

# Structural characterization of interpenetrating network formation of high acyl gellan and maltodextrin gels

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1 **Structural Characterization of Interpenetrating Network Formation of High Acyl Gellan and**  
2 **Maltodextrin Gels**

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8

9 **Abstract**

10 A mixed-gel of high acyl (HA) gellan gum and maltodextrin (MD) (potato DE2) demonstrated a range  
11 of physical properties with a proposed interpenetrating network. Mixed hydrocolloid gels allow for  
12 the development of novel properties that neither polymer alone could create allowing unique  
13 functionality in textures or controlled release. The aim of this work was to identify the type of  
14 network formation by examining material properties and the contribution from of each polymer.  
15 Material properties of quiescently set composite gels were characterized through bulk fracture,  
16 small deformation rheology, DSC, and microscopy. A continuous shift in fracture strain and modulus  
17 were created through mixed gels of the soft and flexible HA gellan with the firm and brittle MD. By  
18 adding MD (from 0 to 40%) at a constant 0.5% gellan, the gel true strain at fracture decreased from  
19 0.50 to 0.18 while the Young's Modulus increased from 3 to 1780 kPa. No indication of phase  
20 separation or chemical complexation was measured. Analysis of the time-dependant MD  
21 contribution and composite material properties hypothesized a gelation mechanism in which HA  
22 gellan forms a network first and MD aggregates within the pores without phase separation. MD  
23 dominated the small deformation rheology while HA gellan appeared to dominate the fracture  
24 point. Material properties were indicative of the type of structural organization in the HA gellan MD  
25 mixed gel network.

26 **Keywords.** Maltodextrin; High Acyl Gellan Gum; Interpenetrating Network; Mixed Hydrocolloids; Gel  
27 Fracture

28

29

30 **1. Introduction:**

31 Mixed biopolymer gel systems have become popular to achieve a wider and more controllable range  
32 of properties than any single gelling system. Applications in the food industry include creation of  
33 novel textures, mimicking of traditional foods with improved nutritional profiles (low and no-fat),  
34 and controlled release functionality. The key to understanding the properties of composite gels lies  
35 in a microstructural understanding of the gel network formation (Norton and Frith, 2001). Mixed gel  
36 networks are divided into three idealized types: phase separated, interpenetrating (IPN), and  
37 coupled (Morris, 1986; Kasapis, 1995; Norton et al., 2014). Interactions between two polymers may  
38 either be segregative or associative based on the enthalpy of interaction of between similar versus  
39 dissimilar polymers (Morris, 2009). A phase separated network originates from segregative  
40 interactions and is often observed as a water-in-water dispersion in which one polymer forms  
41 droplets within a continuous network of the second polymer (Morris 2009, Norton 2014). Both IPN  
42 and coupled are types of associative interactions between polymers. For an IPN gel, both polymers  
43 form networks spanning the gel in one phase and without direct interaction of the polymers  
44 (Kasapis, 1995; Norton et al., 2014). A coupled network additionally involves a chemical association  
45 between the two types of polymer, and this type has only been observed in a few systems (Morris,  
46 1995; Kasapis, 1995; Morris, 2009). A key distinction between these types for polymers that gel by  
47 forming aggregates, which may appear as phase separated, is whether phase separation occurs prior  
48 to gelation (true phase separated by segregative interactions) or the gelation mechanism simply  
49 leads to regions rich in one polymer (micro-phase separated or interpenetrating) (Clark et al., 1999;  
50 Djabourov et al., 2013). Phase separated systems are common because frequently the enthalpic  
51 contribution from like-to-like polymer interactions outweighs the association of dissimilar polymers  
52 and small entropic contribution of staying mixed (Kasapis et al., 1993c; Clark, 1996; Morris, 2009).  
53 Interpenetrating networks occur when the enthalpic contribution of separation is low (such as with a  
54 charged and an uncharged polymer). This work will examine the network formation between high  
55 acyl (HA) gellan and maltodextrin. The first section will examine the type and mechanism of network  
56 formation and the second will examine contributions of each polymer to composite modulus and  
57 fracture behaviour.

58

59 Maltodextrin (MD) gels are formed by dense aggregate regions which form strong brittle gels at high  
60 concentrations (Schierbaum et al., 1992; Chronakis, 1998; Loret et al., 2004; Kanyuck et al., 2019).  
61 This polymer is a carbohydrate created through the hydrolysis of starch into shorter chains  
62 (Chronakis, 1998). A low dextrose equivalent (DE) MD has a lower degree of hydrolysis, and it is the  
63 longer chains of these MDs which exhibit gelling abilities. Gelation occurs by aggregation of double  
64 helix chains of polymer strands and the presence of sufficient long stands that are able to connect  
65 multiple aggregate regions (Reuther et al., 1984; Radosta and Schierbaum, 1990). For the DE 2  
66 potato MD used, critical gelling concentration are reported between 15 and 20% by weight (Kasapis  
67 et al., 1993b; Loret et al., 2004; Kanyuck et al., 2019).

68

69 High acyl (HA) gellan gum forms a soft and flexible gel with a high strain to fracture caused by a  
70 fibrous gel network (Sanderson et al., 1988; Funami et al., 2008). Gelation occurs through double  
71 helix formation of individual chains (Chandrasekaran et al., 1992), and helices are connected by  
72 branching and end-to-end associations (Morris et al., 1999; Noda et al., 2008; Funami et al., 2008).  
73 Unlike many hydrocolloids, there is limited helix aggregation due to the steric hindrance of the  
74 acetyl groups (Noda et al., 2008; Funami et al., 2008). The microbial fermented hydrocolloid is an  
75 acidic carbohydrate comprising of a four-sugar repeated unit with 0.9 glycerate and 0.4 acetate  
76 groups on the first sugar unit and one carboxyl group on the second unit (Kasapis et al., 1999; Sworn,  
77 2009). The more commonly studied variant low acyl (LA) gellan gum has the same carboxyl groups

78 but the deacetylation process removed most of the bulky acyl groups, thus changing the gel  
79 structure and material properties (Morris et al., 2012).

80  
81 MD has been studied in mixed gelling systems previously, and most often for applications in fat  
82 reduction due to the creaminess mimicking abilities of MDs (Chronakis, 1998). Most literature [has](#)  
83 [proposed](#) phase separated mixed gels between a low DE MD and gelatin (Kasapis et al., 1993a; Lorén  
84 and Hermansson, 2000), low methoxy pectin (Picout et al., 2000), agarose (Loret et al., 2006), iota  
85 carrageenan (Wang and Ziegler, 2009), milk protein (Chronakis et al., 1996), locust bean gum, gum  
86 acacia, and high salt carboxymethyl cellulose (Annable et al., 1994). For charged polymers, phase  
87 separation was either not observed with high methoxy pectin (Evageliou et al., 2000) or was only  
88 observed at sufficient levels of added salts with carboxymethyl cellulose (Annable et al., 1994). One  
89 researcher has suggested the presence of an IPN formed with MD in combination with low acyl (LA)  
90 gellan (Clark et al., 1999). Experimentation with HA gellan is limited, although the non-gelling  
91 polymers of chitosan fibers (Liu et al., 2013) and soybean fiber (Chen et al., 2020) have been  
92 proposed to arrange within the pores without phase separation. No published work has examined  
93 the mixed gel of HA gellan and MD, and there is only limited knowledge of the interactions between  
94 HA gellan with other hydrocolloids.

95  
96 The large disparity between the material properties of MD and HA gellan gum allowed a rheological  
97 analysis of the microstructure contribution of each polymer to the mixed gel. This work proposes the  
98 structural organization of the mixed gel is reflective in the physical and chemical properties of the  
99 composite. Materials properties, microscopy, DSC, and modelling of composite gels will be used to  
100 characterize the mixed gel of MD with HA gellan gum. Contributions of each polymer to specific  
101 material properties will also be discussed to examine the structural influence of individual gel  
102 networks.

