Optimisation of a Novel Glass-Alginate Hydrogel for the Treatment of Intracranial Aneurysms

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Abstract

The current gold standard for aneurysm treatment is endovascular coiling. However, recurrence is observed in over 20% of cases. A novel hydrogel has been developed to treat aneurysms. This hydrogel is composed of a polymeric alginate, a novel ion releasing glass and glucono-delta-lactone. This is an internally setting alginate hydrogel, wherein the setting rate can be controlled by both the glass and the alginate chemistry. The aim of this work is to examine the effect of each component of the hydrogel and optimise the composition of the hydrogel, specifically the alginate molecular weight, M/G ratio and concentration. The effects of gamma sterilisation will also be examined. The results show that alginate concentration, chemical composition and molecular weight affect the compressive strength, working time, hardening time and deliverability of the hydrogel. Gamma irradiation of the alginate reduces the molecular weight, which has a negative effect on the usability of this hydrogel.

Keywords

Alginate, molecular weight, chemical composition, concentration, hydrogel, intracranial aneurysm

1. Introduction

An intracranial aneurysm is an irregular out-pouching of a cerebral artery. It is estimated that 1% to 6% of the adult population has an intracranial aneurysm (Brisman, Song, & Newell, 2006). A ruptured aneurysm can lead to stroke, resulting in disability or death. Treatments such as clipping and coiling are currently used to prevent an aneurysm from rupturing; however, there are a number of problems associated with these. Clipping the aneurysm involves a craniotomy and carries the risks of infection and scarring (Brisman et al., 2006). In the United States, the most common treatment method for intracranial aneurysms is coiling. However, recurrence is common, happening in 20.8% of endovascular coiling cases (Crobeddu, Lanzino, Kallmes, & Cloft, 2012), indicating that it is a suboptimal treatment method.

In this study we explore the possibility of delivering a hydrogel which will fill the aneurysm more completely and prevent rupture. This novel hydrogel composite is composed of a polymeric alginate, a novel ion releasing glass and glucono-delta-lactone (GDL).

Ideally, the hydrogel should adhere to the aneurysm wall preventing migration and aneurysm recurrence. Additionally, the hydrogel must be able to withstand a compressive stress of at least 22kPa, which relates to hypertensive blood pressure (Cipolla MJ, 2009). The envisaged hydrogel delivery procedure will involve inflating a compliant balloon adjacent to the aneurysm neck, as with endovascular coiling. The novel hydrogel will then be injected through a micro-catheter into the aneurysm whilst the inflated balloon prevents leakage of the filler into the blood stream (see Figure S 1). To allow for delivery, an optimum working time of between 10 and 30 minutes has been determined by clinical observation. It has been determined that the novel hydrogel must be set within 5 minutes of injection (based on the maximum inflation time for a balloon in the cerebral vasculature. A balloon inflated for greater than 5 minutes may result in cerebral ischemia (Kim Nelson & Levy, 2001). The alginate sterility must also be considered and this can ordinarily be achieved by moist heat sterilization, gamma-irradiation or ethylene oxide sterilization (Munarin, Bozzini, Visai, Tanzi, & Petrini, 2013).

Alginate is a polysaccharide composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G), giving alginate an M/G block structure. Alginate has the ability to gel when cross-linked with multivalent ions. Alginate is typically described in terms of molecular weight and the M/G ratio. G-rich alginates are stiffer and more brittle than M-rich alginates (Morais et al., 2013).

The role of the glass in this hydrogel is to deliver a steady release of multivalent ions, controlling the rate of gelation and the strength of the hydrogel. The more stable the glass the slower the rate of gelation. A glass that releases a higher quantity of ions will form a stronger hydrogel. Bioactive glasses have also traditionally been used to deliver therapeutic ions, which encourage cell growth and extracellular matrix production. This hydrogel formulation could also, in the future, be used to deliver therapeutic ion doses.

GDL is a lactone that hydrolyses in water to form a gluconic acid, its role in the novel hydrogel is to acidify the solution. This in turn releases multivalent ions, contained in the glass, allowing them to cross-link with the alginate. The gelation rate of the hydrogel can be tightly controlled by both the composition of the glass phase and the ratio of constituent components of the gel. An increased amount of GDL results in increased acidity, causing a more rapid glass ion release and a more rapid gelation.

The aim of this work is to individually vary the concentration of two different alginates, with similar viscosities but differing molecular weights and M/G ratios and to examine the effect on the mechanical properties, sample volume conservation, working time, hardening time and deliverability of a hydrogel. We hypothesise that the strength of the hydrogel will increase with increasing alginate concentration but the sample size will reduce over time due to the increased cross-linking density. Although alginate is a Non-Newtonian liquid that undergoes shear thinning, there will likely be a large increase in viscosity with increasing alginate concentration and this will affect the deliverability of the hydrogel. In addition, the effect of a reduction in molecular weight by sterilisation on the most suitable alginate is subsequently examined. Gamma irradiation is known to greatly reduce the molecular weight of alginate and will likely cause a significant reduction in the hydrogel's strength. This will also reduce the viscosity which may help with the deliverability of the hydrogel. Gamma irradiation will be chosen as a possible sterilisation technique for future work, provided there is a positive outcome of these results. This optimisation is designed to improve the novel hydrogel's performance for the treatment of cerebral aneurysms.

