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Research paper

Investigation of the material properties of alginate for the development of hydrogel repair of dura mater

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ABSTRACT

The collagenous dura mater isolates the brain from the external environment and requires a secure closure following invasive neurosurgery. This is typically accomplished by approximation of the dura mater via sutures and adhesives. In selected cases, however, large portions of dura mater require excision, necessitating a tissue replacement patch. The mild reaction conditions and long-term biocompatibility of alginate evince strong candidacy for these applications. This study investigates the potential of diffusion and internally gelled alginates for these applications. Specifically, we quantified the viscosity, gel rate, syneresis level, compressive strength, compressive modulus, complex modulus and loss angle in the context of dura mater repair. The ideal sealant would have a rapid cross-link speed, while the ideal dura mater replacement would have a low level of syneresis. Both applications require a compressive modulus of 20–100 kPa and a complex modulus of 1–24 kPa. The data collected in this study suggests that the use of 1.95 wt% 43 mPa s alginate with 200 mM $CaCl_2$ is sufficient for approximating the dural membrane for closure alone or in conjunction with suture. Alternatively, the use of 1.95 wt% 43 mPa s alginate with 100 mM $CaCO_3$ is sufficient for tissue replacement in large dural defects.

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1. Introduction

Invasive neurosurgery often requires a method to close the collagenous meninges that functionally isolate the brain from the external environment. Typically, adhesive materials can be used as sealants for minor dural defects, while large dural defects require patches. Several different materials have been evaluated for both sized dural defects; however, sufficient drawbacks exist limiting the efficacy of the state-of-the-art approaches.

In the case of minor dural defects, sutures or adhesives are used as dural sealants. Even for minor defect closure, there are sufficient drawbacks to these approaches. Sutures alone are quick but do not provide adequate sealing of

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approximated tissue (Cain et al., 1990). Fibrin glue, the most commonly used adhesive, has been shown to cause both seizures and a significant inflammatory response (Agarwal et al., 1998; Schlag et al., 2000; Schwartz et al., 1973). These drawbacks drive the need for an alternative dural sealant material for minor defects.

Recently, the implantation of neural prosthetic devices such as deep brain stimulators and penetrating cortical electrodes for motor and sensory prostheses have necessitated the creation of large dural defects (Maynard et al., 2000). The remaining tissue surrounding the large defects cannot be approximated, and thus requires a patch to replace the lost tissue and re-isolate the brain from the external environment. Historically, a variety of materials have been used for this application including metal foils (Beach, 1890), various polymers (Barbolt et al., 2001; Cain et al., 1988; Collins et al., 1991; Friedman et al., 2002; Kumar et al., 2003; Park and Tator, 1998; Vinas et al., 1999), cadaveric human tissues (Abbott and Dupree, 1971; Costantino et al., 2000; Thadani et al., 1988), and xenografts (Anson and Marchand, 1996; Filippi et al., 2001; Parizek et al., 1989; Xu et al., 1988; Zeman et al., 1993). These materials have all had deleterious effects, including leaks (Cain et al., 1990; Sawamura et al., 1999; Zeman et al., 1993), seizure activity (Abbe, 1895; Schlag et al., 2000), hematomas (Ohbayashi et al., 1994; Robertson and Menezes, 1997; Schwartz et al., 1973), Creutzfeldt–Jakob disease (Bernoulli et al., 1977; Budka et al., 1995; Thadani et al., 1988), and significant inflammatory response (Agarwal et al., 1998; Barbolt et al., 2001; Cain et al., 1988; Collins et al., 1991; De Vries et al., 2002; Narotam et al., 1995; Park and Tator, 1998). Thus, engineered biocompatible materials need to be developed for dural replacement patches.

Recently, the feasibility of using hydrogels for both applications (dural sealant and dural replacement patch) has been demonstrated in animal models (Alleyene et al., 1998; Preul et al., 2003; Vetter et al., 2003; Williams et al., 2003). Of these, alginate shows promise due to its rapid gel formation, biocompatibility, and mild reaction conditions that allow hydrogel cross-linking while in direct contact with the surrounding meningeal and neural tissue (Aydelotte et al., 1998; Becker et al., 2001, 2005; Le Tallec et al., 1997; Nguyen et al., 2003; Williams et al., 2003). In the presence of divalent cations (e.g. Ca^{2+}, Ba^{2+}, and Sr^{2+}) alginate participates in an accelerated cross-linking reaction that allows for quick sealing of any size dural defect with an alginate-based hydrogel. Furthermore, the alginate-hydrogel components do not induce seizure activity (Becker and Kipke, 2003) or a significant inflammatory response (Becker et al., 2001, 2002, 2005; Draget et al., 1997). Minimal inflammatory response or risk of seizure activity is especially important considering the imminent and prolonged contact of the hydrogel components with the meninges and underlying cortex. Due to these benefits and documented preliminary results (Vetter et al., 2003; Williams et al., 2003), alginate demonstrates promise as either a dural sealant or a dural patch. The goal of these current studies is to determine the optimal alginate properties necessary to maximize the mechanical and chemical viability for both dural sealants and dura mater replacement.

The mechanism of gelation may dictate alginate's success as a dural sealant or patch. Two methods of gelation using calcium to cross-link alginate have been extensively described and used to create alginate hydrogels: diffusion gelling and internal gelling (Becker et al., 2001; Haug and Smidsrod, 1965; Ishikawa et al., 1999; Kuo and Ma, 2001; Liu et al., 2003; Mammarella and Rubiolo, 2003; Shchipunov et al., 2002; Smidsrod and Haug, 1965, 1972; Wang et al., 1993, 1994; Yamagiwa et al., 1995). Calcium chloride ($CaCl_2$) is a commonly used calcium source that relies on diffusion to cross-link alginate versus calcium carbonate ($CaCO_3$), which utilizes internal gelling through the release of calcium ions *in situ*.

In diffusion-based gelling using a $CaCl_2$ solution, dissociated Ca^{2+} cross-links with the first carboxyl groups with which it comes into contact. Due to this rapid reaction, $CaCl_2$ cannot be easily mixed with the alginate volume; rather, when the two fluids are exposed to each other, the Ca^{2+} ions diffuse into the alginate volume. This diffusion and subsequent reaction creates a skin of cross-linked alginate around the volume of liquid alginate. Subsequently, the calcium ions diffuse inward, increasing the skin's thickness as the alginate bolus is cross-linked (Bienaime et al., 2003; Blandino et al., 1999). This makes diffusion an ideal method for approximating minor dural defects where rapid closure is of utmost importance. However, the resulting hydrogel structure is highly variable (Aydelotte et al., 1998; Bienaime et al., 2003) and may lack stability due localized inhomogeneities and potential regions of gel failure, making this approach less ideal for use in repairing large dural defects.