## 103 **2. Material and Methods:**

### 104 **2.1. Materials and Gel Preparation**

105 HA gellan gum (KELCOGEL LT100 lot # 5B1259A) was obtained from CP Kelco (UK). MD (Paselli SA-2)  
106 was purchased from Avebe (Netherlands) produced from potato with a dextrose equivalent (DE)  
107 between 2.7 and 2.9 (Kasapis et al., 1993b; Manoj et al., 1996). The salt content of HA gellan was  
108 19473 ppm potassium, 3615 ppm sodium, 2187 ppm calcium, and 29 ppm magnesium as measured  
109 by ICP-OES (Optima 8000 by PerkinElmer). The DE 10 MD (Paselli SA-10) was purchased from Avebe  
110 (Netherlands), the modified MD originated from waxy corn starch and was modified to increased  
111 branching and lower the molecular weight (Agenanova from Agrana, Austria), and the glucose was  
112 purchased from Sigma Aldrich (UK). DI water was purified with a reverse osmosis milli-Q water  
113 system.

114 Prior to mixing, each polymer was dispersed individually to ensure complete hydration. HA gellan  
115 was mixed into DI water at 90 °C and hydrated for 2 hours with stirring to reach a stock  
116 concentration between 4 and 5% (Cassanelli et al., 2018). MD was slowly dispersed in DI water at 95  
117 °C and stirred for 4 hours to hydrate at stock concentrations ranging from 40% to 60% (Kanyuck et  
118 al., 2019). Glucose and the other MDs (DE 10 and modified) were prepared identically by fulling  
119 hydrating in heated water prior to mixing. Water loss was accounted for by replacing any lost mass  
120 to the sample during heating. Gels were prepared by mixing these heated stock solutions together  
121 with the required amount of heated DI water to achieve the range of concentrations utilized in this  
122 work. Hot solutions were stirred until homogenous (approximately 5 minutes) and quiescently set in  
123 plastic cylindrical molds (20 mm diameter) excluding the samples for DSC which were prepared in

124 specific vessels. Gels for time-dependent measurements were prepared from a single solution to  
125 produce five samples, and at each of the time points one sample was measured and destroyed.

## 126 2.2. Compression and Fracture of Gels

127 Bulk material properties were assessed by compression of gels with a squeeze-flow setup using a  
128 TA.XT.plus texture analyser (Stable Micro Systems, UK). Samples were prepared using a 20 mm  
129 diameter cylindrical mold and cut to a 20 mm height. Gels were compressed with a speed of 2 mm/s  
130 between parallel surfaces of a flat probe (40 mm diameter) and a stationary stage until fracture (up  
131 to a 90% reduction of the initial sample height). Further deformation was not possible with the  
132 instrument and would have resulted in gel material squeezing outside the geometry and losing  
133 contact with the upper plate. From the instrumental measurement of force ( $F$ ) and distance ( $\ell$ ), the  
134 Young's Modulus ( $E$ ), true strain ( $\epsilon_T$ ), and true stress ( $\sigma_T$ ) were calculated:

$$135 \quad E = \sigma/\epsilon \text{ from } \epsilon = 0.01 \text{ to } \epsilon = 0.05 \quad (1)$$

$$136 \quad \epsilon_T = \ln( (\ell_0 + (\ell_0 - \ell)) / \ell_0 ) \quad (2)$$

$$137 \quad \sigma_T = (F/A_0) (1 + \epsilon) \quad (3)$$

138 When ' $\ell_0$ ' is the initial sample height and  $A_0$  the initial contact area of 314 mm<sup>2</sup>.

## 139 2.3. Differential Scanning Calorimetry

140 A  $\mu$ DSC3evo (Setaram Instrumentation, France) was used with a temperature range of 20 to 95 °C  
141 with a heating and cooling rate of 0.2 °C/min with 10 minute isothermal holds at each extreme. A  
142 second heating and cooling step was performed immediately following the first. Only the first  
143 heating and cooling are shown graphically but calculated enthalpies are reported for all steps.  
144 Integration and curve smoothing was performed using CALISTO software (Setaram Instrumentation,  
145 France). Enthalpies were normalized to grams of total sample and curves were compiled after a  
146 manual linear baseline subtraction. Prior to data collection, gels were pre-melted in the DSC by  
147 heating and cooling at 1 °C/min from 20° to 95° C with a 10 minute hold at 95° to ensure a  
148 homogenous sample over the bottom of the vessel. For adequate measurement of the MD  
149 contribution to the gel, all samples were held at room temperature for 4 days prior to the reported  
150 DSC analysis (Kanyuck et al., 2019). Gels were added hot with a mass of  $0.8 \pm 0.1$  g and a matching  
151 mass of DI water. Sample and reference cells had a volumetric capacity of  $\sim 0.9$  mL and were sealed  
152 vessels of Hastelloy material.

## 153 2.4. Modelling by Polymer Blending Laws

154 The isostress and isostrain equations are often used to model physical properties of phase separated  
155 gel systems based on a composite of the individual polymers. Theory and models were adapted from  
156 E. R. Morris (1992). The equations predict the composite gel modulus ( $G_c$ ), using the phase volume  
157 of HA gellan gum ( $\phi_{HAG}$ ) and MD ( $\phi_{MD}$ ) and calculated modulus at the effective concentration of HA  
158 gellan gum ( $G_{HAG}$ ) and MD ( $G_{MD}$ ):

$$159 \quad \text{Isostrain } G_c = \phi_{HAG}G_{HAG} + \phi_{MD}G_{MD} \quad (4)$$

$$160 \quad \text{Isostress } 1/G_c = \phi_{HAG}/G_{HAG} + \phi_{MD}/G_{MD} \quad (5)$$

161 Application of these models to biopolymer gels relied on several assumptions that required  
162 modification; no polymer volume, complete phase separation, and a mathematically model-able  
163 modulus (Morris, 1992). Firstly, the assumption of no polymer volume is invalid in this system with  
164 MD concentrations up to 40% (Manoj et al., 1996). To account for this, effective concentrations of

165 each polymer were expressed as polymer per water (w/w) ( $\text{Mass}_{\text{polymer}} / \text{Mass}_{\text{water}}$ ) to calculate the  
166 modulus. This modification allows consideration to the concentrating effect that a 30% MD solution  
167 would have on the effective HA gellan gum concentration through a reduction in the amount of  
168 water in the total mixture. For example, a 1% HA gellan gum with 30% MD would be calculated using  
169 the water amount of 69% and the effective concentration becomes 1.4%. Secondly, the concept of  
170 calculating phase volumes ( $\phi$ ) became difficult without observation of any separation so typical  
171 methods of measuring volume of each water phase were not applicable to this system. Relaxation  
172 rates ( $1/T_2$ ) of single polymer gels and mixed gels were used to approximate the proportional water  
173 binding by shifts in the composite relaxation rates. Addition of 1% HA gellan caused a shift along the  
174 entire curve approximately equal to 10% MD, and thus a 1% HA gellan to 10% MD equivalence point  
175 was estimated (Supplement Figure 1). Previous effective concentration measurements for LA gellan  
176 and MD were modelled non-linearly but values were of a similar ratio (Clark et al., 1999). Variations  
177 above and below this ratio were tested in the models, but similar fits to the reported values were  
178 obtained. We acknowledge many assumptions were made from this simplification, and this value  
179 merely represents the closest possible approximation of a value we propose is meaningless in the  
180 mixture. Effective concentration models (Figures 4C and 4D) did not utilize the assumption of phase  
181 volumes. Concentration curves of individual polymers were used to estimate the contribution of  
182 each individual polymer. Polynomial equations from predictive models were written for calculations  
183 of modulus at any effective concentration ( $G_{\text{HAG}}$  and  $G_{\text{MD}}$ ).

