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# Modified gellan gum hydrogels for tissue engineering applications

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## Abstract

Gellan gum is an anionic linear polysaccharide well known for its use as a multi-functional gelling, stabilising and suspending agent in a variety of foods and personal care products. In this Highlight, we explore the recently established directions for gellan gum hydrogels as materials for applications in tissue engineering. We highlight that modified gellan gum will be well suited for this purpose, providing that a number of remaining challenges are addressed.



## Keywords

tissue, engineering, applications, hydrogels, modified, gum, gellan


## Disciplines

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# Modified gellan gum hydrogels for tissue engineering applications

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Gellan gum is an anionic linear polysaccharide well known for its use as a multi-functional gelling, stabilising and suspending agent in a variety of foods and personal care products. In this Highlight, we explore the recently established directions for gellan gum hydrogels as materials for applications in tissue engineering. We highlight that modified gellan gum will be well suited for this purpose, providing that a number of remaining challenges are addressed.

## 1. Introduction

The biopolymer gellan gum (GG) was identified in 1978 by CP Kelco (San Diego, USA) during a large scale screening operation to identify polysaccharides from soil and water bacteria with useful rheological properties[1]. Since then it has found wide application mainly as a multi-functional gelling, stabilising and suspending agent in a variety of foods and personal care products, and has received both US FDA and EU (E418) approval for these purposes.

Gellan gum is an anionic extracellular bacterial polysaccharide produced in high yield by the non-pathogenic strain *Sphingomonas elodea*[2] (ATCC 31461, formerly classified as *Pseudomonas elodea*). It consists of tetrasaccharide repeat units containing β-D-glucose, β-D-glucuronic acid and α-L-rhamnose monomers in the molar ratio 2:1:1[3]. In its native form, usually referred to as high-acyl GG (HAGG), *O*-acetate and L-glycerate substituents are attached to one glucose residue, with an average of 1 glycerate and 0.5 acetate substituents per repeat unit[4] (Fig. 1A). In most commercial products, however, these substituents are removed by alkali treatment to yield low-acyl GG (LAGG, Fig. 1B), which contains very few if any acyl groups. The average molecular mass of LAGG is ~ 2-5x10$^5$ Da. GG is sold commercially under a number of product names depending on the application area. For example, the CP Kelco company sells Gelzan™ as an alternative to agar for microbiological media. The LAGG in Gelzan™ has been extensively purified to remove any residual endotoxin material remaining after synthesis.

The usefulness of GG pertains largely to its gelation properties, which have been discussed in detail in a number of review articles[1,7,8]. Briefly, GG dissolves readily in water, adopting a disordered conformation (random coil) at higher temperatures (> ~ 40 °C) which subsequently undergoes a disordered-to-ordered transition on cooling. X-ray diffraction studies on GG fibres have shown that the ordered conformation is a threefold, left-handed, parallel double helix[9]. This conformational transition has been observed and characterised extensively by techniques including rheology[10,11], light scattering[12,13], nuclear magnetic resonance (NMR) spectroscopy[14,15], and circular dichroism (CD) spectroscopy[13]. These studies have revealed that the conformational transition temperature is dependent on a number of factors including the concentration and molecular weight of the polymer [16,17], cation concentration[18,19] and pH[20]. While helical ordering at low temperatures may impart weak gel characteristics, the formation of a true hydrogel network is achieved through cation-mediated association of helices[21,22] (Fig. 2). This association can be facilitated through either monovalent or divalent cations, although divalent cations produce stronger gels. Divalent cations act as direct bridges by site binding between pairs of carboxyl groups, while monovalent cations induce aggregation by suppressing electrostatic repulsions[23].

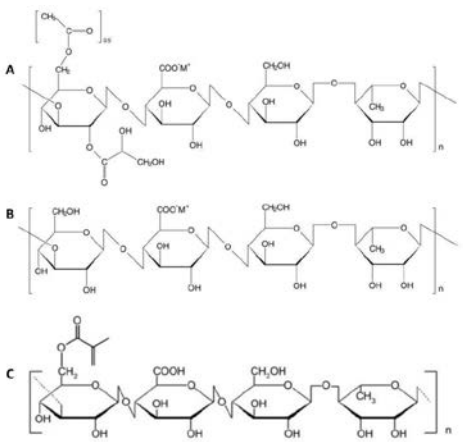


**Fig. 1.** Structure of the tetrasaccharide repeat unit in commercially available high acyl (A) and low acyl (B) gellan gum, as well as methacrylated gellan gum (C). A and B reproduced from reference 5, C reproduced from reference 6.