Alternatively, with internal gelling, poorly soluble Ca^{2+} in the form of $CaCO_3$ is homogeneously mixed with alginate and then the Ca^{2+} is solubilized by the addition of a catalyst, such as D-glucono-δ-lactone (GDL). GDL slowly acidifies the alginate:$CaCO_3$ solution, driving the release of Ca^{2+} ions (Draget et al., 1991; Shchipunov et al., 2002). Calcium ions bind to the nearest available carboxyl groups, cross-linking the hydrogel in a spatially uniform manner. This method can be time intensive due to the time required for the hydrolysis of GDL and subsequent calcium release; however, the resultant hydrogel has a more uniform and reproducible structure (Draget et al., 1991; Kuo and Ma, 2001; Shchipunov et al., 2002). This may make *in situ* gelling a better method for creating a more stable patch for repairing larger dural defects.

In this study, we manipulated the conditions for diffusion and *in situ*-based gelation of alginate (concentration and molecular weight of ingredients) and measured (1) the rate of gel formation, (2) the syneresis levels, (3) the compressive modulus, (4) the complex modulus, and (5) the loss angle. Based on our hypotheses, these measurands are important in specific manifestations. The rate of gel formation should be sufficient to rapidly close a dural defect (small or large), thus minimizing cerebrospinal fluid leaking and brain swelling. The syneresis level is an indicator of how well the alginate hydrogel will maintain its original volume. A low syneresis level is important such that a dural patch will maintain its original volume, preserving the seal with the surrounding meningeal tissue. After application of the dural sealant or dura mater replacement, the gel is subjected to a constant intracranial pressure (approximately 1 kPa) as well as pulsation of the brain with heart rate and respiratory rate that compresses the gel between the brain and skull. Thus, both the compressive and complex moduli of alginate and the underlying brain

tissue should match in order to decrease the occurrence of trauma due to a mechanical mismatch.

The goals of this study were to investigate gel formation and the material properties of alginate hydrogels created by diffusion and *in situ*-based gelling in the context of dura mater repair. Additionally, we sought to elucidate how these properties are influenced by alginate molecular weight, alginate concentration, and $CaCl_2$ or $CaCO_3$ concentration. More specifically, the goal was to determine the combinations of these components that (1) most closely approximated the material properties of the tissues (meningeal and brain) which the alginate will contact *in vivo*, and (2) meet the design requirements, as previously discussed, for use as a dural sealant or dural replacement patch. The resulting data were used to determine the individual roles of each component and the relationships between the components.

2. Materials and methods

2.1. Alginate preparation

Batches of sodium alginate, having average molecular weights of 125–205 kDa, and apparent viscosities of 43–200 mPa s, were acquired from Novamatrix (Pronova UP LVG, Drammen, Norway). The apparent viscosity (mPa s) of each batch of alginate was determined by creating a 1 weight % (wt%) solution of alginate dissolved in water and measuring its viscosity at 20 °C. The apparent viscosity is highly dependent on the molecular weight of the alginate sample (Haug, 1964; Smidsrod, 1970), and Novamatrix supplies the alginate based on apparent viscosity; thus, this unit is used synonymously with molecular weight in these experiments. All of the alginates used were of a high G content (65%–68 %) as determined through NMR conducted by the manufacturer (Grasdalen, 1983). The purified, high G content alginates used in these experiments came in a range of molecular weights, which affected the usable concentration range and final viscosity of the liquid alginate solution. Different units were utilized to denote the difference between the apparent viscosity and final liquid viscosity. The apparent viscosity, which is used as the name of the alginate samples, is dependent on the molecular weight and is quantified in mPa s. The liquid viscosity is dependent on the concentration, and is quantified in cP. Unreacted alginate was tested for liquid viscosity changes with respect to concentration of the sample and shear using a 2° conical rheometer shear plate (AR1000 Rheometer, TA Instruments, New Castle, DE) at room temperature (25 °C). The rheometric testing was repeated four times at four concentrations of alginate: 0.5, 1, 1.5, and 2 wt%. Averages and standard errors were determined.

We used exponential regression to model the relationship of each alginate's liquid viscosity relationship to alginate molecular weight and concentration. These equations allowed for interpolation of equivalent liquid viscosity matching with respect to concentration of the alginates of different molecular weight in subsequent experiments. The liquid viscosities of 1.5 wt% 43 mPa s, 1.5 wt% 116 mPa s, and 1.5 wt% 200 mPa s alginate were chosen for viscosity matching of the four different alginates.

Each of the four alginate types were mixed at three different concentrations and reacted with one of three different $CaCl_2$ concentration solutions (50, 200, and 680 mM) or three different $CaCO_3$ concentrations (20, 50, or 100 mM). This resulted in 72 testable combinations of alginate molecular weight, alginate concentration, and $CaCl_2$ or $CaCO_3$ concentration. These combinations can be seen in Table 1 ($CaCl_2$) and Table 2 ($CaCO_3$).

There was variation in the conditions for diffusion and *in situ*-gelled alginate due to the differences in gelation mechanism. $CaCO_3$ can be thoroughly mixed with alginate before gelation begins but $CaCl_2$ cannot due to the rapid cross-link reactions. The diffusion-gelled alginate conditions were as follows: (1) gels used for mechanical testing and syneresis were created by reacting 10 ml of alginate solution with 10 ml of $CaCl_2$ solution in a 60 mm Petri dish for 24 h; (2) gels used for viscoelastic testing were created by reacting 1 ml alginate solution with 1 ml $CaCl_2$ solution in a 30 mm diameter glass beaker for 24 h. The *in situ*-gelled alginate conditions were as follows: (1) gels used for mechanical testing and syneresis were created by mixing 10 ml of alginate solution with $CaCO_3$ and GDL powder at the specified concentration in a 60 mm Petri dish and allowed to set for 24 h; (2) gels used for viscoelastic testing were created by mixing 1 ml alginate solution with $CaCO_3$ and GDL powder at the specified concentration in a 30 mm glass beaker and allowed to set for 24 h.

2.2. Gel rate — diffusion-based gelation

To initiate gelation, alginate samples were reacted with calcium chloride dihydrate (Sigma, St. Louis, MO) at concentrations of 50–680 mM. The gel rate studies were all conducted at room temperature, since there is no significant difference in gelation rate due to a temperature change from 25 to 37 °C (Kuo and Ma, 2001). The gel rate was characterized by recording the time for murexide, a calcium:alginate complex indicator, to change from dark-red to yellow in 50% of the hydrogel (Bienaime et al., 2003). Briefly, murexide was added to the alginate solution at a final concentration of 0.01 wt%. A volume of 200 μl of the alginate:murexide solution was reacted with 500 μl of $CaCl_2$ to maintain an excess of calcium ions and to mimic potential conditions of surgical use. Alginate was pipetted onto a Petri dish and $CaCl_2$ was dropped onto the alginate bolus. The gelation phenomenon was visually monitored and images recorded at 10 s intervals using a color camera (Coolsnap-Pro cf, Roper Scientific, Photometrics, Tucson, AZ) on a stereomicroscope (Leica MZFLIII, Leica Microscopy Systems Ltd., Heerbrugg, Switzerland). Images were processed offline using custom software that utilized the image analysis toolbox in Matlab 7.0 (Mathworks, Natick, MA) to identify the regions of cross-linked (yellow) and unreacted (red) alginate in each image based on a colorimetric scale. A full factorial design was employed to determine the effect of $CaCl_2$ concentration and alginate molecular weight and concentration. Five trials of each combination were completed, and the average gel times and standard errors were calculated.