## 184 2.5 Scanning Electron Microscopy (SEM)

185 SEM measurements were acquired using a JEOL 6060LV (Tokyo, Japan). Samples were cut into 1.5 x  
186 1.5 mm cubes and suspended 1 cm above liquid nitrogen for one minute and then immersed in  
187 liquid nitrogen for one minute. An aluminium sample stage and carbon tab were cooled via  
188 immersion in liquid nitrogen for one minute. Images were acquired using back scatter electrons  
189 (BSE), a low vacuum of 50 Pa, an accelerating voltage of 10 kV, and a 10 mm working distance.

## 190 2.6 Statistical Analysis

191 Figures show the average of at least three samples and error bars represent the standard deviation.  
192 Statistical lettering was used in Table 2 to distinguish statistically different ( $p < 0.05$ ) samples after  
193 using an ANOVA and all pairwise comparison test with the Student-Newman-Keuls method  
194 (SigmaPlot 12.5). In reference to the discussion of Figure 8 and Table 1, the claim of “no change with  
195 time” was based on a comparison of means for each concentration of MD and a p-value of 0.01 to  
196 account for the number of pairwise comparisons.

## 197 3. Results and Discussion:

### 198 3.1 Network Characterization

199 Fracture mechanics, lack of continuous phase discontinuity, disagreement with phase-separated  
200 models, cumulative bond energies, and microscopy will be discussed to support the proposition of  
201 an interpenetrating network of HA gellan and MD. Above critical gelling concentrations of both  
202 polymers, a true IPN network was suggested and was consistent with the mechanism proposed for  
203 LA gellan and MD by Clark et al. (1999) whereby the MD aggregated within a porous gellan network  
204 without phase separation. Below the gelling concentration of MD (approximately 20%), a non-  
205 segregative gel was comprised of MD aggregates within a continuous HA gellan network.

#### 206 3.1.1 Material Properties

207 Material properties of the gels were measured by compression of cylindrical shaped samples using a  
208 texture analyzer. The compression curves were measured as force over distance and converted to  
209 true stress and true strain using equations 1 and 2 (Section 2.2) with examples of representative  
210 curves in Figure 1. Arrows point out the fracture point for each sample. Strain at fracture, stress at  
211 fracture, and modulus were observably different by examining the shape of compression profiles  
212 between individual gels of MD and gellan (solid lines). MD alone at a concentration of 30% formed a  
213 firm gel with a large modulus and showed brittle fracture at 0.13 true strain (Figure 1). Conversely,  
214 the 1% HA gellan was soft and flexible with fracture only at 0.56 true strain. The composite gels  
215 resulted in intermediate strain at fracture and modulus (Figure 1). For the composite gels, only one  
216 fracture point was observed.

217 Fracture points of the composite material and the contribution of polymer concentrations are  
218 displayed in Figure 2. HA gellan gum was very flexible with high strains to fracture (0.49 to 0.59)  
219 while MD alone fractured at low strains (0.14-0.18). Mixtures showed fracture points varying  
220 between the two individual polymer values. A common indication of a phase separated gel is a large  
221 shift in behaviour (discontinuous) from switching between continuous phases (Norton and Firth,  
222 2001). A phase separated system would be expected to show strain values determined by the  
223 continuous polymer (with some modification from the interface) and a large shift when the  
224 continuous phase changed (Norton and Frith, 2001). This was not observed and the incremental  
225 change in fracture strain of the composite gel (non-discontinuous) suggests there is not bulk phase  
226 separation. Increasing levels of MD also increased the Young's Modulus of the mixed gel (Figure 3).  
227 All samples of HA gellan without the inclusion of MD resulted in comparatively low moduli (2500 –  
228 5000 Pa). The curve shapes of the Young's Modulus of mixed gel closely followed the MD curve (□)  
229 with a small increase from greater HA gellan concentrations (Figure 3). HA gellan had a much smaller  
230 modulus than MD, by one to two orders of magnitude, so the minimal contribution was not  
231 unexpected.

232 MD independently did not form a gel below 20%, and at 15% the solution was still pourable with  
233 visibly increased light scattering from chain aggregation (Kasapis et al., 1993b; Loret et al., 2004;  
234 Kanyuck et al., 2019). Below the gelling concentration, aggregates are proposed to be adding  
235 inhomogeneity to the HA gellan network as well as overall rigidity to the composite by acting as filler  
236 particles. The formation of aggregated regions of MD within a rearranged HA gellan network are  
237 consistent with the deswelling and "elongated configurations" proposed when co-gelling with LA  
238 gellan (Kasapis et al., 1999). Below the gelation point of MD, the decreased strain to failure is  
239 suggested to have originated from inhomogeneities in the HA gellan network created by the MD. For  
240 most food gels, the fracture point occurs when the applied force becomes greater than the cohesive  
241 bonds and the concentration of polymer does not actually effect the strain at fracture (Zhang et al.,  
242 2005). The failure point is expected to be characterized by the microstructure defects and energy  
243 dissipation mechanism, which initiate and propagate fracture respectively (Van Vliet and Walstra,  
244 1995). Aggregation of MD into large dense regions within the HA gellan network is feasible to have  
245 created cracks or inhomogeneities. This aggregation is proposed to modify the loose HA gellan  
246 network into less flexible regions. These heterogeneities within the network act as cracks and focus  
247 the stresses to cause a more brittle fracture (Van Vliet and Walstra, 1995). Above the gelling  
248 concentration (20%) which coincides with the largest changes in strain, MD is proposed to reach a  
249 critical number of aggregates to form a second continuous network. Only at this point is the mixed  
250 gel network hypothesized to create two interpenetrating networks. Below the gelation point of MD  
251 (20%), MD appeared to act as a filler particle without formation of a secondary gel network. Bulky  
252 aggregates appeared to have sterically hindered compression by decreasing mobility of the HA  
253 gellan network in the mixed gel.

### 254 3.1.2. Microstructure Analysis

255 Theoretical models can be utilized to analyse the relationship between microstructure and function.  
256 Phase separated biopolymers have commonly been understood by modelling individual  
257 contributions to isostrain and isostress models by considering effective concentrations and assuming  
258 complete phase separation. A phase separated system is suggested when predicted values from  
259 these models are similar to the measured values, although minor deviation is always expected  
260 (Kasapis, 2008). Utilizing the models refined by Morris (1992), the equations were modelled for  
261 0.5%, 1% and 1.5% HA gellan with increasing concentrations of MD (Figure 4). Even without phase  
262 separation, changes in effective concentrations of each polymer should be expected (Clark et al.,  
263 1999). All estimates have been modified to effective concentrations (considered a percentage of the  
264 water mass in the sample) to account for the high solid content of MD, comparable to the method of  
265 Manoj et al. (1996). True Isostrain and isostress predications are shown in Figure 4A, 4B, and 4C  
266 which takes into account the effective concentrations after an assumed phase separation of the  
267 polymers. Alternatively, Figure 4D, 4E, and 4F only adjusts the concentration based on the total  
268 water within a sample and assumes no liquid-liquid demixing. Both models utilize the in-series  
269 model (isostress) and the in-parallel (isostrain) models for the contribution of each polymer  
270 (equations 4 and 5). Other concentrations of HA gellan were also modelled and showed equivalent  
271 trends and quality of fit and thus are not included for redundancy.

272 The phase-separated models (Figure 4A-C) were not a good fit for the observed composite moduli.  
273 At most concentrations of MD (below 25%), the measured modulus was weaker than predicted.  
274 When liquid-liquid mixing occurs, higher effective concentrations of each polymer occur in both  
275 phases. In this system, no indication of phase separation by increases in modulus from higher  
276 effective concentrations was observed. Additionally, at higher MD concentrations neither phase-  
277 separating model could predict the measured composite modulus effectively. The prediction from  
278 the non-phase separating models (Figure 4D-F) were closer to the measured values. At low  
279 concentrations (0-10% MD), none of the models predict a deviation far from that of HA gellan alone  
280 and was consistent with measured values (Figure 4D-F). The rise in modulus with increasing MD was  
281 best accounted in the single phase isostrain model, which is consistent with the compositional  
282 theory of two networks spanning the length of the gel (Ross-Murphy, 1995). Similarity of models in  
283 Figure 4D-F to the measured values suggested a lack of phase separation which could be consistent  
284 with either an IPN or coupled network.