The presence of the acyl substituents in HAGG does not change

the overall helical structure, but changes the binding (cross-linking) sites for the cations. It has been suggested that this change is responsible for the loss of cation-mediated aggregation between the HAGG helices. The result of this difference in aggregation behaviour is that LAGG forms hard (non-elastic) and brittle gels, whereas HAGG gels are soft (elastic) and non-brittle[1].

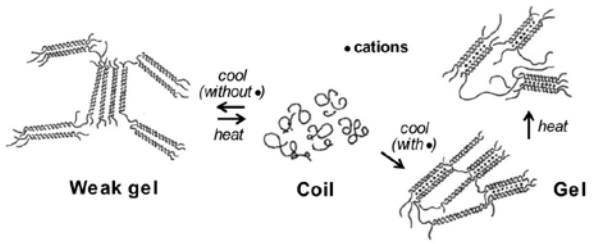


**Fig. 2.** Schematic model of the conformational transitions and gelation of low acyl gellan gum through temperature changes with and without added cations. Adapted from reference 1.

Aside from its widespread application in food and cosmetics, GG's unique suspending (dispersing), gelation and rheological properties have been utilized for biomedical purposes and in the processing of conducting fillers. For example, GG has been employed as a versatile encapsulating agent and active ingredient in numerous controlled drug delivery systems for nasal, ocular, gastric and colonic drug delivery applications[24-33], as implants for insulin delivery[34] and for wound healing applications[35-38]. In addition, we and others have used GG to disperse carbon nanotubes in aqueous media which enables subsequent processing into useful architectures by film casting[39-42], vacuum filtration[43], inkjet printing[44] and extrusion printing[45]. GG has also been used as a dopant in the synthesis of polypyrrole electrode coatings for neural devices[46] and to stabilise nanoparticles[47].

The remainder of this article explores an emerging new direction for GG as a material for applications in tissue engineering (TE). In particular, we have highlighted gellan gum which has been modified either chemically (covalent functionalisation) or physically (e.g. interpenetrating network formation). This is a multidisciplinary field that draws primarily on principles from the engineering and life sciences to develop biological substitutes that restore, maintain or improve tissue function[48]. Typically, this is achieved through some combination of living cells with synthetic or natural biomaterials. These biomaterials act as a surrogate for the natural extracellular matrix (ECM), with the primary aim of engineering functional constructs that recapitulate the complex characteristics of natural tissues and organs.

## 2. Why consider gellan gum?

Gelation of GG, as described previously, is preceded by a conformational transition from coil to double helix, and association of these helices in junction zones is facilitated through either monovalent or divalent cations. Consequently, GG hydrogels may be formed at low concentrations of divalent cations, or even in the presence of monovalent cations alone[1]. This could be advantageous in TE applications and 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[49]. In addition, GG formed a gel on contact with tear fluid[50], which is advantageous for ophthalmic drug delivery. GG hydrogels are therefore also stable during long-term culture in standard media and do not suffer from unwanted dissolution due to ionic exchange[51]. In addition to these gelation properties, GG's excellent optical clarity could prove advantageous in analysis of encapsulated cells[7]. It has been shown that GG scaffolds can be made porous using straightforward fabrication methods[52]. Furthermore, GG appears not to inhibit polymerase chain reaction (PCR) analysis[53] and is suitable as an injectable material[54-56].

A further attractive characteristic of GG for TE are the mechanical similarity to the elastic moduli of common tissue. The mechanical characteristics of GG depend on type (LAGG or HAGG) and concentration as well as the type and amount of physical cross-linker[57]. Other strategies involve methacrylation of the GG chain (Fig. 1C) followed by physical and/or chemical cross-linking[58]. These approaches can be used to tune the elastic modulus of GG hydrogels to that comparable with a wide range of human soft tissues such as muscle, liver and cartilage[59].