Table 1 – Summary of experimental factorial design and the resultant average measurements for each measurand. Diffusion-based gelation of alginate.

Alginate molecular weight (mPa s)	Alginate conc. (wt%)	$CaCl_2$ conc. (mM)	Gel rate (min)	Syneresis level (%)	10% Compressive strength (kPa)	60% Compressive strength (kPa)	Compressive modulus (kPa)	Complex modulus (kPa)	Loss angle (degrees)
43	1.5	50	20.23 ± 1.03	41.25 ± 1.89	2.26 ± 1.08	48.17 ± 6.78	22.95 ± 11.48	22.50 ± 3.30	9.87 ± 0.22
43	1.5	200	6.63 ± 0.46	55.00 ± 1.08	4.74 ± 1.51	85.49 ± 10.44	46.64 ± 14.79	14.55 ± 2.70	9.29 ± 0.24
43	1.5	680	3.10 ± 1.36	58.00 ± 1.14	6.32 ± 1.24	108.10 ± 10.13	63.33 ± 11.82	16.10 ± 2.66	9.62 ± 0.32
43	1.95	50	22.97 ± 4.28	27.00 ± 1.47	2.74 ± 0.85	25.13 ± 7.01	32.01 ± 12.13	25.37 ± 1.55	9.38 ± 0.08
43	1.95	200	8.23 ± 1.74	52.25 ± 1.03	5.42 ± 1.64	111.30 ± 12.54	54.37 ± 16.32	21.62 ± 3.97	9.30 ± 0.24
43	1.95	680	4.13 ± 0.73	57.13 ± 2.47	8.72 ± 2.85	145.38 ± 31.67	88.73 ± 29.22	25.78 ± 2.76	9.75 ± 0.20
43	2.27	50	57.57 ± 6.17	17.06 ± 3.72	1.78 ± 0.30	18.79 ± 2.24	17.57 ± 3.06	28.30 ± 3.37	9.41 ± 0.15
43	2.27	200	15.73 ± 3.50	50.80 ± 1.66	6.01 ± 1.39	112.48 ± 19.01	59.02 ± 12.84	25.91 ± 3.95	9.56 ± 0.31
43	2.27	680	4.87 ± 0.41	57.20 ± 1.03	6.80 ± 2.03	183.78 ± 26.92	68.29 ± 19.30	45.67 ± 15.29	10.55 ± 0.88
64	1.36	50	24.13 ± 2.39	48.88 ± 1.59	2.34 ± 0.74	41.08 ± 7.15	23.87 ± 7.99	15.17 ± 2.18	9.47 ± 0.15
64	1.36	200	6.47 ± 0.71	60.63 ± 0.80	3.55 ± 0.84	74.47 ± 8.15	36.56 ± 9.24	15.05 ± 1.08	9.35 ± 0.10
64	1.36	680	2.80 ± 0.31	63.00 ± 0.71	4.57 ± 0.54	81.63 ± 10.48	46.72 ± 5.97	12.90 ± 0.41	9.50 ± 0.10
64	1.79	50	38.03 ± 3.62	38.38 ± 2.84	5.46 ± 1.27	48.86 ± 7.26	57.19 ± 14.05	23.93 ± 1.62	9.58 ± 0.17
64	1.79	200	11.00 ± 1.97	56.00 ± 0.41	6.46 ± 1.94	112.37 ± 6.40	65.24 ± 19.95	25.47 ± 3.00	9.55 ± 0.18
64	1.79	680	3.60 ± 0.36	57.83 ± 0.59	9.07 ± 2.38	150.03 ± 43.12	89.21 ± 23.14	19.20 ± 2.69	9.63 ± 0.29
64	2.11	50	39.67 ± 6.24	25.20 ± 3.09	2.13 ± 0.67	19.80 ± 3.41	21.94 ± 6.81	33.00 ± 3.53	9.79 ± 0.19
64	2.11	200	9.07 ± 1.05	56.70 ± 1.18	5.48 ± 1.04	115.48 ± 5.06	54.76 ± 9.91	37.73 ± 5.27	10.18 ± 0.33
64	2.11	680	3.37 ± 0.37	57.40 ± 2.58	5.30 ± 0.97	141.72 ± 12.45	53.71 ± 9.64	42.22 ± 4.81	10.26 ± 0.24
116	1.08	50	23.23 ± 5.43	53.38 ± 1.14	2.73 ± 0.71	51.71 ± 7.32	27.16 ± 7.03	10.70 ± 2.07	9.26 ± 0.25
116	1.08	200	5.63 ± 1.04	63.50 ± 1.67	1.44 ± 0.40	43.54 ± 8.27	14.48 ± 3.83	7.55 ± 1.11	9.08 ± 0.18
116	1.08	680	2.30 ± 0.39	65.88 ± 2.11	3.62 ± 0.46	71.02 ± 4.67	36.58 ± 5.56	8.88 ± 2.35	9.28 ± 0.35
116	1.5	50	42.07 ± 4.28	43.25 ± 2.99	5.57 ± 1.20	55.74 ± 10.20	56.90 ± 11.95	16.32 ± 2.82	9.55 ± 0.17
116	1.5	200	10.13 ± 1.65	57.75 ± 1.36	7.50 ± 1.89	118.34 ± 14.91	76.37 ± 18.49	14.84 ± 3.11	9.52 ± 0.29
116	1.5	680	3.57 ± 0.34	58.88 ± 0.66	6.85 ± 1.63	114.78 ± 16.57	71.02 ± 15.91	14.12 ± 2.37	9.42 ± 0.22
116	1.8	50	36.53 ± 2.72	38.30 ± 3.92	2.88 ± 1.43	30.02 ± 3.62	29.32 ± 15.31	18.21 ± 2.68	9.50 ± 0.18
116	1.8	200	10.57 ± 1.61	59.10 ± 1.19	5.04 ± 1.11	106.33 ± 16.49	53.50 ± 10.22	17.25 ± 2.67	9.40 ± 0.18
116	1.8	680	4.03 ± 0.19	62.10 ± 0.75	7.43 ± 1.78	145.64 ± 13.84	75.60 ± 17.96	20.74 ± 3.63	9.72 ± 0.25
200	0.9	50	17.90 ± 1.97	62.88 ± 1.48	1.83 ± 0.31	49.91 ± 11.15	18.02 ± 2.79	6.44 ± 1.81	9.00 ± 0.25
200	0.9	200	3.77 ± 0.56	67.00 ± 1.22	3.71 ± 0.47	61.04 ± 4.07	37.31 ± 4.87	6.10 ± 1.67	8.98 ± 0.28
200	0.9	680	2.30 ± 0.32	66.88 ± 2.13	3.57 ± 0.44	50.50 ± 2.21	35.93 ± 6.34	6.04 ± 1.68	9.23 ± 0.35
200	1.25	50	30.40 ± 3.69	55.13 ± 1.76	6.72 ± 1.49	74.49 ± 18.19	67.91 ± 15.00	8.68 ± 2.29	9.06 ± 0.22
200	1.25	200	8.10 ± 0.77	61.83 ± 0.82	7.64 ± 2.00	121.36 ± 15.59	77.86 ± 20.13	7.35 ± 1.43	9.12 ± 0.27
200	1.25	680	3.40 ± 0.33	62.13 ± 0.97	7.08 ± 1.00	95.45 ± 6.72	74.74 ± 9.67	7.10 ± 1.54	9.20 ± 0.22
200	1.5	50	26.80 ± 0.55	51.80 ± 1.93	4.87 ± 1.93	60.37 ± 13.77	48.71 ± 19.58	11.79 ± 2.57	9.15 ± 0.35
200	1.5	200	7.70 ± 1.19	60.80 ± 0.78	5.04 ± 1.63	88.28 ± 13.10	51.21 ± 15.83	9.29 ± 1.92	9.29 ± 0.33
200	1.5	680	4.30 ± 0.30	64.50 ± 1.64	5.09 ± 1.06	104.35 ± 10.49	53.60 ± 10.69	10.15 ± 2.26	9.28 ± 0.29