285 Glucose-based polymers of various sizes were substituted at equivalent concentrations with 1% HA  
286 gellan in an attempt to separate the aggregation (solid filler) and solvent quality effects of MD from  
287 the network effects. Material properties of these gels are summarized in Table 1. The DE 2 MD is the  
288 one used throughout this paper. The DE 10 MD is also from potato and further hydrolysed so that it  
289 can form aggregates but is unable to form a gel network. The modified MD (with increased  
290 branching) and glucose are unable to form aggregates or a gel network. Comparison of these three  
291 analogues to the DE 2 MD allowed for determination of the influence of network formation  
292 specifically. Changes in solvent quality or water of hydration (mimicked by glucose or the modified  
293 MD) only showed a small (less than 10%) change in composite modulus at fracture strain and  
294 highlighted the importance of DE 2 gelation on composite fracture and modulus (an increase of  
295 6,000 %). The contribution of MD to the mixed gel originates in network structure formation beyond  
296 just a small influence from changes to the solvent. Fracture strain was only decreased with the DE 2  
297 and DE 10 MDs (Table 1) which both form aggregates (Kasapis et al., 1993b). The DE 10 MD is able to  
298 form aggregates but is thought to not have enough long chains to gel, although the composite  
299 flexibility was still decreased. Aggregation of these MDs can also be seen in the time dependence (a



300 change in modulus over 14 days, which is discussed in section 3.2) while no effect was seen for the  
 301 modified MD nor the glucose. In these mixed gels, aggregates appear to have created enough  
 302 structural inhomogeneities in the HA gellan to increase brittleness of the composite gel. A network  
 303 formation by the DE 2 MD was indicated by the increase large increase in modulus that not even the  
 304 non-aggregating MD exhibited.

305

306 Table 1. Comparison of material properties of 1% HA gellan gels with 30% carbohydrate additives.  
 307 Time dependence refers to a significant change ( $p < 0.05$ ) in Young's Modulus or true strain at  
 308 fracture over the period of 1 and 14 days.

<b>Carbohydrate Type</b>	<b>Young's Modulus (Pa)</b>	<b>Strain at Fracture</b>	<b>Stress at Fracture (kPa)</b>	<b>Time Dependence</b>
MD DE 2	235,707 ± 42,000	0.32 ± 0.01	122 ± 3	Yes
MD DE 10	3,300 ± 200	0.47 ± 0.01	25 ± 2	Yes
Modified MD (non-aggregating)	3,300 ± 250	0.57 ± 0.01	207 ± 16	No
Glucose	3,600 ± 90	0.57 ± 0.01	184 ± 11	No
No Additive (1.5%)	3,870 ± 82	0.58 ± 0.01	283 ± 29	No
No Additive (1.0%)	3,200 ± 100	0.56 ± 0.01	114 ± 18	No

309

310 Gel networks were further compared by differential scanning calorimetry (DSC) to check for  
 311 associations between polymers indicative of a coupled network. Heat flow of gels melting (A) and  
 312 cooling (B) are shown in Figure 5. The range of melting temperatures (between peak onset and  
 313 offset) from both polymers was quite broad and has been attributed to low cooperation between  
 314 aggregates (Morris et al., 1996; Mazen et al., 1999). Individually, the 1% HA gellan showed gelling  
 315 and melting temperatures of 63 °C and 60 °C respectively (Figure 5) and was consistent with the  
 316 small hysteresis usually reported because of the minimal aggregation of helices (Morris et al., 1996;  
 317 Kasapis et al., 1999; Mazen et al., 1999; Morris et al., 2012). Heating curves for MD showed a broad  
 318 breaking of the network during melting, but no observable bonds were measured during the cooling  
 319 process (Figure 5B). Although unusual for most hydrocolloids, this is consistent with the long gelling  
 320 time of multiple days for MD (Loret et al., 2004; Kanyuck et al., 2019). The slight upward movement  
 321 of the MD curve approaching 25 °C could be from the beginning of detection of MD aggregation.  
 322 There was indication that some bonds slowly reformed evidenced by the melting enthalpy of 27% of  
 323 the original during the second heating cycle (Table 2).

324 Melting endotherms and enthalpy were additive without indication of increased enthalpy or bonds  
 325 between polymers. Enthalpy values were not different than a pure summation of the two polymers  
 326 (Table 2) and no new bonds were suggested by new peaks at different melting temperatures  
 327 (Kasapis, 2008). The first melting cycle includes both MD aggregates and HA gellan gel helices  
 328 melting as samples were held for 4 days prior to analysis. In cooling, only the HA gellan was observed  
 329 to gel within the experimental timeframe (which is consistent with the proposed mechanism of slow  
 330 MD gelation). A slight increase in gelling temperature between HA gellan with or without MD can be  
 331 attributed to an increase in effective concentration of the HA gellan component. Due to the 20% MD  
 332 in the formation, less water resulted in an effective concentration of 1.25% which was consistent  
 333 with a melting temperature of 66 °C (Supplementary Figure 2). The enthalpy respective to gelation  
 334 of the HA gellan was unchanged by the MD ( $p > 0.05$ ). Additive bond energies were consistent with an  
 335 IPN or phase separated gel, and inconsistent with a coupled network. Preceding analysis has  
 336 eliminated the phase separated model and jointly the results are only consistent with an IPN.

337 Table 2. Calculated enthalpy values (J/g) from DSC heating and cooling cycles based on grams of  
 338 total sample. Values are reported as the average with one standard deviation for three replicates.  
 339 Different letters indicate significantly different sample means.

340

	1 <sup>st</sup> Cycle (4 day gelation)		2 <sup>nd</sup> Cycle	
	Heating	Cooling	Heating	Cooling
HA Gellan (1%)	0.13 <sup>A</sup> ± 0.04	0.12 <sup>A</sup> ± 0.04	0.10 <sup>A</sup> ± 0.01	0.09 <sup>A</sup> ± 0.01
MD (20%)	1.74 <sup>D</sup> ± 0.09	**	0.47 <sup>B</sup> ± 0.07	**
Measured HA Gellan (1%) + MD (20%)	1.92 <sup>E</sup> ± 0.16	0.12 <sup>A</sup> ± 0.01	0.59 <sup>C</sup> ± 0.12	0.13 <sup>A</sup> ± 0.01
Summation of HA Gellan (1%) + MD (20%)	1.87	0.12	0.57	0.09

341 \*\* peaks could not be resolved from baseline

342 Microscope images of a 30% MD gel and a mixed gel of 25% MD and 1% HA gellan are shown in  
343 Figure 6. Images were representative of the entire gel that was viewed and no signs of macroscopic  
344 phase separation were observed. It was not possible to image the HA gellan component due to  
345 visible cracking from evaporating/melting at low magnification in a 1% HA gellan sample.  
346 Additionally, the lack of aggregation of double helix strands means the gel structure would be very  
347 fine and therefore difficult to observe. MD gelation occurs through the formation of large crystalline  
348 regions of aggregated double helices (Chronakis, 1998). The gelation mechanism can appear as  
349 phase separated with a microscopy technique, but the lack of demixing prior to gelation excludes  
350 the applicability of phase-separated descriptions (Clark et al., 1999). TEM images of MD aggregate  
351 networks have shown microspheres of 20-30  $\mu\text{m}$  (Schierbaum et al., 1984) made of smaller comb-  
352 like structures of 1-4 nm (Schierbaum et al., 1984; Clark et al., 1999; Kanyuck et al., 2019). Evidence  
353 of the smaller aggregates were seen in gels with or without HA gellan (Figure 6A and 6B). The  
354 arrangement of the microcrystalite aggregates showed differences with the presence of HA gellan,  
355 but continuous networks were observed in each. Smaller and more regular microcrystalites were  
356 observed in the mixed gel, while larger rod-like structures were formed of the aggregates for MD  
357 alone (Figure 6C and 6D). As the MD aggregated within the pores of the HA gellan network, it  
358 appears that formation of very large aggregates were prevented. This is also consistent with the  
359 images of MD with LA gellan observed by Clark et al. (1999). Although the HA gellan strands could  
360 not be seen, the modification to the MD network were consistent with an interpenetrating network.

