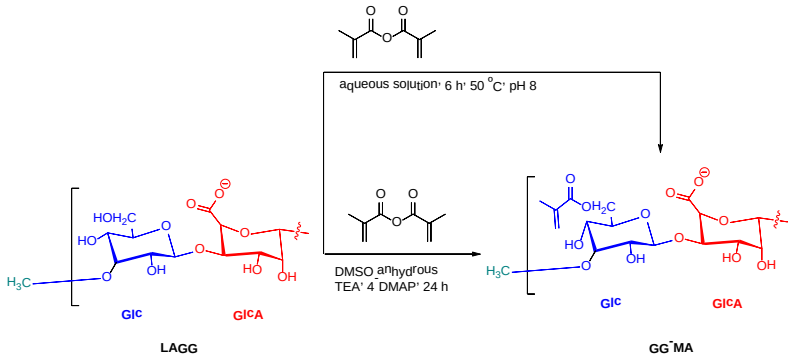
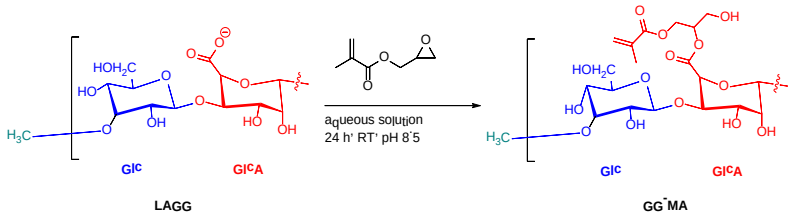
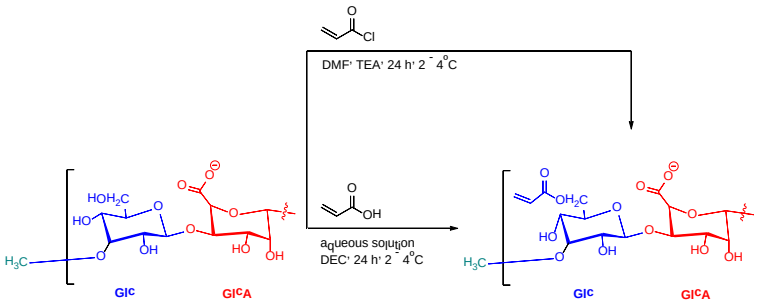
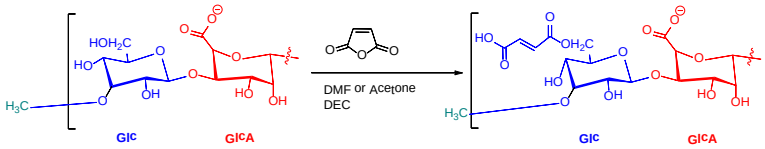
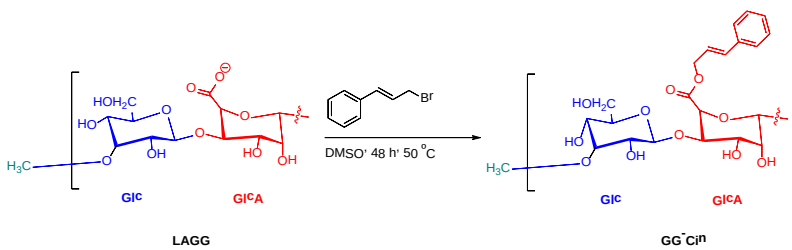
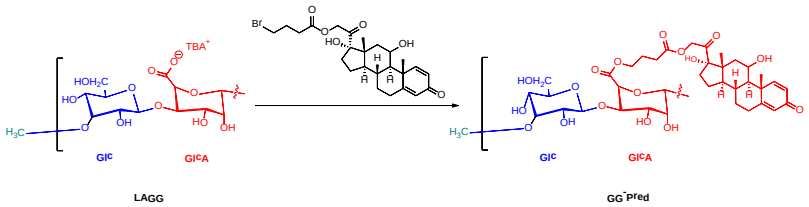
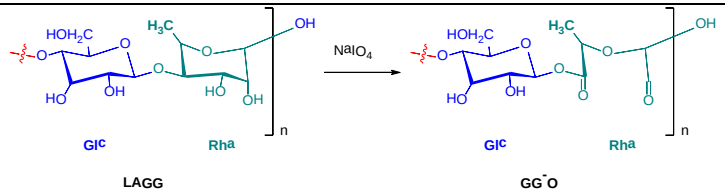
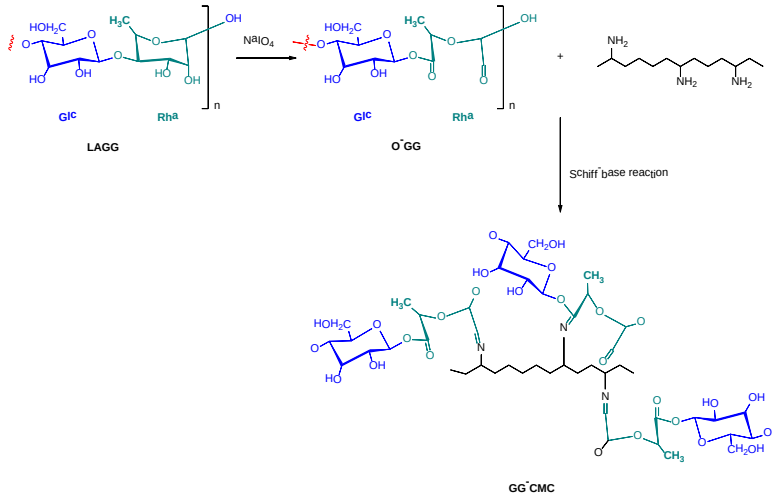
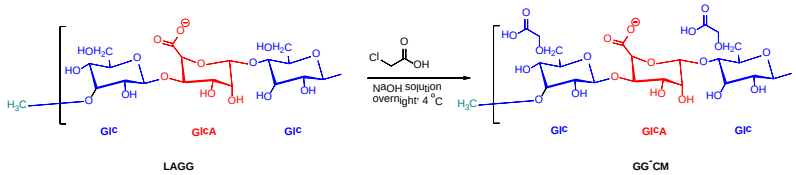
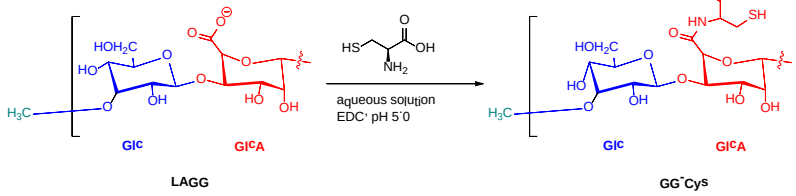
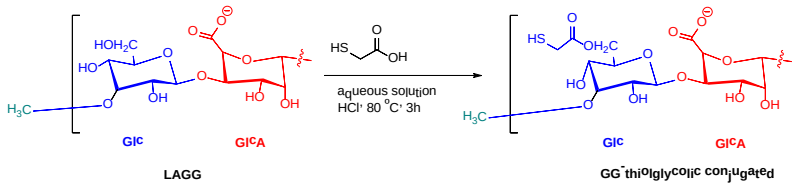
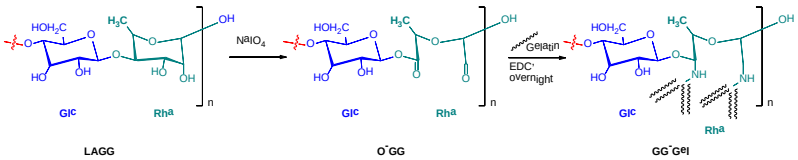
ENTRY	TYPE OF REACTION	REACTION SCHEME/REACTION CONDITIONS	PROPERTIES	REF.
1	**Methacrylation**		GG-(meth)acrylate vs GG	
	• With methacrylic anhydride (MA)		✓ Mechanical and degradation properties improved: improvements in the Young's modulus values between 0.15 and 148 kPa for GG-MA ✓ Possibility of photo-initiated crosslinking	31, 32
	• With glycidyl methacrylate (GMA)			23