Table 2 – Summary of experimental factorial design and the resultant average measurements for each measurand. *In situ*-based gelation of alginate.

Alginate molecular weight (mPa s)	Alginate conc. (wt%)	$CaCO_3$ conc. (mM)	Gel rate (min)	Syneresis level (%)	10% Compressive strength (kPa)	60% compressive strength (kPa)	Compressive modulus (kPa)	Complex modulus (kPa)	Loss angle (degrees)
43	1.5	20	30.02 ± 1.85	0.02 ± 0.02	1.92 ± 0.22	19.08 ± 1.70	17.07 ± 1.14	4.24 ± 0.80	5.31 ± 0.32
43	1.5	50	19.94 ± 1.28	2.84 ± 0.96	5.70 ± 0.88	94.86 ± 17.46	59.32 ± 6.30	15.91 ± 2.72	8.34 ± 0.26
43	1.5	100	13.38 ± 0.19	10.75 ± 0.95	7.32 ± 1.81	148.02 ± 13.42	92.63 ± 4.64	22.75 ± 5.04	8.99 ± 0.49
43	1.95	20	40.96 ± 3.87	0.00 ± 0.00	2.83 ± 1.13	20.93 ± 3.64	15.07 ± 3.02	4.58 ± 1.14	4.99 ± 0.29
43	1.95	50	30.08 ± 0.36	1.26 ± 0.17	6.50 ± 1.02	102.50 ± 8.16	65.37 ± 9.59	19.96 ± 3.13	7.88 ± 0.40
43	1.95	100	19.28 ± 0.84	3.55 ± 0.68	7.86 ± 1.36	132.21 ± 4.90	85.34 ± 7.50	29.60 ± 4.45	8.88 ± 0.42
43	2.27	20	46.86 ± 1.95	0.00 ± 0.00	2.93 ± 1.77	22.05 ± 0.88	11.80 ± 3.30	3.96 ± 0.76	4.68 ± 0.21
43	2.27	50	36.62 ± 1.46	0.24 ± 0.07	7.86 ± 1.24	123.86 ± 7.88	79.41 ± 12.61	25.30 ± 5.58	8.07 ± 0.42
43	2.27	100	24.34 ± 1.33	0.91 ± 0.34	11.64 ± 1.20	140.35 ± 9.92	129.47 ± 15.57	29.01 ± 4.36	8.50 ± 0.36
64	1.36	20	31.56 ± 1.34	0.63 ± 0.47	2.71 ± 0.30	30.24 ± 3.72	26.28 ± 3.82	6.95 ± 1.26	6.51 ± 0.32
64	1.36	50	21.12 ± 1.61	11.83 ± 1.14	6.20 ± 1.02	103.85 ± 15.16	55.55 ± 10.55	16.77 ± 3.43	8.34 ± 0.38
64	1.36	100	12.56 ± 0.75	16.83 ± 1.24	8.58 ± 0.48	154.50 ± 16.87	84.77 ± 4.41	22.17 ± 3.63	9.18 ± 0.30
64	1.79	20	33.48 ± 2.36	0.00 ± 0.00	2.57 ± 0.43	43.73 ± 1.41	22.15 ± 1.58	5.19 ± 1.82	5.05 ± 0.46
64	1.79	50	23.88 ± 1.21	6.19 ± 1.14	7.32 ± 0.42	117.19 ± 12.51	77.94 ± 0.91	19.68 ± 3.50	8.34 ± 0.37
64	1.79	100	16.18 ± 0.75	8.11 ± 1.12	6.91 ± 1.19	137.62 ± 8.95	72.65 ± 13.15	26.68 ± 3.92	9.19 ± 0.20
64	2.11	20	38.72 ± 1.57	0.00 ± 0.00	2.45 ± 0.65	47.87 ± 4.51	17.95 ± 0.80	6.01 ± 0.86	5.18 ± 0.24
64	2.11	50	27.50 ± 1.26	3.31 ± 0.80	9.01 ± 0.32	139.77 ± 7.96	85.66 ± 1.87	18.92 ± 3.03	7.85 ± 0.21
64	2.11	100	20.92 ± 2.01	6.37 ± 1.34	9.27 ± 0.62	180.82 ± 9.22	93.64 ± 10.73	23.47 ± 3.80	8.26 ± 0.28
116	1.08	20	27.86 ± 2.52	7.25 ± 0.48	4.20 ± 1.37	55.32 ± 3.34	30.57 ± 5.62	7.83 ± 1.53	7.19 ± 0.17
116	1.08	50	15.96 ± 1.24	18.88 ± 1.83	6.55 ± 0.84	101.5 ± 12.29	59.65 ± 3.44	14.59 ± 2.83	8.82 ± 0.29
116	1.08	100	8.26 ± 0.89	24.88 ± 1.09	8.02 ± 0.96	136.00 ± 8.07	70.93 ± 3.18	16.89 ± 2.97	9.66 ± 0.38
116	1.5	20	35.16 ± 1.74	1.25 ± 0.25	3.22 ± 0.18	65.18 ± 3.10	33.75 ± 3.64	8.43 ± 1.33	6.90 ± 0.42
116	1.5	50	21.60 ± 2.09	12.75 ± 1.11	10.09 ± 2.35	125.97 ± 7.21	80.04 ± 4.81	16.46 ± 2.59	8.97 ± 0.30
116	1.5	100	12.08 ± 0.49	18.50 ± 0.65	6.07 ± 1.65	161.10 ± 19.41	76.27 ± 12.46	21.59 ± 4.58	9.54 ± 0.50
116	1.8	20	35.88 ± 1.73	0.05 ± 0.03	3.69 ± 0.95	61.08 ± 2.77	24.16 ± 3.22	6.32 ± 0.87	5.54 ± 0.22
116	1.8	50	27.36 ± 2.03	8.55 ± 0.87	7.06 ± 1.91	150.17 ± 9.62	88.32 ± 3.96	21.75 ± 4.52	8.91 ± 0.34
116	1.8	100	17.50 ± 1.26	10.40 ± 1.08	8.97 ± 0.19	185.69 ± 12.38	89.60 ± 4.61	24.73 ± 4.61	8.88 ± 0.20
200	0.9	20	17.98 ± 1.47	18.25 ± 2.72	3.95 ± 1.58	49.31 ± 5.97	24.52 ± 5.62	6.01 ± 1.70	7.08 ± 0.25
200	0.9	50	11.76 ± 0.97	27.50 ± 1.55	3.88 ± 0.98	83.90 ± 8.41	45.36 ± 9.78	10.57 ± 2.61	9.51 ± 0.44
200	0.9	100	7.52 ± 0.53	30.50 ± 1.94	4.55 ± 1.37	100.68 ± 6.71	58.15 ± 9.29	11.80 ± 2.64	9.67 ± 0.25
200	1.25	20	26.34 ± 0.77	4.50 ± 0.96	5.66 ± 2.23	67.70 ± 1.59	34.52 ± 1.63	6.39 ± 1.30	6.27 ± 0.53
200	1.25	50	19.12 ± 2.27	20.25 ± 2.29	6.97 ± 0.52	119.81 ± 13.24	69.99 ± 6.51	17.15 ± 3.09	9.21 ± 0.23
200	1.25	100	10.16 ± 0.90	23.25 ± 0.95	7.08 ± 0.36	150.46 ± 15.31	79.87 ± 6.78	18.41 ± 2.97	9.54 ± 0.19
200	1.5	20	36.72 ± 1.56	1.29 ± 0.31	3.08 ± 0.40	73.42 ± 4.73	31.53 ± 3.49	8.05 ± 1.43	5.85 ± 0.53
200	1.5	50	24.50 ± 1.86	15.00 ± 1.02	8.12 ± 0.27	134.06 ± 9.77	75.52 ± 3.66	26.27 ± 2.71	9.22 ± 0.15
200	1.5	100	13.22 ± 0.59	15.74 ± 1.31	6.95 ± 0.56	160.52 ± 3.95	66.84 ± 5.00	25.60 ± 2.15	9.23 ± 0.10