### 361 3.2. Polymer Contributions

362 Compression profiles and microstructure analysis have suggested interpenetrating networks. The  
363 presence of two continuous networks naturally leads to a questioning of the specific contribution of  
364 each polymer. Utilization of the time dependence of MD aggregation allowed measurement of the  
365 direct contribution of this aggregation. The small deformation behaviour of the composite appeared  
366 most similar to the MD while the HA gellan network appeared dominant in the fracture. This was  
367 consistent with the relative magnitudes of individual properties. Composite material properties were  
368 not simply additive and showed an interaction between components during network formation. The  
369 presence of another polymer was proposed to affect the distribution and microstructure of each  
370 respective network but not the actual chemistry of the network (consistent with no changes in the  
371 enthalpy, Table 2).

372 Previous experiments (Figure 3) showed the concentration dependence of the composite Young's  
373 Modulus to be more strongly effected by MD than HA gellan. Considering the modulus of individual  
374 components, this was not surprising as the modulus of MD is almost a hundred times higher than  
375 that of HA gellan. It was of interest to understand the effects of MD aggregation on the composite  
376 modulus. A unique behaviour of low DE MDs is increasing gel strength, following a logarithmic  
377 function, over the time frame of weeks, (Loret et al., 2004; Kanyuck et al., 2019) and shown by in  
378 Figure 7. For gels containing MD at concentrations above (30 and 25%) and below (20, 15, and 10%)  
379 the gelation point, an increase in Young's Modulus followed a natural log function (Figure 7). HA  
380 gellan alone had the lowest Young's Modulus and was unchanged over the measured time period of  
381 1 to 14 days. Lack of change in the HA gellan sample and the characteristic natural log curve shape  
382 suggested the increase was caused by aggregation of MD. Thus, the direct influence of MD  
383 aggregation was able to be observed. MD concentrations below the critical gelation level resulted in  
384 a modulus at an order of magnitude lower. However, both above and below the expected gelation  
385 point of MD the aggregation kinetics were the same (natural log curve shape). Aggregates of MD  
386 thus influenced the composite gel firmness even at points when a true network had not formed. As  
387 initially proposed in section 3.1.1, the aggregates added bulk and resistance to deformation. At

388 concentrations sufficient to form a MD gel network, MD is the major contributor to the resistance in  
389 small deformations as seen from the similarity in modulus with or without HA gellan (Figure 7 and  
390 Figure 3).

391 While MD aggregation caused an increase in the Young's Modulus of the composite gel, the strain  
392 and stresses at fracture exhibited behaviour more similar to HA gellan. The next experiment  
393 examined the change in fracture strain by the aggregation of MD through utilization of the time-  
394 dependence discussed previously. Concentrations of each polymer expected to form two continuous  
395 interpenetrating networks were selected for this study. The fracture of the composite is caused by  
396 breakage of the HA gellan network, while the MD is thought to modify the stress propagation  
397 (section 3.1). At the composite fracture point the MD network had already fractured. A decrease in  
398 the strain at fracture for all three concentrations of HA gellan tested corresponded to an increasingly  
399 brittle gel with aggregation of MD (Figure 8). For comparison, a 30% MD alone is also shown with  
400 fracture at a low strain (0.14 true strain). Alone a 1% HA gellan did not fracture until 0.56 true strain  
401 (Figure 2). The shifting in strain at fracture between these two extremes is proposed as an indication  
402 of inhomogeneities forming and growing in the HA gellan network by aggregation of MD. The stress  
403 at fracture was not changed ( $p > 0.01$ ) over the 14 days (data not included). Decreasing in fracture  
404 strain was indicative of the changing distribution of HA gellan aggregates and consistent with the  
405 proposed mechanism of network heterogeneities (distribution of polymer chains and junction zone  
406 size) (Van Vliet and Walstra, 1995) and microscope images (Figure 6). As the MD aggregates grew  
407 larger, the HA gellan bends to allow the large and bulky aggregates which decreased stress  
408 propagation during compression. The influence on composite fracture also supports the proposal of  
409 MD aggregating within the pores of the HA gellan network without phase separation (IPN model). If  
410 phase separation had occurred, a continued aggregation within an excluded MD phase should not  
411 have an impact on the continuous HA gellan phase.

412 Due to the great difference in individual properties, each polymer was a major contributor to  
413 different composite properties. With a brittle gel and large Young's Modulus from many dense  
414 aggregates, the MD dominated the small deformation behaviour. The flexible and soft network from  
415 HA gellan dominated the fracture behaviour of the composite. Importantly, each polymer impacted  
416 the distribution (microstructure) of the other but not the chemistry of associations. The aggregation  
417 of MD made the composite stronger from the bulky aggregates and also more brittle by introducing  
418 heterogeneities into the HA gellan network.

### 419 3.3. Gelation Mechanism

420 The following mechanism is proposed for development of the interpenetrated networks and is  
421 illustrated in Figure 9. Initially upon cooling, HA gellan forms a network with negligible impacts from  
422 the presence of MD in solution. As the gelation is very quick, the HA gellan network has been set  
423 prior to aggregation of MD (which is consistent with DSC cooling curves). As the slow gelation of MD  
424 begins, aggregates form within pores of the HA gellan network. Growth of these regions condenses  
425 and introduces structural defects in the formerly loose and flexible HA gellan chains. Formation of  
426 these MD aggregates within the pores of HA gellan inhibits the growth of very large microcrystallites,  
427 but not the smaller helix aggregate structure. The distribution of the gellan network was also  
428 impacted by the MD, while on a chemical level the helix formations and aggregations remained  
429 unchanged (DSC confirmed the enthalpy stays the same for both polymers). Aggregates of the MD  
430 network contribute a resistance to deformation to the flexible HA gellan network. The more flexible  
431 HA gellan network was responsible for the ultimate fracture of the gel. Both polymers thus  
432 contribute to the composite physical properties and complex interactions between the networks  
433 resulted in a broad range of physical properties.

434 Formation of this IPN is likely caused by an associative interaction from a thermodynamic  
435 unfavourability of separation. Self-association of gellan would need to overcome the charge  
436 repulsion of the carboxylic acid groups and entropy of counterions. Concentrating similar charges  
437 into one phase is thermodynamically unfavourable and the charge repulsion theory has been  
438 proposed for other interpenetrating networks (Amici 2002). In similar cases with one ionic polymer,  
439 single phase mixtures are favoured from the higher entropy of counterions within a concentrated  
440 system (Piculell et al., 1995). The IPN suggested between MD and LA gellan supports the charge  
441 repulsion theory also occurs for the HA variant (Clark et al., 1999). It is possible that gelation of HA  
442 gellan could kinetically trap the mixed system before separation, but no indication of segregative  
443 interactions was observed in this work. Many phase separated polymers are thought to have a  
444 thermodynamic equilibrium in complete phase separation, but the gelation kinetically traps into  
445 smaller regions of separation (Kasapis et al., 1993c; Lorén et al., 1999). Future work should  
446 investigate whether segregative interactions could occur in other regimes such as controlling the  
447 gelling rates or effects of salt or pH to neutralize charge repulsion and would allow a better  
448 understanding of mechanism, but is outside the scope of this paper.