Finally the degradation behaviour of GG can be controlled. There are a number of human enzymes such as lysozyme, amylase and trypsin which are known to degrade common polysaccharides[60]. These enzymes are commonly found in tears, mucus, milk and the stomach and degrade polysaccharides through hydrolysis. It has been reported that the enzymatic degradation of GG containing hydrogels resulted in a mass loss of 20% and 30% over 7 days with lysozyme and trypsin, respectively[61]. However, most studies with TE in mind have focussed on the degradation behaviour of GG in ionic solutions. For example, we recently investigated the mass loss of LAGG, HAGG and LAGG/HAGG blended hydrogels for up to 168 days in phosphate buffered saline (PBS, pH 7.4) at 37 °C [51]. It was observed that all three types of gels degraded for 28 days and then did not degrade any further for the additional 140 days of the testing period. Mass loss was smallest for LAGG (5.3 ± 0.7 %), largest for HAGG (12.1 ± 0.6), and intermediate for the blend. The degradation behaviour of GG gels in culture media in the presence and absence of bone marrow cells has also been investigated using rheological measurements[62]. Other work using methacrylated LAGG has shown that the degradation rate (in 0.1 mM NaOH, at 37 °C) can be influenced by the cross-linking mechanism, i.e. physical or combined physical and chemical[58].

## 3. Gellan gum in tissue engineering

Smith and co-workers were the first group to demonstrate that GG hydrogels could be used to encapsulate viable mammalian cells[49]. They showed that GG could be crosslinked by the addition of cell culture media alone, owing to the gelation of GG at milliMolar concentrations of divalent cations which are present in most media formulations, to form self-supporting hydrogels (Fig. 3A). This enabled a mild encapsulation process for rat bone marrow cells (Fig 3B), which remained viable in the GG hydrogels for 21 days in culture. In our initial work on applications of GG to TE, hydrogels were produced with and without added CNTs and surface topographical features, and these were shown to support and guide the growth of L929

fibroblast cells[54].

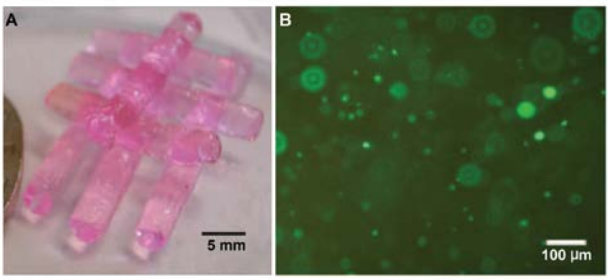


**Fig. 3.** (A) GG hydrogel cylinders produced by extruding 1% w/v GG solution into culture medium. (B) Calcein-stained rat bone marrow cells in GG hydrogel after 10 days in culture. Figures adapted from reference 49. (See web version of this article for a colour version of this figure.)

Reis and co-workers at the 3B's Research Group (University of Minho, Portugal) have explored the use of GG hydrogels in TE applications[63-65]. Chondrocytes were shown to remain viable when encapsulated in GG hydrogels[63] and exhibited ECM production when implanted subcutaneously in nude mice[66]. Injectable delivery of these chondrocyte-laden gels was also investigated, taking advantage of the ability of GG to form a gel under physiological conditions[67]. GG hydrogels were also used to encapsulate oligodendrocyte-like cells within the centre of a tubular structure fabricated by 3D extrusion printing of starch[68]. This group has also demonstrated that GG can be methacrylated to introduce the possibility of photo-initiated crosslinking[58], thus enhancing the range of mechanical and degradation properties that can be tailored in GG hydrogels. These materials have been studied for application as cellular or acellular artificial nucleus pulposus implants in the treatment of intervertebral disk degeneration[69-71]. Du and co-workers took an alternative route to chemical crosslinking by thiolation of GG to produce a stable injectable system[55].