2.3. Gel time — in situ-based gelation

Alginate samples were reacted with 20–100 mM calcium carbonate (Sigma, St. Louis, MO) and 80 mM GDL (Sigma, St. Louis, MO) to initiate gelation. The gel time was determined by reacting 0.75 ml of alginate with the addition of $CaCO_3$ and GDL powder at the specified concentration. Measurements were collected with a rheometer (RA 550, TA Instruments, New Castle, DE) via a time sweep program using a 40 mm 2° steel cone oscillating at a frequency of 1 Hz and a 1% strain at 25 °C. The gel times were determined as the time required for the storage modulus (G') to exceed the loss modulus (G'') after adding both the $CaCO_3$ and GDL to the alginate solution as described by Shchipunov et al. (2002). The gel time studies were conducted at room temperature to maintain consistency with the other experiments in this study. A full factorial design was employed to determine the effect of $CaCO_3$ concentration, alginate molecular weight, and alginate concentration. Five specimens of each condition were tested, and the averages and standard errors were calculated.

2.4. Syneresis

Syneresis is macroscopically characterized by a slow, time-dependent de-swelling of a gel, resulting in an exudation of liquid (Draget et al., 2001). The percentage of syneresis was determined by measuring the amount of fluid remaining in the gel mold 24 h after mixing the alginate and $CaCl_2$ solutions or $CaCO_3$ and GDL powders at room temperature using Eq. (1):

$$\text{Syneresis} = 100 - 100 \times \frac{V_r}{V_t}, \tag{1}$$

where V_r is the volume of fluid removed and V_t is the total initial volume of alginate and $CaCl_2$ combined (Becker et al., 2001). A full factorial design was employed to determine the effect of $CaCl_2$ or $CaCO_3$ concentration, alginate molecular weight, and alginate concentration. Four specimens of each condition were tested, and the averages and standard errors were calculated.

2.5. Mechanical properties: compressive strength and compressive modulus

The mechanical properties of the alginate gels were tested using uniaxial compression (Instron 4502, Instron Corporation, Canton, MA). The compressive strength and modulus were tested at room temperature, as it has previously been shown that there is no significant difference in either measurement over the range 25–37 °C (Andresen and Smidsrod, 1977). Compressive testing was completed on 8 mm thick gels using a crosshead speed of 4.8 mm/min, compressing the samples to 95%. Individual compressions did not exceed a maximum force of 2200 N (6800 kPa) (Becker et al., 2001; Kuo and Ma, 2001). All samples had a surface area greater than that of the cylindrical load cell so that a resistive pressure could be calculated by dividing the force reading by the load cell surface area. The diameter of the load cell was 31.7 mm, and the diameter of the samples was a minimum of 32 mm. The compressive strength was determined graphically from the resistive pressure versus compression (%) for each sample to directly compare the various alginate samples (Becker et al., 2001). The compression was calculated via Eq. (2):

$$\text{Compression (\%)} = 100 \times \frac{t_i - t_f}{t_i}, \tag{2}$$

where t_i is the initial gel thickness and t_f is the gel thickness after compression (Becker et al., 2001).

In this study, 10% and 60% compression were chosen as the values for comparison. These compression values were chosen because: (1) alginate has a high elasticity and typically exceeds intracranial pressure (1 kPa) at the 10% compression point without degrading, (2) although alginate does not exhibit a pronounced fracture point due its high elasticity and water content, 60% compression was found to be at or near the elastic limit of most alginates. A full factorial experimental design was used to test all of the gelling conditions thoroughly. Four specimens were tested for each condition combination. The average compressive strengths at 10% and 60%, and respective standard errors were calculated.