#### 449 **4. Conclusions:**

450 An interpenetrating gel network of MD and HA gellan has been characterized. The mixed gel showed  
451 complex interactions between the polymers based on modification to microstructural distribution  
452 and crack propagation to fracture. Opposite textures of individual polymers have allowed an  
453 understanding of the contribution of each through analysis of the composite properties. Through  
454 microstructure modification, material properties of the composite gel shifted over a continuous  
455 range from the firm and brittle MD to the soft and flexible HA gellan. The time-influence of MD  
456 aggregation was shown to increase the modulus of the composite, but not change the fracture  
457 stress. MD aggregation showed signs of increasing brittleness through introduction of stiffness and  
458 inhomogeneities to the HA gellan network. The quick gelation and charge repulsion of HA gellan  
459 provide a thermodynamic justification for an associative interaction. The Young's Modulus,  
460 appearing almost entirely dependent on the MD contribution, supports previous claims of the  
461 limited utility of small deformation to predict texture of mixed biopolymer gels (Van den Berg et al.,  
462 2007). Small deformations and bulk fracture were needed to describe the physical properties of this  
463 IPN gel. Characterization of mixed gels from these polymers have brought a greater understanding of  
464 the physical property to microstructure relationships for multicomponent systems.

465  
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#### 468 **CRedit authorship contribution statement.**

469 **Kelsey M. Kanyuck:** Conceptualization, Methodology, Formal analysis, Investigation, Writing -  
470 original draft. **Tom B. Mills:** Supervision, Writing - review & editing. **Abigail B. Norton-**  
471 **Welch:** Supervision, Funding acquisition, Writing - review & editing. **Ian T. Norton:** Supervision,  
472 Funding acquisition, Writing - review & editing.

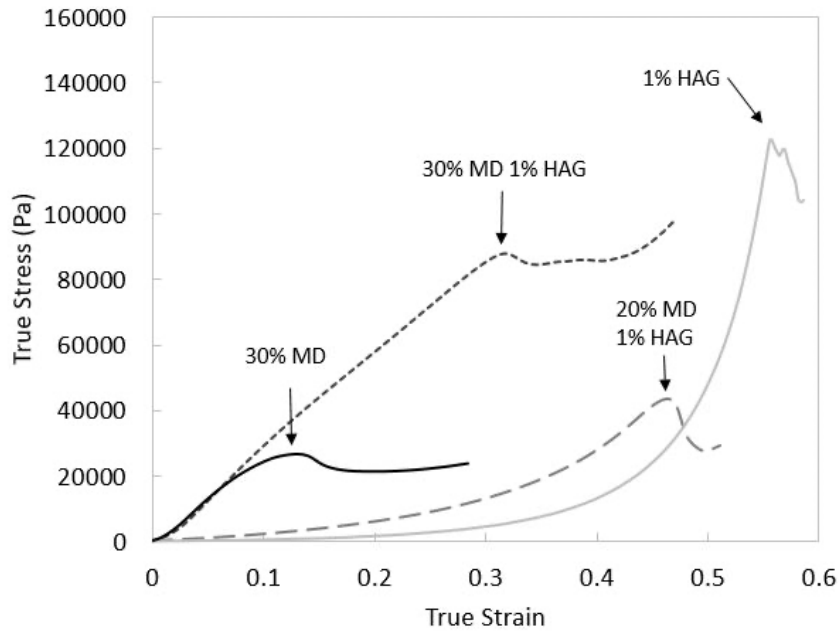
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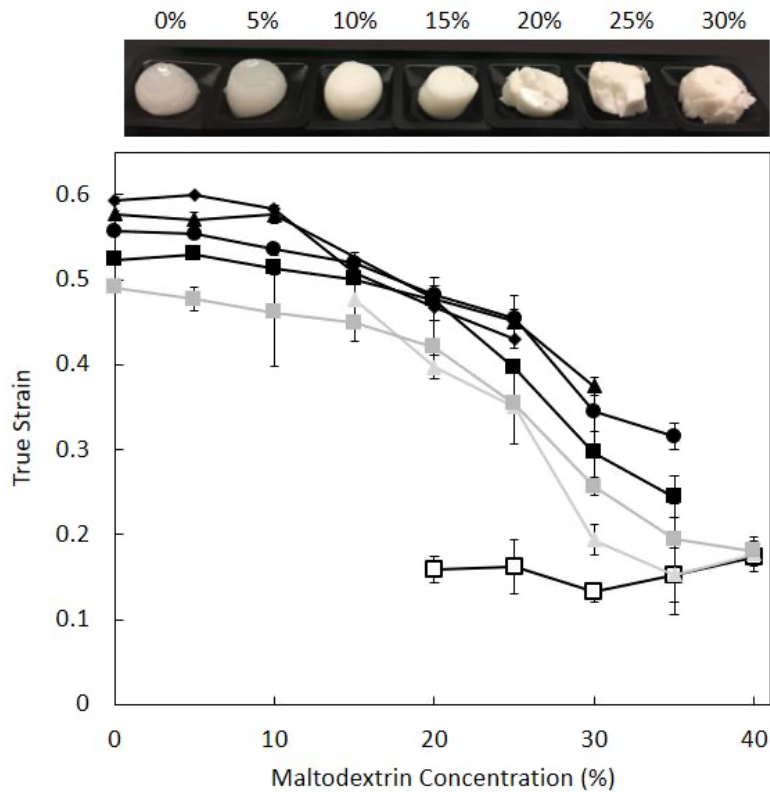
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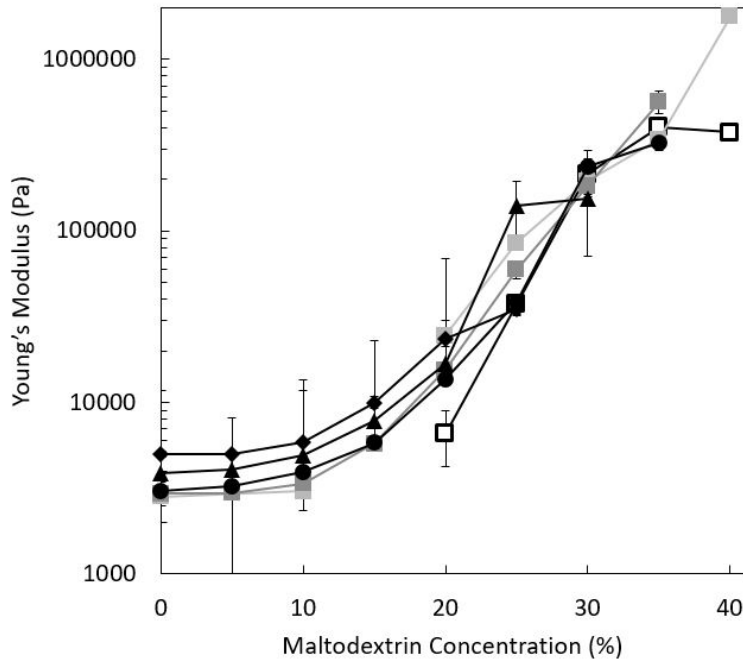
591 Figure 1. Compression curves for 30% MD and 1% HA gellan (HAG) (- - -) with comparison to 30%  
 592 MD (—), 1% HA gellan (—), 20% MD and 1 % HA gellan (HAG) (—) using calculated true stress and  
 593 true strain values. Arrows show the points of fracture for each gel.