2. Material and Methods

2.1. Materials

2.1.1. Alginate Purification

All reagents used were purchased from Sigma-Aldrich (Wicklow, Ireland), unless stated otherwise. Two different techniques were used to produce the two different potassium alginates; a medium viscosity high-G content alginate (MVG) and a medium viscosity high-M content alginate (MVM). The hydrogel was purified from a sodium salt and alginate to a potassium alginate to reduce the endotoxin levels found in the supplied alginate (Dusseault et al., 2006). All acid/base solutions were made up in 20 mmol/L of NaCl in deionised water (DI), unless stated otherwise.

MVG was produced by dissolving 8g of a sodium salt from brown algae in 400 mL of DI. The alginate pH was raised to 7.0 by adding a 0.5 M potassium hydroxide (KOH). The alginate was then precipitated by adding 200 mL of methanol per 100 mL of alginate. The alginate was filtered through a 500 µm sieve after 10 minutes. The alginate was then freeze-dried.

MVM was produced by dissolving 9 g of sodium alginate was dissolved in 900 mL of 1 mmol/L sodium EGTA. The solution was then filtered through 11 µm and 2.5 µm filter paper respectively. The alginate was then precipitated on ice by reducing the pH to 1.5 using a 2 M hydrochloric acid (HCl). The alginate was decanted through a 500 µm stainless sieve and stirred 30 minutes in 200 mL of a 0.01 M HCl solution and decanted again. This stirring and decanting was repeated three times. To remove proteins the alginate was stirred for 30 minutes in 100 mL of a 0.01 M HCl solution with 20 mL of chloroform and 5 mL of 1-butanol, and collected in a 500 µm stainless steel sieve. This washing and collecting was repeated three times. 350 mL of DI was added and the pH was raised to 7.0 by adding a 0.5 M KOH. The alginate was stirred in a solution of 20 mL chloroform and 5 mL of 1-butanol per 100 mL of alginate and centrifuged at a rate of 5,000 rpm for 5 minutes. The alginate was then separated using a pipette from the chloroform/1-butanol solution. This washing and centrifuging was repeated once. Finally, the alginate was precipitated by adding 200 mL of ethanol per 100 mL of alginate and filtered after 10 minutes. The alginate was then freeze-dried.

A sample of the porous freeze-dried solid MVM alginate was gamma irradiated at Synergy Healthcare (Westport, Ireland) by exposing the sample to a cobalt 60 source until a final irradiation dose of 25kGy was achieved.

The required amount of freeze-dried alginate, to make the required alginate concentration, was then added to 12 mL of DI.

2.1.2. Glass

The glass had a mole fraction composition of $0.33SiO_2 \cdot 0.18Ga_2O_3 \cdot 0.23CaO \cdot 0.11P_2O_5 \cdot 0.15CaCl_2$. A glass frit of this composition was produced by melting the appropriate raw materials in a platinum 10% rhodium crucible at 1480 °C for 1 hour. The molten mixture was then shock quenched into water. A glass powder was then produced by grinding 30 g of glass frit using 15 mm diameter zirconia balls in a ball mill (Pulverisette 6 classic Mono planetary ball mill, Fritsch GmbH, Germany) at 500 rpm for 10 minutes. Particles over 500 µm were removed by sieving the glass powder through a 500 µm sieve. 7.5 g of the <500 µm particles were ground in 22.5 mL of DI using 5 mm zirconia balls in a ball mill at 500 rpm for 10 minutes. The glass mixture was dried in the oven at 130 ºC.

2.1.3. Glucono-Delta-Lactone

D-(+)-Gluconic acid δ-lactone was purchased from Sigma Aldrich. The GDL particle size was reduced by grinding 30 g at 500 rpm for 5 minutes using a ball mill. Particle size analysis is shown in Figure S 2.

2.1.4. Hydrogel preparation

The required amount of freeze-dried alginate was dissolved in 1.2 mL of DI. The novel hydrogel was produced by mixing 4.6% of glass powder, 50 mg of GDL with the 1.2 mL of the alginate solution for 1

minute, unless otherwise stated. Design Expert 9 (Stat-Ease, Minneapolis, USA) was used to determine the four alginate concentrations to be tested; 0.5%, 2.5%, 4.5% and 6.0%.

2.2. Methods

2.2.1. Alginate Characterisation

Gel permeation chromatography (GPC) and nuclear magnetic resonance spectroscopy (^{1}H NMR) were carried out as follows in order to characterise the alginates produced.

GPC was carried out using a liquid chromatography system (Agilent 1200, Agilent, USA) equipped with a Suprema Linear GPC column (PSS, Germany). The mobile phase used consisted of 0.1 M disodium hydrogen phosphate containing 0.5 g/L of sodium nitrate buffered to pH 9. All samples were injected at a concentration of 1 mg/mL, at a flow rate of 0.5 mL/min. Pullulan standards were used to construct the calibration curves as alginate standards are not available. This is not an ideal standard as it can overestimate the molecular weight (Andersen, Strand, Formo, Alsberg, & Christensen, 2012). However, they are commonly used in determining molecular weights of alginates with a refractive index detector (Barbetta, Barigelli, & Dentini, 2009)(Ding, Zhou, Zeng, Wang, & Shi, 2017)(Aida, Yamagata, Watanabe, & Smith, 2010).