Wang's group at Nanyang Technological University (Singapore) have also implemented GG hydrogels in cartilage engineering. The gelation temperature of GG hydrogels was optimised (to 37.5 °C) by controlling the GG molecular weight through oxidative cleavage[72]. Chondrocytes encapsulated in these hydrogels showed expression levels of collagen that outperformed cells in agarose. Furthermore, rabbit mesenchymal stem cells encapsulated in GG hydrogels and cultured in chondrogenic medium were shown to express both chondrocytic genes and cartilaginous matrix[73]. Lee and co-workers also attempted to optimise the physical parameters of GG hydrogels for cartilage applications by blending low-acyl and high-acyl GG[74]. They found that increasing the HAGG:LAGG ratio resulted in a decrease in the gel's stiffness, and that gels of 2% (w/v) LAGG were most suitable for fibro-cartilage applications. Blending GG with other types of biomolecules (e.g. polysaccharides and enyzmes) for TE applications have also been considered[75-81].

## 4. Modified gellan gum

GG is a relatively bio-inert material. This has been demonstrated through the lack of cell infiltration and angiogenesis observed when implanting GG hydrogels in vivo[70] and through evaluation of the behaviour/response of anchorage dependent cells encapsulated in GG hydrogels[58,68]. Thus application has so far been largely limited to anchorage-independent cells like chondrocytes[63,72]. Therefore, in order to function as a useful artificial ECM for anchorage-dependent cell types, GG must be modified. Previously, GG microspheres produced by a water-in-oil emulsion process have been covalently functionalised with gelatin through redox-mediated crosslinking to encourage the attachment of human dermal fibroblasts and human fetal osteoblasts[82]. Photo-crosslinkable variants of both GG and gelatin have also been combined in a novel double-network hydrogel with enhanced mechanical properties[6]. More recently, GG hydrogels were modified with RGD-containing peptides to enhance interaction with encapsulated neural stem/progenitor cells[83] (Fig. 4). Another interesting development is the modification of GG with surfactants to function as a bio-ink for cell printing applications[84].

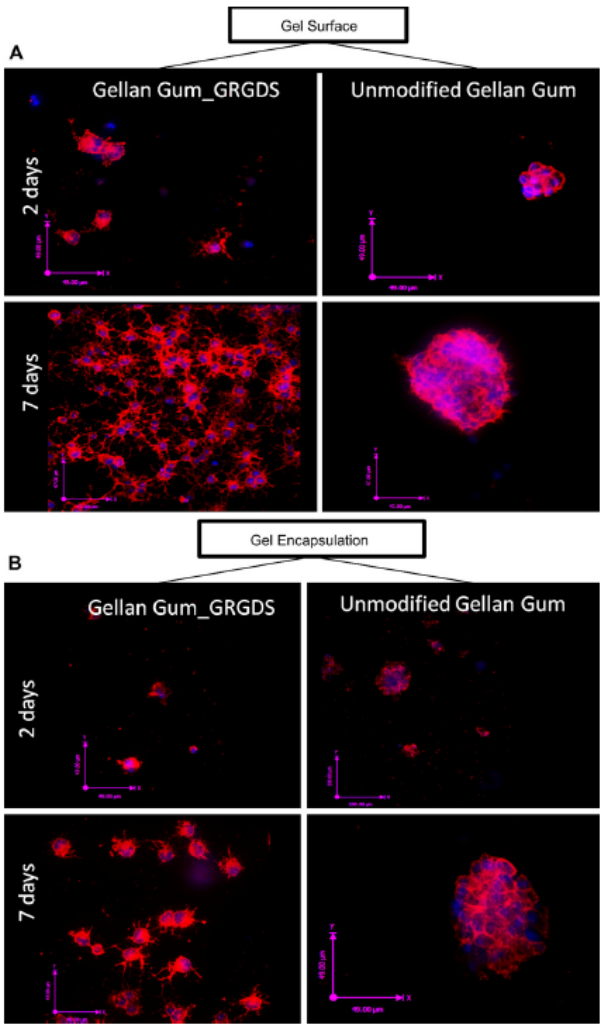


**Fig. 4.** Morphology and dispersion of neural stem/progenitor cells (NSPCs) on the gellan gum hydrogel modified with the cell-adhesive peptide (GG-GRGDS). Confocal analyses revealed substantial differences in NSPC morphology when cultured either (A) on the surface or (B) encapsulated within the GG-GRGDS vs. unmodified GG gel. Cell spreading and visible cytoplasmatic extensions were only observed in the GG-GRGDS. In the unmodified GG, NSPCs proliferated as neurospheres. The cytoplasm was stained with the anti-F-actin/phalloidin (red) and nuclei counterstained

with DAPI (blue). (See web version of this article for a colour version of this figure.) Reproduced from reference 83.