The compressive modulus of the alginate was calculated from the slope of the linear regions of the compressive strength curves for each gel condition. A full factorial experimental design was used to test all of the gelling conditions thoroughly. Four specimens were tested for each condition combination. Average compressive moduli and respective standard errors were calculated.

2.6. Viscoelasticity: complex modulus and loss angle

The viscoelastic behavior of the cross-linked alginate was tested with a parallel plate rheometer (AR 550, TA Instruments, New Castle, DE) using a 25 mm plate at 25 °C. The alginate samples were compressed to 60% and subjected to a 1% strain across a frequency sweep of 1–100 rad/s at each level. Sandpaper (150-grit) was placed on the plate surfaces to minimize the occurrence of slip at the plate/sample interface. The storage (G') and loss moduli (G'') were calculated by the rheometer and recorded for further analysis.

The complex modulus (G^*), which represents the frequency-dependent stiffness of the hydrogel, was obtained from (G') and (G'') as seen in Eq. (3):

$$G^* = \sqrt{G'^2 + G''^2}. \tag{3}$$

The loss angle, δ, which provides a relative measure of viscous effects to elastic effects in a material, was obtained from Eq. (4):

$$\delta = \tan^{-1}\left(G''/G'\right). \tag{4}$$

Low values of δ indicate minimal internal damping, a result of energy dissipation and internal friction in deformation cycles ($\delta = 0°$, elastic solid; $\delta = 90°$, Newtonian viscous fluid) (LeRoux et al., 1999). Alginate's internal cross-links and entrapped entanglements likely contribute to the elastic behavior of the gel while other physical mechanisms, such as slippage at the gel–plate interface, can contribute to viscous behaviors. The storage and loss moduli, and therefore G^* and δ, typically depend on frequency (LeRoux et al., 1999). For application as a dural sealant, the frequency range of 1–5 Hz was chosen to

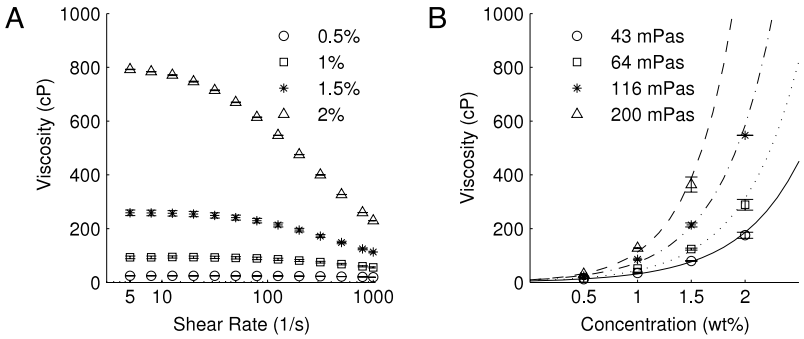

Fig. 1 – The liquid viscosity of an alginate solution is a function of molecular weight, concentration, and shear rate. (A) Alginate solutions exhibit shear thinning. Results for 64 mPa s alginate (0.5–2 wt%) over a shear rate range of 5–1000 s^{-1}. (B) The liquid viscosity increases with both alginate apparent viscosity and concentration. Liquid viscosities at a shear rate of 125 s^{-1}. Results for all alginates 43, 64, 116, and 200 mPa s) at all concentrations tested (0.5, 1, 1.5, and 2 wt%). All results are reported as the average liquid viscosity ± standard error, $n = 6$.

represent the typical heart rate frequency range of humans and rats. A full factorial designed experiment was employed and five specimens were tested for each condition. Average complex moduli and loss angles and standard errors were calculated at 1 Hz.

2.7. Statistics

ANOVA was utilized to determine the relative contributions of each independent variable. Computations were performed using the R statistics program (R Development Core Team, Vienna, Austria) (R Development Core Team, 2005) to determine the significance of the effect of each of the testing variables (alginate molecular weight, alginate concentration, and $CaCl_2$ or $CaCO_3$ concentration) on the measured outcomes. The effects of each variable as well as their interactions were calculated, and the resulting statistics are reported in this study.

The experimental variance of the compressive response of $CaCO_3$-reacted alginate was compared with that of $CaCl_2$-reacted alginate. By experimental design, each experimental condition was replicated four times. We hypothesized that the variance within each replicate is lower for the $CaCO_3$-reacted alginate than for the $CaCl_2$-reacted alginate. In order to ensure that the variances were not artificially inflated by differences in means (Montgomery et al., 1998), the data for each experimental condition were normalized by dividing the standard deviation of the four replicates of the condition by the mean of the replicates. These normalized data were then analyzed for a significant difference between the $CaCO_3$-reacted and $CaCl_2$-reacted alginates via a *t*-test.

3. Results

3.1. Rheology

The liquid viscosity of each alginate was dependent on both the molecular weight and the concentration of the alginate solution. All of the alginates tested exhibited shear thinning for increasing shear rates (Fig. 1(A)). The various alginate solutions were compared at a shear rate of 125 s^{-1} to determine the effect of alginate molecular weight and concentration on the viscosity (Fig. 1(B)). The following exponential equations were fit to each data set (Eqs. (5)–(8)):

$$V_{43} = 5.43e^{1.766*C}, \quad (5)$$

$$V_{64} = 6.45e^{1.94*C}, \quad (6)$$

$$V_{116} = 9.51e^{2.06*C}, \quad (7)$$

$$V_{200} = 9.65e^{2.46*C}, \quad (8)$$

where V is the liquid viscosity, the subscript numbers refer to the alginate apparent viscosity, and C represents the alginate concentration in wt%. The values resulting from Eqs. (5)–(8) all strongly correlate to the raw data ($R^2 = 0.99$). The liquid viscosity curves allowed for viscosity matching of the four different alginates in subsequent experiments. The viscosities resulting for 1.5 wt% solutions of 43, 116, and 200 mPa s were 87.9, 208.6, and 386.7 cP, respectively. These liquid viscosities were chosen to determine the equivalent concentrations of each alginate batch to use in subsequent experiments (43 mPa s: 1.5, 1.95, and 2.27 wt%; 64 mPa s: 1.35, 1.79, and 2.11 wt%; 116 mPa s: 1.08, 1.5, and 1.8 wt%; 200 mPa s: 0.9, 1.25, and 1.5 wt%).

3.2. Gel rate — diffusion-based gelation

The gel rate was used to determine how quickly the hydrogel would seal a dural defect. The rate of gel formation was determined colorimetrically using the calcium chelator, murexide (Fig. 2(A)). The gel time was dependent on the $CaCl_2$ concentration ($p < 10^{-15}$, Fig. 2(B)), the alginate concentration ($p < 0.05$, Fig. 2(C)), and the alginate molecular weight ($p < 10^{-4}$, Fig. 2(D)). A significant interaction between the $CaCl_2$ concentration and the alginate concentration was also found ($p < 0.005$). The mean 50% gel time and standard error for all combinations are reported in Table 1.