^{1}H NMR analysis of the potassium alginate was carried out using a modified version of the standard ASTM F2259–03. The alginate solution was prepared by mixing the alginate to a 0.1% (w/v) in DI. HCl was used to bring the alginate pH to 5.6 and the alginate solution was stored in a water bath at 100 °C for 1 hour. HCl was used to further adjust the pH of the alginate to 3.8. The solution was stored again in a water bath at 100°C for 30 minutes. The pH was then raised to 7 using NaOH and the alginate was freeze dried. The alginate was then dissolved in 5 mL of 99% D_2O and freeze dried overnight. 12 mg of alginate was dissolved in 1 mL of D_2O and placed in a NMR tube. The NMR of the alginate was tested using a Bruker Advance 400 (Bruker, Massachusetts, USA) at 80°C. 64 scans were carried out using a 2s relaxation delay. The M-block, G-block and alternating block sequences content were then calculated as per the equations in ASTM F2259-03 using the produced spectrum.

2.2.2. Viscosity

The viscosity of each alginate at varying concentrations was determined at 24ºC using a SV-10 tuning forks Vibro Viscometer (A&D Company, Japan) running a sine wave formation at a constant frequency of 30Hz and amplitude of less than 1 mm. The viscometer measures up to 10,000 mPa.s.

2.2.3. Volume Conservation

To examine whether the 1.2 mL hydrogel volume was conserved over time, the samples volumes were measured after storage in 20 mL of DI for 1, 3 and 7 days. The hydrogel was mixed and poured into a

cylindrical mould (10 mm diameter and 14 mm height). The hydrogel was left to set for 1 hour and incubated in DI at 37°C for the required amount of time. After the required time the hydrogel was removed from the DI and dimensions were measured using callipers. 'Volume conservation' was calculated by calculating the change in volume compared to the original (pre-incubated) volume.

2.2.4. Compression testing

To examine the mechanical properties of the novel hydrogel, compression testing was carried after storing the novel hydrogel for 7 days in DI. The compression testing samples were made as described above. After the required amount of time the hydrogel sample was compressed using a mechanical testing machine (Z005, Zwick Roell, Germany) equipped with a 5 kN load cell. A 0.005 N pre-load was applied. The samples were compressed up to 70% strain at a crosshead speed of 2 mm/min.

2.2.5. Working and Hardening Time

The working and setting time of each alginate was determined using a modified version of ISO 9917. The setting time was found by mixing the hydrogel and placing a circular indenter (diameter: 6 mm, weight: 20 g) on the sample every 60 seconds. The hydrogel was considered set when it held the indenter without causing an indentation in the hydrogel.

The working time was determined by stirring the hydrogel every 1 minute until the hydrogel would not return to its original shape.

The hardening time is defined as the difference between the setting time and working time of the hydrogel.

2.2.6. Deliverability

The deliverability of each alginate was tested by injecting the hydrogel by hand at the varying alginate concentrations through a 3F micro catheter, using a quick stop syringe.

The optimum hydrogel was then injected through a 2.7F micro-catheter into a silicone side wall aneurysm, (neck size of 2.5 x 6 mm, model H+N-S-A-005, Elastrat, Switzerland). A pulsatile blood pump (1423, Harvard Apparatus, Mass., USA) was used to pump a 36:64 water:glycerol solution through the aneurysm and to provide physiologically correct blood pressure (140/80 mmHg) and flow rates (700 mL l/min). The pressure was measured at the aneurysm inlet and outlet using pressure transducers (DTX Plus Disposable Pressure Transducer, Argon) which was amplified using a bridge analogue input (NI9237, National Instrument) and the pressure was monitored using LabView. A 5 mm diameter balloon (Trek, Abbott) was inflated adjacent to the aneurysm neck, to facilitate placement.

2.2.7. Statistical analysis

Student T-tests ($p<0.05$) and ANOVA with Bonferroni's post hoc test was carried out using IBM SPSS (IBM, Armonk, NY).

3. Results

3.1.Effect of alginate molecular weight and chemical composition

3.1.1. Alginate Characterisation

GPC was carried out to determine the molecular weight (MW) of each alginate and ^{1}H NMR spectra of the alginates were used to determine the guluronic acid (F_G), mannuronic acid (F_M) and alternating block (F_{GM}) fractions, which were calculated as per ASTM F2259 – 03.

Table 1 gives the calculated results of the alginates produced; see Figure S 3 and Figure S 4 for graphs. MVG produced a low molecular weight (60kDa) alginate with a high-G content. MVM produced a high molecular weight (700kDa) alginate with a high-M content.

Table 1 *Alginate chemical composition*

Sample	Molecular weight	F_G	F_M	M/G	F_{GG}	F_{MM}	F_{GM}	$\bar{N}_{G>1}$
MVG	60kDa	0.52	0.48	0.92	0.38	0.33	0.14	7.08
MVM	700kDa	0.37	0.63	1.7	0.18	0.45	0.18	3.59

3.1.2. Volume Conservation

The volume of the samples with a low alginate concentration reduced in size, see Figure 1. A 4.5% alginate concentration approximately maintains its size or slightly expands (<5%) whilst the samples at the 6.0% alginate concentration expanded after 7 days.