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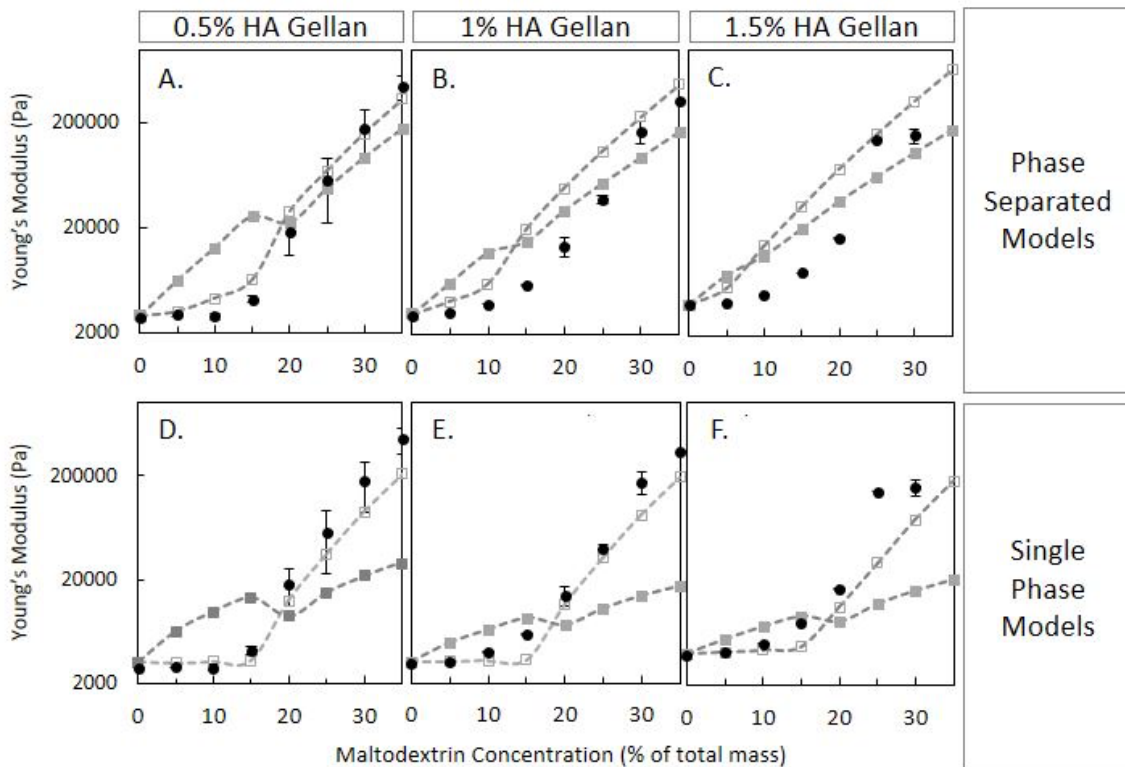
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596 Figure 2. Comparison of the strain at failure for increasing levels of MD with HA gellan  
 597 concentrations of 0 % (□), 0.25% (▲), 0.5% (■), 0.75% (■), 1% (●), 1.5% (▲), and 2% (◆). Images  
 598 show the appearance of 1% HA gellan with maltodextrin after compression.



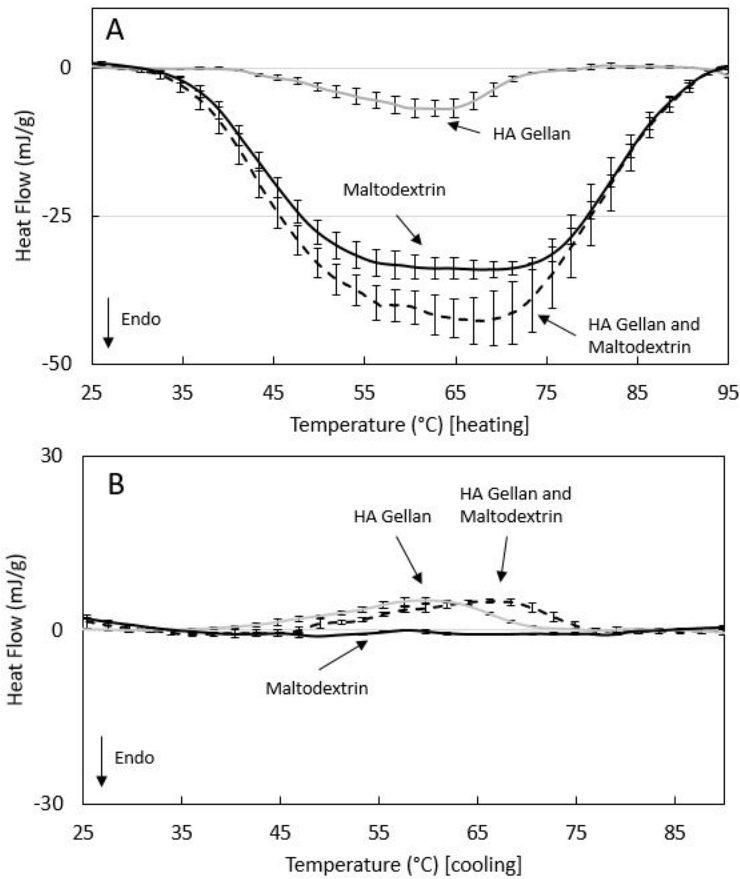
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600 Figure 3. Small deformation mechanics before failure as indicated by the Young's Modulus for HA  
 601 gellan concentrations from 0 % (□), 0.25% (▲), 0.5% (■), 0.75% (■), 1% (●), 1.5% (▲), and 2% (◆) at  
 602 increasing levels of MD.



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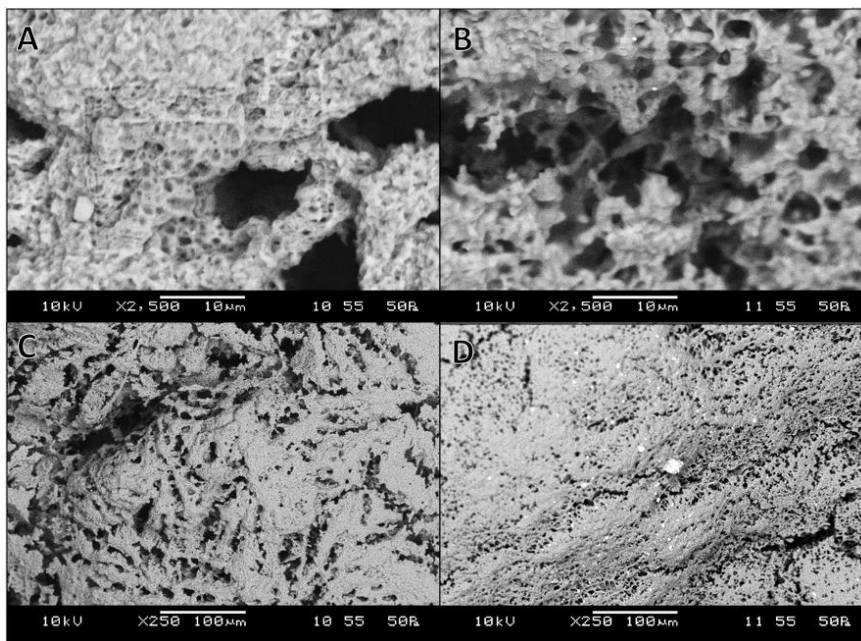
604 Figure 4. Comparison of measured network modulus of (A) 0.5% HA gellan, (B) 1% HA gellan and (C)  
 605 1.5% HA gellan with added MD (●) to calculated moduli from the isostrain (□) and isostress (■)  
 606 phase separation models and (D) 0.5% HA gellan, (E) 1% HA gellan and (F) 1.5% HA gellan for models  
 607 utilizing one phase concentrations.