3.3. Gel time — in situ-based gelation

The gel time was highly dependent on the $CaCO_3$ concentration ($p < 10^{-15}$, Fig. 3(B)), the alginate concentration ($p < 10^{-15}$, Fig. 3(C)), and the alginate molecular weight ($p < 10^{-15}$,

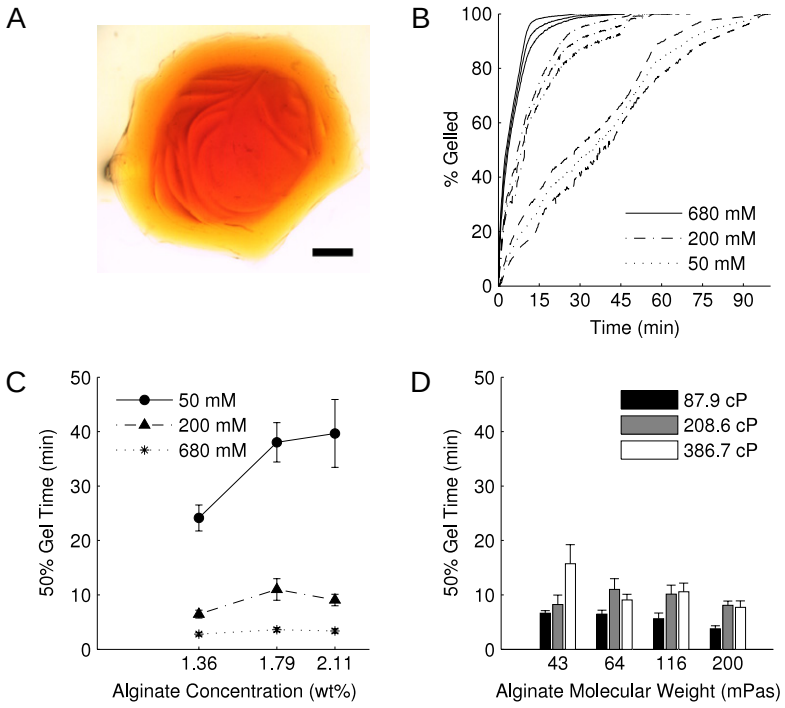

Fig. 2 – The gel time is dependent on the $CaCl_2$ concentration, the alginate concentration, and the alginate molecular weight. (A) Murexide is an indicator of an alginate:calcium complex and can be used to determine the gel rate as seen in the micrograph demonstrating murexide as an indicator of alginate cross-linked with calcium ions. The unreacted alginate is a dark-red color that changes to yellow as the carboxyl groups interact with the free calcium ions. The scale bar represents 1 mm. (B) The gel time is dependent on the $CaCl_2$ concentration ($p < 10^{-15}$). Resulting gel curves for 1.25 wt% 200 mPa s alginate reacted with $CaCl_2$ (50, 200, and 680 mM). The lines above and below each curve represents the standard error, $n = 5$. (C) The gel time is dependent on the alginate concentration ($p < 0.05$). Results for 64 mPa s alginate (1.36, 1.79, and 2.11 wt%) reacted with $CaCl_2$ (50, 200, and 680 mM). (D) The gel time is dependent on the alginate molecular weight ($p < 10^{-4}$). Results for all alginates (43, 64, 116, and 200 mPa s) at concentrations that viscosity match the samples at 87.9, 208.6, and 386.7 cP and reacted with 200 mM $CaCl_2$. All results are reported as the average 50% gel time ± standard error, $n = 5$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3(D)). The interaction between the $CaCO_3$ concentration and the alginate concentration was significant ($p < 0.05$), as well as the three-way interaction between the alginate molecular weight, the alginate concentration, and the $CaCO_3$ concentration ($p < 0.05$). Fig. 3(A) shows a representative gel curve. The gel times for each experimental combination of alginate molecular weight, alginate concentration, and $CaCO_3$ concentration are reported in Table 2.

3.4. *Syneresis*

Diffusion-gelled alginate hydrogels exhibited high and varying levels of syneresis dependent on the experimental parameters. The syneresis was dependent on the $CaCl_2$ concentration ($p < 10^{-15}$, Fig. 4(A)), the alginate concentration ($p < 10^{-14}$, Fig. 4(B)), and the alginate molecular weight ($p < 10^{-8}$, Fig. 4(C)). Significant interactions between the alginate molecular weight and the $CaCl_2$ concentration ($p < 10^{-4}$) and the $CaCl_2$ concentration and the alginate concentration ($p < 0.001$) were also found. The average syneresis level and standard error for all diffusion-gelled hydrogel experimental combinations are reported in Table 1.

In situ-gelled alginate showed a low but varying amount of syneresis dependent upon the various alginate and $CaCO_3$ parameters. The syneresis was dependent on the $CaCO_3$ concentration ($p < 10^{-15}$, Fig. 5(A)), the alginate concentration ($p < 10^{-15}$, Fig. 5(B)), and the alginate molecular weight ($p < 10^{-15}$, Fig. 5(C)). We found significant interactions between the alginate molecular weight and the $CaCO_3$ concentration ($p < 10^{-4}$), the alginate molecular weight and the alginate concentration ($p < 10^{-6}$), and the $CaCO_3$ concentration and the alginate concentration ($p < 10^{-4}$), as well as a three-way interaction between the alginate molecular weight, the alginate concentration, and the $CaCO_3$ concentration ($p < 0.001$). The average syneresis level and standard error for all *in situ*-gelled hydrogel experimental combination are reported in Table 2.

3.5. *Mechanical properties: compressive strength and compressive modulus*

The dependences of the compressive strength of diffusion-gelled alginate on the $CaCl_2$ concentration, alginate concentration, and alginate molecular weight are seen in Fig. 6.