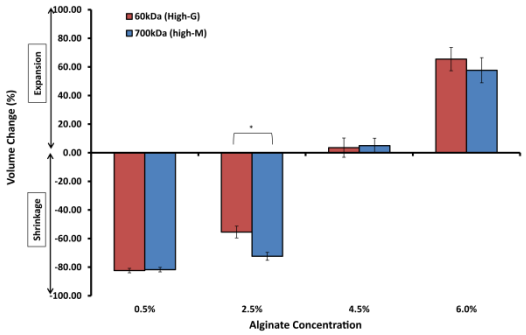

Figure 1 *Volume conservation after storage for 7 days in DI at 37°C (n=5)*

3.1.3. Viscosity

Both alginates have a similar viscosity at each concentration and the viscosity increases with increasing alginate concentration (Figure S 5). Both the alginates have viscosity greater than 10,000 mPa.s at the 6.0% alginate concentration.

3.1.4. Compression testing

Both hydrogel compositions at each concentration far exceed the minimum compressive strength (22 kPa) required.

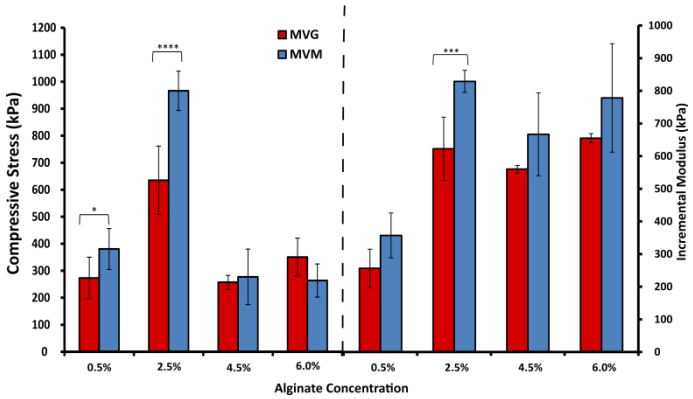

Figure 2 *(a) Compressive stress up to 70% strain and (b) Incremental modulus (30 to 50% strain) of the alginates at the 4 concentrations after storage for 7 days in DI at 37°C*

The strength of the hydrogel was observed to increase from 0.5% to 2.5% alginate concentration. This was expected to continue with increased alginate concentration, as observed elsewhere (Draget, Skjåk Bræk, & Smidsrød, 1994; C K Kuo & Ma, 2001; Becker, Kipke, & Brandon, 2001), however, this is not observed here. The decreased strength observed at 4.5% and 6.0% may be caused by shortage of cross-linking cations. As there is no increase in GDL or glass content there will be no increase in ion availability to cross-link with increasing number of chains provided by the increased alginate concentration.

Figure 3 shows that increasing the glass content increases the modulus and strength of the hydrogel at a 4.5% alginate concentration. Increasing the glass content from 4.6% to 9.2% more than doubles both the strength and modulus of the hydrogel, with no further increase being observed with further glass addition (13.8%). This may be increased further with an increase in GDL content.

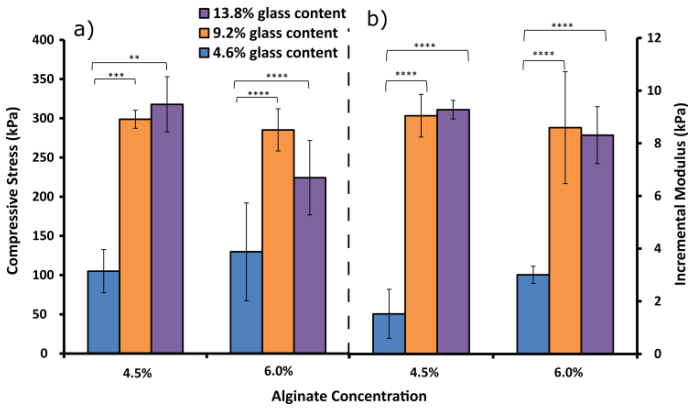

Figure 3 *(a)Compressive stress up to 70% strain and (b) Incremental modulus (30 to 50% strain) of 4.5% and 6.0% MVM alginate with 50 mg GDL and an increased glass content after storage for 1 day in DI at 37°C (n=5)*

3.1.5. Working Time and Hardening Time

Figure 4 shows the working and hardening time of each composition. For both alginates only the 4.5% and 6.0% alginate concentrations are within the required working and hardening time. There is an approximately linear decrease in working time for the MVG and MVM alginate (R^2 = 0.91 and R^2 = 0.94, respectively) and hardening time for the MVM alginate (R^2 = 0.93) with increasing alginate concentration. As there is no significant difference between the 0.5% and 2.5% hardening time ($p < 0.05$), there is not a linear reduction for the MVG alginate.