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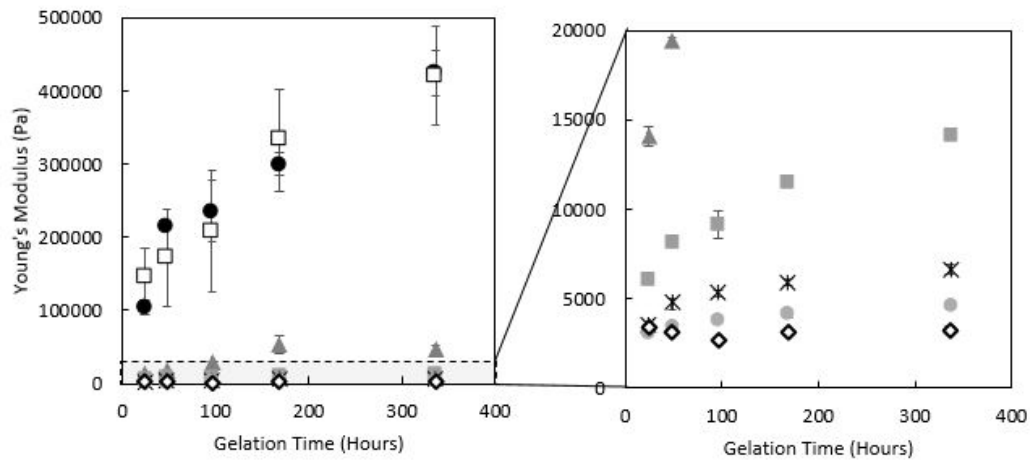
609 Figure 5. DSC heating (A) and cooling (B) curves for gels of 1% HA Gellan (—), 20% MD (---), and 1%  
 610 HA Gellan with 20% MD (- - -) after four days of gelation. After baseline subtraction, the error bars  
 611 represent a standard deviation of triplicate samples.

612



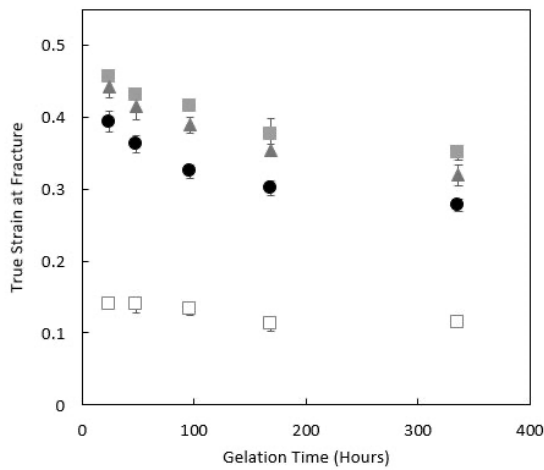
613

614 Figure 6. SEM images of 30% MD (A and C) and 1% HA gellan with 25% MD (B and D).



615

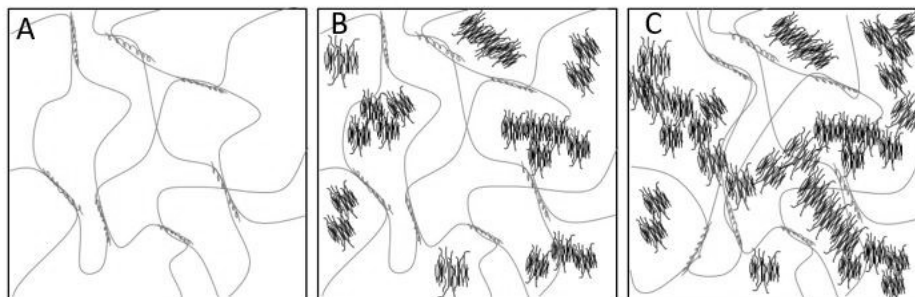
616 Figure 7. Contribution of MD aggregation to the composite Young's Modulus at 0% (◇), 10% (●),  
 617 15% (X), 20% (■), 25% (▲), and of 30% (●) to a 1% HA Gellan network and 30% MD without HA  
 618 gellan (□) from 24 hours to 14 days.



619

620 Figure 8. Contribution of MD aggregation to the true strain at fracture for mixed gels with 30% MD  
 621 and 1% (●), 0.75% (■), 0.5% (▲), and 0% (□) HA gellan from 24 hours to 14 days.

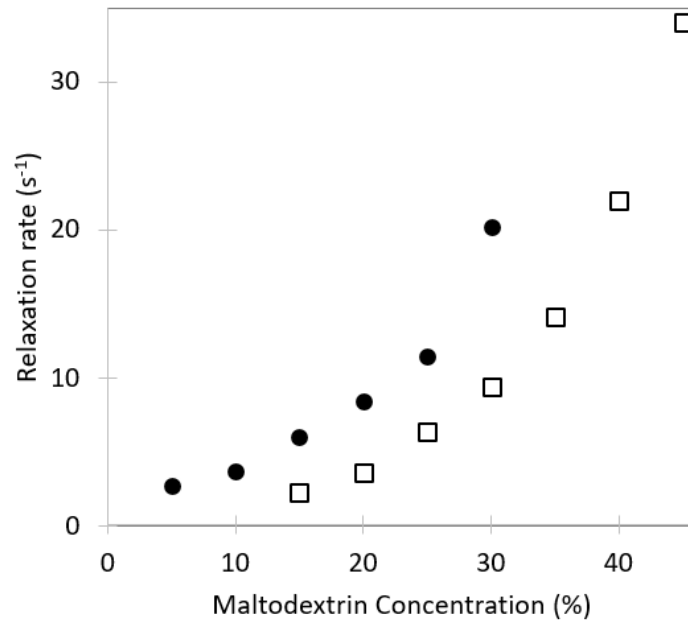
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623

624 Figure 9. Proposed schematic for HA gellan network alone (A) and contributing to an  
 625 interpenetrating network with MD (aggregates) below (B) and above (C) the critical gelation  
 626 concentrations of MD.

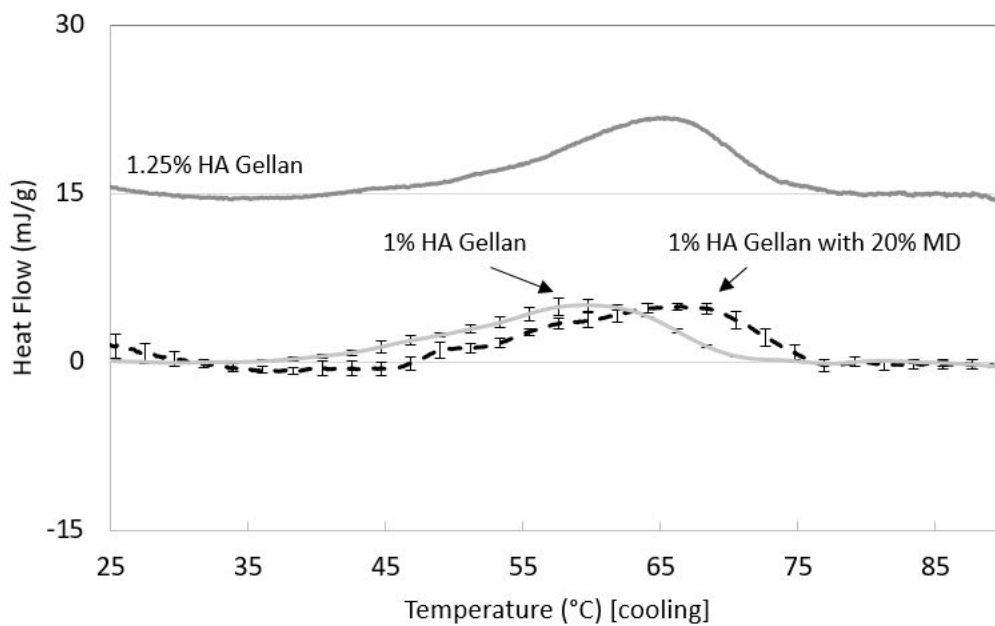
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628

629 Supplementary Figure 1. NMR relaxation rates ( $1/T_2$ ) used to estimate phase separation volumes for  
 630 MD (□), MD with 1% HA gellan (●). A Bruker mq20 minispec benchtop NMR was used for the  
 631 measurement with a CPMG pulse sequence and tau of 0.25 ms.

632



633

634 Supplementary Figure 2. DSC curves comparing melting temperature of 1% HA gellan gum with and  
 635 without 20% MD (from Figure 5) to 1.25% HA gellan gum.