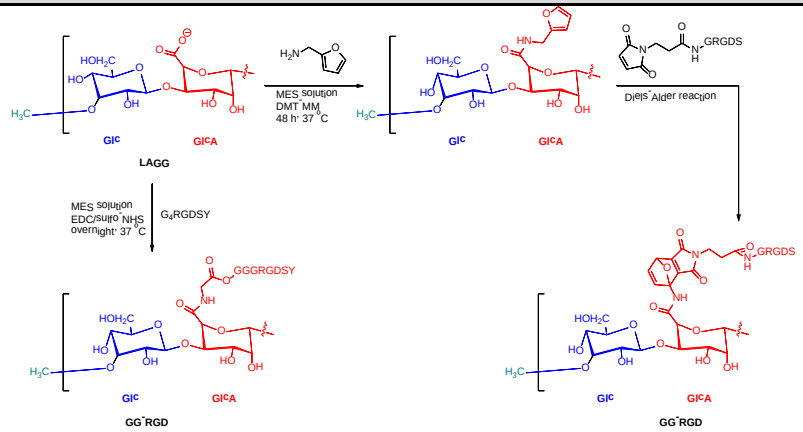
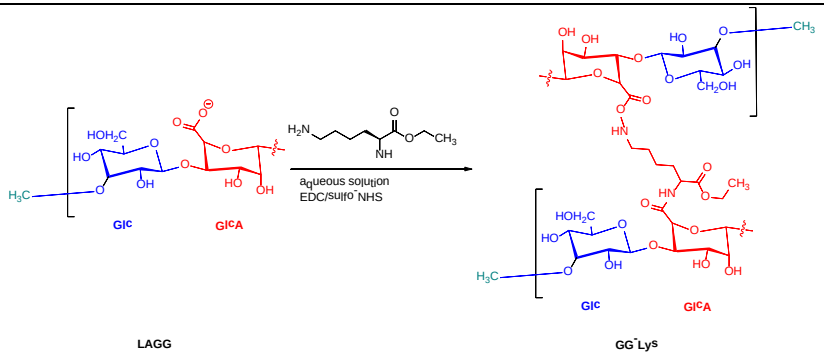
ENTRY	TYPE OF REACTION	REACTION SCHEME/REACTION CONDITIONS	PROPERTIES	REF.
2	**Esterification**		A-GG/MA-GG vs GG:	
	• With organic acids derivatives (acryloyl chloride and acrylic acid)		✓ Polymerization under mild conditions ✓ Thermo- and pH-stimulable hydrogels	36
	• With maleic anhydride			36