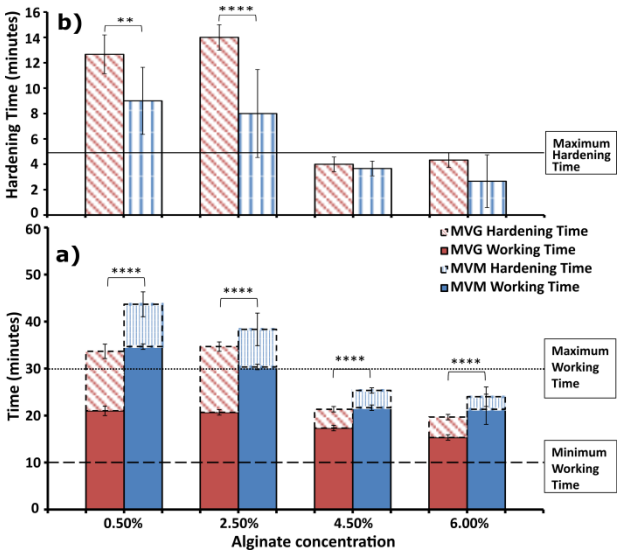

Figure 4 *Alginate a) working and b) hardening time (n=5)*

3.1.6. Deliverability

The alginate concentration of 0.5% (either MVG or MVM) gave insufficient viscosity (41-44mPas) to remain in the aneurysm when blood flow was applied. The 2.5% and 4.5% alginate concentration of both MVG and MVM alginates (<10,000 mPa.s) would inject through the micro-catheter easily, up to 20 minutes after mixing. The MVG and MVM alginates at 6.0% alginate concentration (>10,000 mPa.s) would not inject through the micro-catheter, blocking it 2 minutes after mixing the hydrogel.

As the 4.5% concentration of the MVM alginate had the correct strength, sufficient volume conservation, and correct working and hardening times it was selected to inject into the aneurysm model. With the balloon inflated and the flow pump on, the hydrogel was injected into the 10 mm aneurysm with a 2.5x2.5 mm neck two minutes before the end of the working time (22 minutes after mixing). The hydrogel was fully injected by the working time and the balloon remained inflated for two minutes. The hydrogel stayed within the aneurysm and remained in the aneurysm with no perceptible erosion once the balloon was deflated for the time tested (30 minutes).

3.2. Effect of Gamma Irradiation

As previously stated, the MVM alginate was the more suitable of the two alginates examined for treatment of cerebral aneurysms and hence was selected for gamma irradiation. A sample of the alginate

was gamma irradiated by Synergy Healthcare (Westport, Ireland) at 25kGy to examine the effects of a change in molecular weight alone and for sterilisation purposes.

3.2.1. Alginate Characterisation

GPC and ^{1}H NMR showed that gamma irradiation causes a reduction in alginate molecular weight to 180kDa without a change to the alginate chemical composition (Table 1).

3.2.2. Volume Conservation

Figure 5 shows that, again, the lower irradiated alginate concentrations shrink in volume whilst the 6.0% alginate concentration increases in volume over 7 days for both alginates. The 4.5% alginate concentration has minimal expansion in volume.

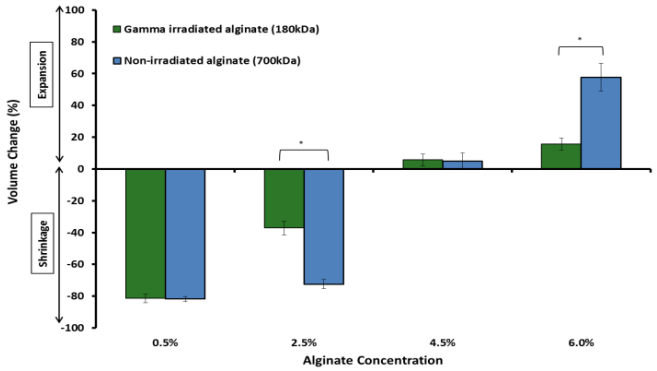

Figure 5 *Volume conservation after storage for 7 days in DI at 37°C (n=5)*

3.2.3. Viscosity

The results show that gamma irradiation reduces alginate viscosity, with values ranging from 2.83 to 86.43 mPa.s. The viscosity increases by approximately 6.25 mPa.s for each 1% increase in alginate concentration for the gamma irradiated alginate, see Figure S 8.

3.2.4. Compression testing

Reduction in molecular weight caused by gamma irradiation greatly affects the compressive stress and incremental modulus of the hydrogel compared to the non-irradiated sample ($p > 0.05$). Although the gamma irradiated alginate has a compressive strength that exceeds the limit discussed in the introduction (22 kPa), the incremental modulus of the alginate decreases with increasing alginate concentration and at each concentration has a decreased strength compared to the non-irradiated alginate, see Figure 6.

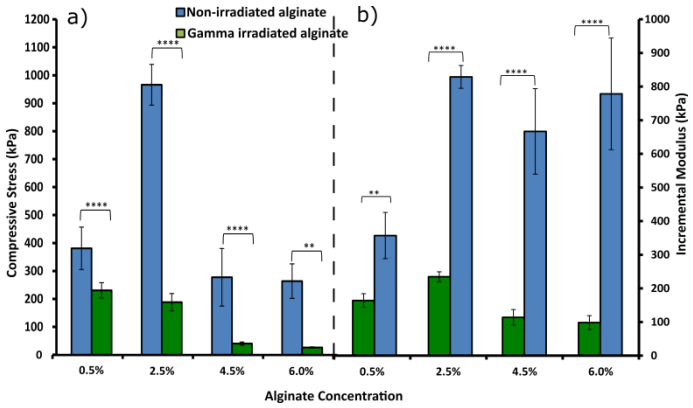

Figure 6 *(a) Compressive stress up to 70% strain and (b) Incremental modulus (30 to 50% strain) of irradiated and non-irradiated alginate compositions after storage for 7 days in DI at 37°C*

3.2.5. Working Time and Hardening Time

The reduction in molecular weight of the gamma irradiated alginate significantly increases the working and hardening time ($p > 0.05$), see Figure 9. The working and hardening times decrease linearly with increasing alginate concentration for both irradiated and non-irradiated samples, i.e. R^2 = 0.91, 0.91 and R^2 = 0.91, 0.92, respectively. However, at each concentration test, the gamma irradiated alginate is not within the time limits for its intended application.