Although it is straightforward to prepare GG hydrogels with elastic moduli similar to that of tissue, matching the toughness and load tolerance of mammalian tissue is not. For example, LAGG and HAGG gels can be prepared with elastic moduli (kPa range) similar to that of liver, fat, muscle or cartilage[58,85]. But the compressive stress at failure of these gels (kPa range) is orders of magnitude lower than tissues such as cartilage (MPa range)[58,86]. In other words GG hydrogels are mechanically weak, which is a generally recognised drawback of hydrogel materials under consideration for tissue engineering[87].

A number of strategies have been adopted to address the mechanical weakness and/or load intolerance. Hydrogels based on chemically/physically cross-linked methacrylated GG resulted in improvements in the magnitude of compressive stress at failure (up to 0.9 MPa)[58]. It is not known if these gels would be able to recover from damage (load tolerance). Progress towards the latter has been made by building on the pioneering research on toughening gels by J.P. Gong[88-90]. These gels exhibit excellent mechanical performance such as, for example, compressive stress at failure values of up to 60 MPa[90]. The toughening of hydrogels is achieved using an interpenetrating polymer network (IPN) approach which results in so-called "double network" (DN) hydrogels with mechanical properties that are significantly improved compared with either one of the parent networks[88-90]. The two polymer networks in the DN approach are chemically (covalently) cross-linked, and the toughening mechanism arises from efficient energy dissipation due to fragmentation of the first (brittle) network thereby allowing the second (ductile) network to facilitate large deformations[89,90]. The DN approach has also been adopted for methacrylated GG in combination with methacrylated gelatin[91]. The resulting DN gels exhibited compressive stress at failure values of close to 7 MPa. DN gels are extremely tough, but due to the irreversible, permanent fracture of the chemical cross-links[89,90] DN gels are not able to recovery from significant loading and have poor fatigue resistance.

Recently, it has been demonstrated that preparing IPN gels combining one network with reversible physical (non-covalent) bonds and one network with irreversible covalent networks results in gels that are tough but can recover from damage[92-94]. For example, it was demonstrated that hydrogels consisting of ionically cross-linked LAGG and covalently cross-linked poly(acrylamide) exhibited double network behaviour, i.e. improved mechanical properties compared to their respective single network hydrogels[93]. These so-called ionic-covalent entanglement hydrogels exhibited self-recovery of 53 ± 4 % within 80 min from the first compressive cycle (Fig. 5).

## 5. Recommendations

This article has highlighted modified gellan gum as a suitable material for tissue engineering applications. The remaining challenges are to prepare gellan gum materials that achieve one or all of the following (depending on the intended application): (i) improved toughness and extensibility so that these gels can function as tissue mimics. In particular, gels that have the appropriate mechanical characteristics of mammalian tissue so that they can recover from strain and absorb impact without permanent damage (this is important for cartilage tissue engineering); (ii) attachment and function of encapsulated anchorage-dependent cells; and (iii) suitable degradation behaviour (important for regenerative tissue engineering). In conclusion, it is clear that modified gellan gum offers great opportunities as a material for tissue engineering, but a number of challenges remain to be addressed.

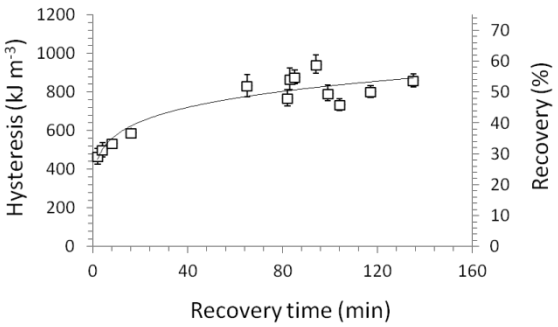


**Fig. 5.** The dissipated energy ($U_{hist}$, hysteresis of loading/unloading cycle 2) and recovery as a function of recovery time between cycles 1 and 2 for hydrogels consisting of ionically cross-linked LAGG and covalently cross-linked poly(acrylamide). The line is a fit to the data to guide the reader's eye. Figure reproduced from reference 93.


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## Notes and references


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