ENTRY	TYPE OF REACTION	REACTION SCHEME/REACTION CONDITIONS	PROPERTIES	REF.
3	• With cinnamate		GG-Cin vs GG: ✓ Novel anti-adhesion film with high gel contents and suitable mechanical properties ✓ Photosensitivity was achieved ✓ Anti-inflammatory effect in rats.	57
4	• With prednisolone		GG-Pred vs GG ✓ Enhancing bioavailability of poorly water soluble drugs ✓ Efficient prednisolone carriers	58
5	**Oxidation** • With sodium periodate		GG-O vs GG ✓ Gelation point was optimised to 37.5 ºC by	59

ENTRY	TYPE OF REACTION	REACTION SCHEME/REACTION CONDITIONS	PROPERTIES	REF.
			controlling the GG molecular weight ✓ Encapsulated chondrocytes stayed viable, had normal morphology and proliferated (150 days)	
6	**Schiff-base** • With carboxymethyl chitosan		GG-CMC vs GG ✓ Stabilization of gellan sctruture with carboxymethyl chitosan ✓ Improved gelation temperature ✓ Increased compressive modulus of 278 kPa ✓ Ability to return to the original shape	60

ENTRY	TYPE OF REACTION	REACTION SCHEME/REACTION CONDITIONS	PROPERTIES	REF.
7	**Carboxymethylation** • With chloroacetic acid		GG-CM vs GG ✓ Improve the in situ gelling properties ✓ Improve aqueous solubility (soluble at RT up to 10%)	61
8	**Thiolation** • With cysteine		GG-Cys vs GG ✓ Stable injectable system ✓ Improvement in the in situ gelling properties ✓ Strong enhanced rheological properties ✓ Decrease gelation temperature while keeping its physical gelation capacity	62, 75

Entry	Type of reaction	Reaction scheme/Reaction conditions	Properties	Ref.
9	• With thioglycolic acid		GG-thiolglycolic acid conjugate vs GG ✓ Improvement in mucoadhesive properties ✓ Degree of crystallinity was increased ✓ Ion gelation decreased	64
10	**Amidation** • With gelatin		GG-Gel ✓ Facility ADC adhesion, proliferation and differentiation ✓ Attachment of human dermal fibroblasts and human fetal osteoblasts	65

ENTRY	TYPE OF REACTION	REACTION SCHEME/REACTION CONDITIONS	PROPERTIES	REF.
11	• With RGD (arginine-guanine-aspartic acid)-sequence peptides		GG-RGD vs GG ✓ Enhanced cell adhesion and interaction ✓ Cells (NSPCs, BM-MSCs, C2C12) presented higher proliferation and metabolic activity	66, 68
12	• With lysine ethyl ester		GG-Lys ✓ Bioavailability of poorly water soluble anti-inflammatory drugs is enhanced	17

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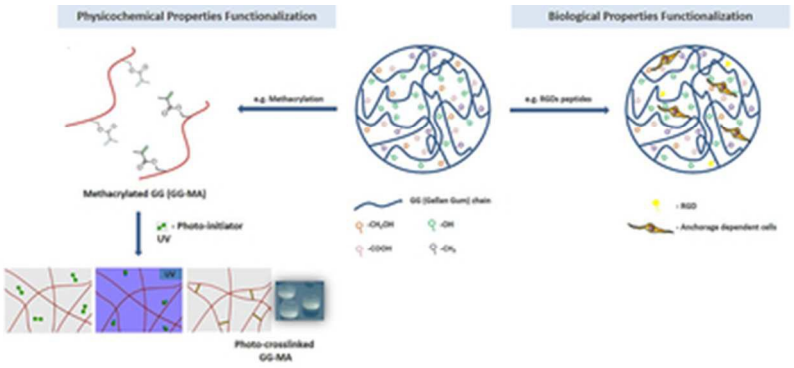

35x15mm (300 x 300 DPI)

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Gellan gum and its functionalized derivatives present a wide range of applications that open-up new possibilities in tissue engineering and regenerative medicine