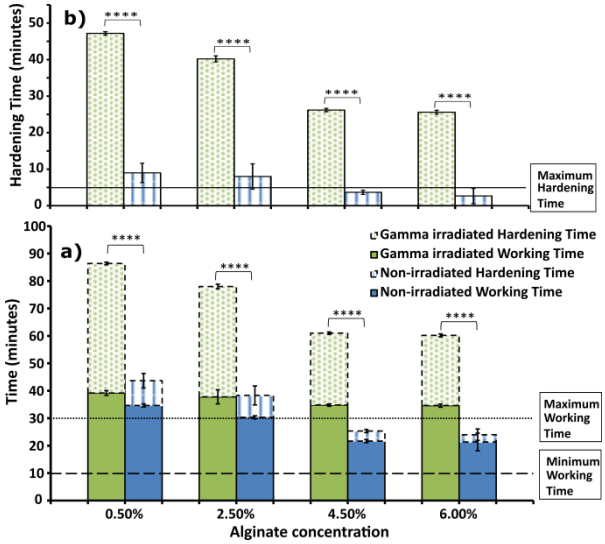

Figure 7 *Hydrogel a) working and b) hardening time (n=5)*

3.2.6. Deliverability

None of the gamma irradiated alginate concentrations had sufficient viscosity to remain in the aneurysm when blood flow was applied, similar to that of the 0.5% alginate concentration of the non-irradiated alginate.

4. Discussion

From the GPC and ^{1}H NMR results we can see that two different alginates were produced, a MVM with a high molecular weight and a MVG with a low molecular weight. Though the molecular weight of the

MVG alginate is over ten times less than that of the MVM alginate, the viscosities are similar (Figure S 5). This is not typical but may be due to differences in M/G ratios and G-block length, with higher G content and length alginates being typically stiffer and more viscous compared to alginates with a high-M content (Smith & Miri, 2011)(Nedovic & Willaert, 2013)(Jothisaraswathi & Rengasamy, 2006)(Schmid, Fariña, Rehm, & Sieber, 2016). It has been shown that varying the alginate purification method results in alginates with significantly different viscosities. The varying alginates and purification methods used here may have an additional effect on the viscosity of the alginate due variances in residual salts and impurities and the resulting pH of the alginate solution (Dusseault et al., 2006; K. Y. Lee & Mooney, 2012; McHugh, 2003).

It is clear from the results that the cross linking density of the hydrogel is important to control the strength and volume conservation. At the 0.5% alginate concentration there is likely a shortage of alginate chains and an excess of cations. At a 4.5% and 6.0% alginate concentration there is a shortage of cross-linking ions and, as a result, there are alginate chains that are not optimally cross-linked.

The sample volume conservation exhibits results similar to those described by Kuo *et al* (Catherine K Kuo & Ma, 2008). At a 0.5% and 2.5% alginate concentration the samples shrink and the 4.5% and 6.0% alginate concentration samples expand (Figure 1). This may be due to the hydrogel's cross-linking density decreasing with increasing alginate concentration as a result of the glass content, and hence ion content, remaining constant. The lower concentration alginates have an increased cross-linking density. This increased cross-linking increases the elastic forces that can resist the swelling caused by water molecules diffusing into the hydrogel. As the concentration increases the cross-linking density decreases allowing water to diffuse through the hydrogel and swell. Neither significant shrinkage nor expansion is desired, as shrinkage may contribute to aneurysm recurrence and excessive sample expansion may cause aneurysm rupture. Samples produced from 4.5% alginate concentration exhibit minimal swelling, which suggests that this alginate concentration provides a cross-linking density that provides a high strength while conserving sample volume.

For both the MVG and MVM alginate the incremental modulus and compressive strength increase from 0.5% to 2.5% alginate concentration (Figure 2). The incremental modulus and compressive strength then decreases with further increased alginate concentration (≥2.5%). The decrease above 2.5% alginate was unexpected and may be due to a shortage of cross-linking ions. This shortage of ions may reduce the cross-linking density and result in an inhomogeneous hydrogel that is prone to fracture. To explore this further, the glass content was increased. This increased the strength for the 4.5% and 6.0% alginate concentration whereby the increase in cations provided a higher cross-linking density (Figure 3). For each of the concentrations, the MVM alginate had the highest modulus and compressive strength for each

alginate concentration. Typically, the high-G alginate would have the higher strength (Draget et al., 1994); however, the increased molecular weight of the MVM alginate may compensate for the lower G-block content and provides increased entanglements.

Though the MVG alginate has the shortest working time, the MVM alginate has the shortest hardening time at each alginate concentration (Figure 4). This indicates that the working time is governed mostly by the chemical composition of the alginate with a high-G content providing a decreased working time. The hardening time of the alginate is governed mainly by the molecular weight of the alginate with a high molecular weight alginate having decreased hardening time. Increasing the alginate concentration of each alginate provides an increased number of ionic cross-linking locations and increased likelihood of chain entanglements, causing working and hardening time to reduce in each hydrogel.

The deliverability of the hydrogel can be determined from the viscosity, with ungelled alginates up to 9,000 mPa.s being injectable through a micro-catheter. For the intended applications of this hydrogel; the strength, conservation of sample volume, working time and hardening time, along with the deliverability of this hydrogel must be considered. The 4.5% concentration of the MVM alginate met each of the requirements and was tested in an aneurysm model with physiological pressures. This hydrogel remained in the aneurysm without migrating.

Gamma irradiation of the MVM alginate was shown to have no effect on the M/G ratio of the alginate. However, it does cause scissions of the glycosidic bonds, which reduces the molecular weight of the alginate.

As expected, viscosity was seen to decrease with gamma irradiation of the MVM alginate, as expected, due to decreased chain entanglements (C K Kuo & Ma, 2001; Dusseault et al., 2006; Rendevski & Mahmudi, 2012; Ouwerx, Velings, Mestdagh, & Axelos, 1998)

From the alginate working and hardening time data it can be seen that the gamma irradiated alginate has the longest working and hardening time due to the high-M content and low molecular weight having a reduced number of ionic cross-linking locations (Popeski-Dimovski et al., 2012). Although usability of the gamma irradiated alginate may be improved by increasing alginate concentration and optimising the glass and GDL content, an alternative method of sterilization may be more efficient, such as sterilisation by filtration in a sterile manufacturing environment.

A range of injectable polymer formulations have been developed for soft tissue applications but many contain toxic monomers, activators and free radicals. The gelation rate of the hydrogel described here is

slow compared to other alginate hydrogels (Larsen, Bjørnstad, Pettersen, Tønnesen, & Melvik, 2015; Lee et al., 2003), which allows the hydrogel to be delivered and set, within a clinically applicable time.

Though GDL can be used with alginate to produce acid gels, this is likely not the case with the current hydrogel due to the high strengths and relatively rapid gelation rates observed. The addition of GDL results in a reduced pH during setting, which may affect the hydrogel's biocompatibility. The pH of the storage medium drops by 0.50 with the 4.5% high-M alginate. However, the presence of alkali ions in the bioactive glass has a neutralizing effect over 24 hours. It should be noted that this temporary reduction in pH, as occurs with many synthetic biomaterials, may result in an increased inflammatory and fibrotic response *in vivo* (Edgar et al., 2016).

For an aneurysm filler to be effective it should have sufficient strength in order to behave similarly to native cerebral tissue; diverting flow and withstanding haemodynamic stresses caused by arterial expansion and contraction and blood flow forces (static, dynamic and shear). A low elastic modulus is required to insure stress caused by blood flow is not transferred to the damaged tissue, causing aneurysm rupture. A calcium alginate gel that was designed for embolization was reported to have a compressive strength of 124kPa at 60% strain (Becker et al., 2001). Onyx®, is a non-adhesive material approved for the treatment of cerebral aneurysms in Europe, has been observed to have a maximum compressive strength of 3 MPa (Ohyama, Ko, Miura, Iwata, & Taki, 2004). However, Onyx® sets rapidly when in contact with blood, which combined with the slow injection rate, is not ideal for placement.

The optimised hydrogel in this study exhibits a compressive strength of 280 kPa. Although it is not equal in strength to Onyx®, it may act to support the existing tissue in its function, without transferring excessive stress to the damaged tissue. The optimised hydrogel reported herein has higher incremental modulus and compressive strength compared to those of other ionically cross-linked alginate hydrogels described in the literature at similar alginate concentrations and chemical compositions (Becker et al., 2001; C K Kuo & Ma, 2001). This novel material reported herein likely has a greater mechanical integrity than the currently used coil technology that can compact with blood flow, which though not optimal, continues to function in this mechanical environment (Gallas et al., 2009; Sluzewski et al., 2004).

Agglomerates were clearly visible in the hydrogel both during and after setting, which may affect consistency of strength and setting, therefore, future testing will be carried out to minimise these agglomerates. The adhesive nature of the hydrogel will also be examined as this is important to reduce aneurysm recurrence. Further optimisation of the sterilisation techniques for each hydrogel component of the hydrogel will also need prior to commercial application.

5. Conclusion

From the results it can be seen that alginate concentration, molecular weight and chemical composition affect the sample volume conservation, viscosity, strength, deliverability, working and hardening time of the novel hydrogels. An alginate with an increased molecular weight and high-M content provides a hydrogel with an increased working time and decreased hardening time while providing the required strength which is advantageous for a cerebral aneurysm filler. Alginates with a viscosity between 2,000 mPa.s and 9,000 mPa.s can be injected through a micro catheter while providing sufficient viscosity to remain within an aneurysm without migration.

Sterilisation by gamma irradiation causes a reduction of molecular weight which decreases the alginate's viscosity and strength and increases the hydrogel's working and hardening time. This is undesirable for the treatment of cerebral aneurysms. For this application, an alternative, less aggressive method of sterilisation will be required.

Acknowledgements

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Supplementary Material

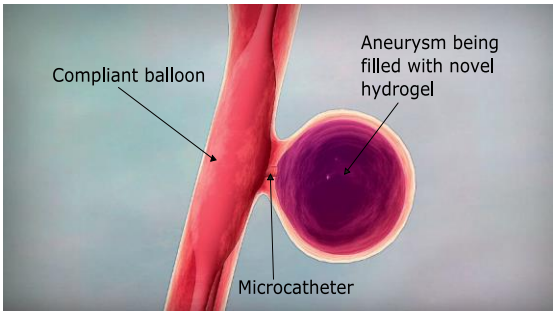


Figure S 1 Schematic of novel hydrogel being injected into a cerebral aneurysm (Clarkin, 2017)

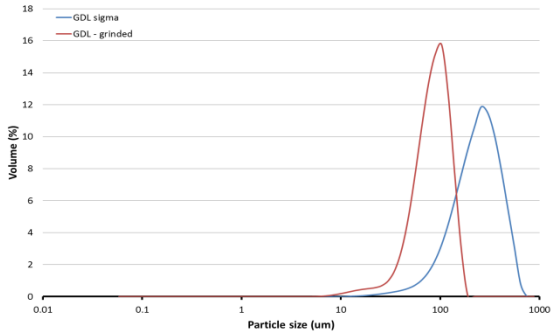


Figure S 2 *Particle Size Analysis of glass purchased from sigma before and after grinding*

Effect of alginate molecular weight and chemical composition

Alginate Characterisation

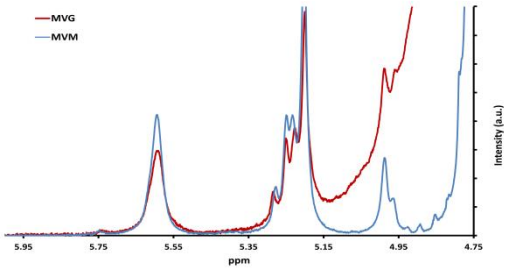
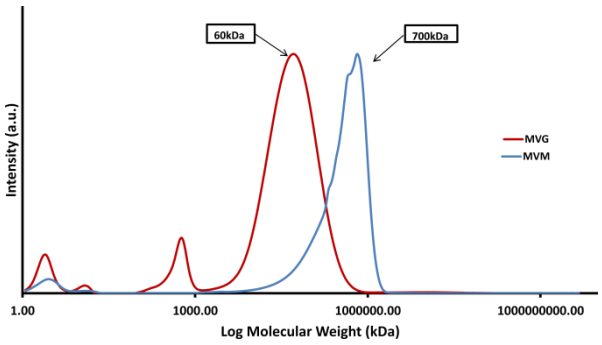
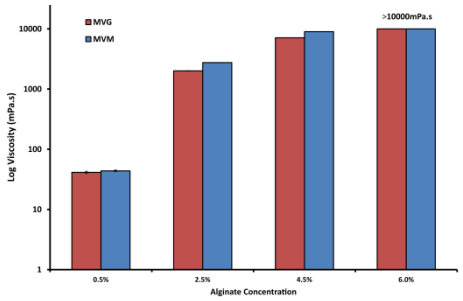


Figure S 3 *^{1}H NMR of MVG and MVM alginate*



513

514 ***Figure S 4*** *GPC of MVG and MVM alginate*

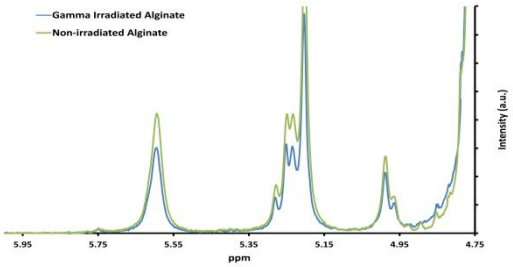


515

516 ***Figure S 5*** *Viscosity of the alginates at each of the four concentrations (n=5)*

517 **Effect of Gamma Irradiation**

518 **Alginate Characterisation**



519

520 ***Figure S 6*** *^{1}H NMR of gamma irradiated and non-irradiated alginate*

521 The GPC, Figure S 7, shows that gamma irradiation causes scissions of the glycosidic bonds, reducing the
522 molecular weight of the alginate.

523

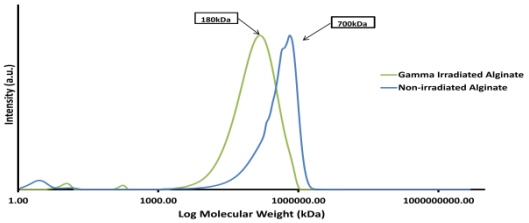


Figure S 7 *GPC of gamma irradiated and non-irradiated alginate*

Using Equation S1 it was calculated that gamma irradiation causes approximately 3.3 chain breaks per molecule.

$$N = \frac{M_o}{M} - 1$$

Equation S 1 Calculating chain breaks per molecule

Where M_o is the original molecular weight and M is the molecular weight of the alginate following irradiation (D. W. Lee et al., 2003).

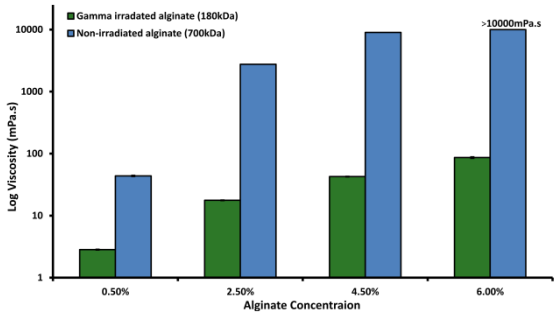


Figure S 8 *Viscosity of alginates at each of the four concentrations (n=5)*