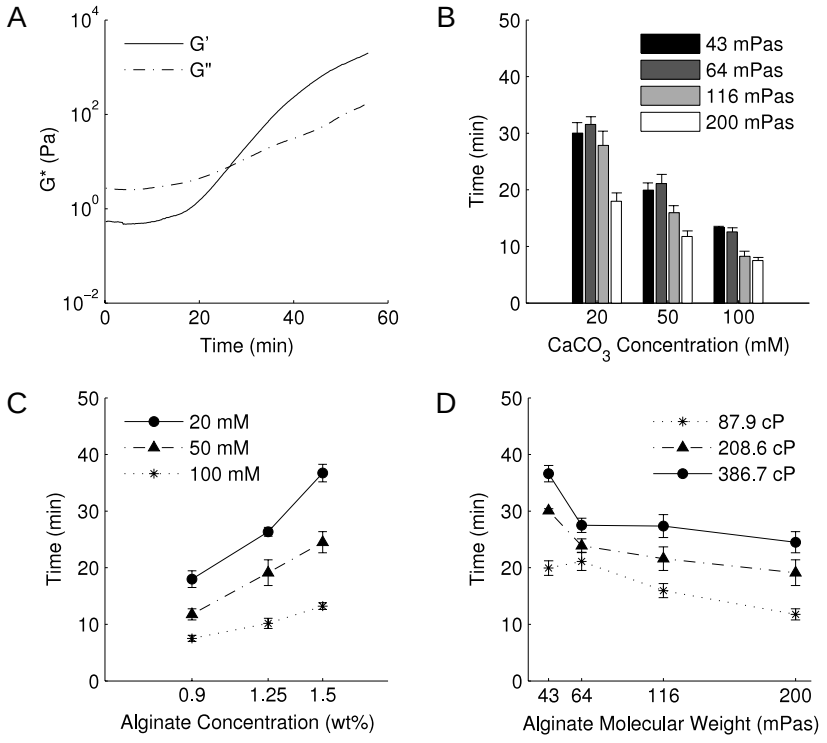

Fig. 3 – The gel time is dependent on the $CaCO_3$ concentration, the alginate concentration, and the alginate molecular weight. (A) The gel time was determined as the point when G' exceeds G'' under a shear rate of 1 Hz and 1% strain as seen in the representative gelation curve for 1.5 wt% 200 mPa s alginate with 50 mM $CaCO_3$. (B) The gel time decreases with increasing $CaCO_3$ concentration ($p < 10^{-15}$). Results for all alginate molecular weights (43, 64, 116, and 200 mPa s) viscosity matched at 87.9 cP and reacted with $CaCO_3$ (20, 50, and 100 mM). (C) The gel time increases with increasing alginate concentration ($p < 10^{-15}$). Results for 200 mPa s alginate (0.9, 1.25, and 1.5 wt%) reacted with $CaCO_3$ (20, 50, and 100 mM). (D) The gel time decreases with increasing alginate molecular weight ($p < 10^{-15}$). Results for all alginates (43, 64, 116, and 200 mPa s) at concentrations that viscosity match the samples at 87.9, 208.6, and 386.7 cP and reacted with 50 mM $CaCO_3$. All results are reported as the average gel time ± standard error, $n = 5$.

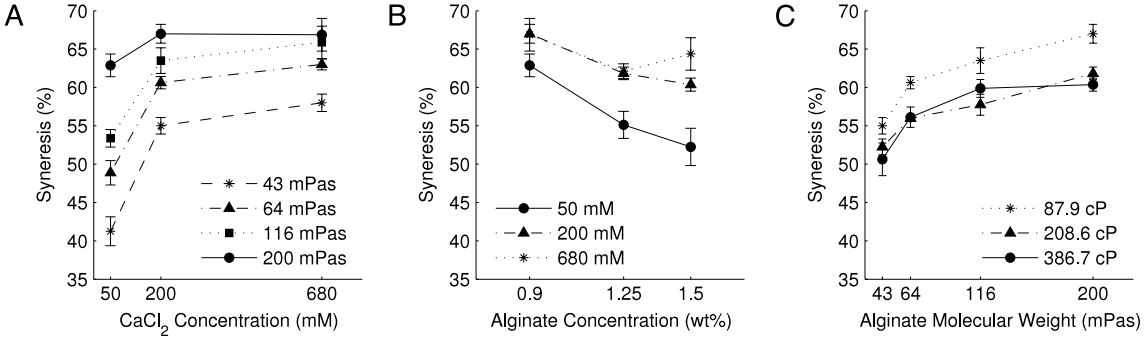

Fig. 4 – The syneresis of diffusion-gelled alginate hydrogels is dependent on the $CaCl_2$ concentration, the alginate concentration, and the alginate molecular weight. (A) The syneresis is dependent on the $CaCl_2$ concentration ($p < 10^{-15}$). Results for all alginate molecular weights (43, 64, 116, and 200 mPa s) viscosity matched at 87.9 cP and reacted with $CaCl_2$ (50, 200, and 680 mM). (B) The syneresis is dependent on the alginate concentration ($p < 10^{-14}$). Results for 200 mPa s alginate (0.9, 1.25, and 1.5 wt%) reacted with $CaCl_2$ (50, 200, and 680 mM). (C) The syneresis is dependent on the molecular weight ($p < 10^{-8}$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9, 208.6, and 386.7 cP and reacted with 200 mM $CaCl_2$. All results are reported as the average syneresis level ± standard error, $n = 4$.

The gel strength was dependent on the $CaCl_2$ concentration ($p < 10^{-4}$, Fig. 6(A) and (D)) and the alginate concentration ($p < 0.01$, Fig. 6(B) and (E)), but the molecular weight did not have a statistically significant effect ($p = 0.89$, Fig. 6(C), and (F)). A significant interaction between the alginate molecular weight and the $CaCl_2$ concentration ($p < 0.05$) was found

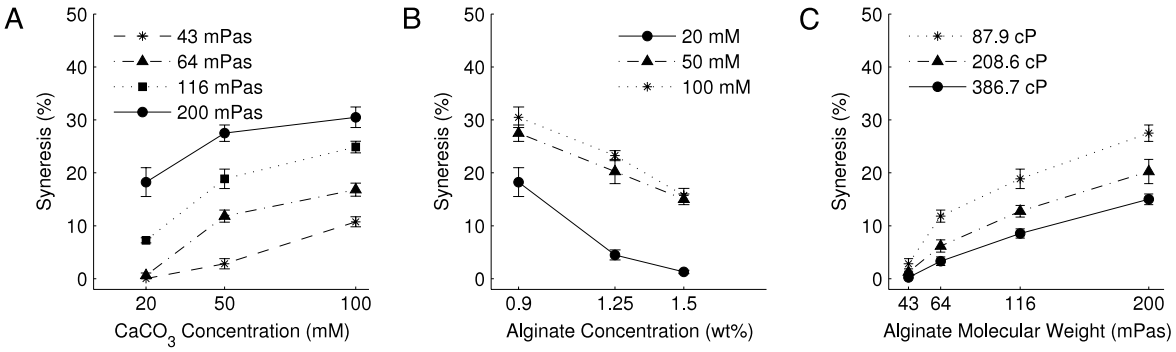

Fig. 5 – The syneresis of *in situ*-gelled alginate hydrogels is dependent on the $CaCO_3$ concentration, the alginate concentration, and the alginate molecular weight. (A) The syneresis increases with increasing $CaCO_3$ concentration ($p < 10^{-15}$). Results for all alginate molecular weights (43, 64, 116, and 200 mPa s) viscosity matched at 87.9 cP and reacted with $CaCO_3$ (20, 50, and 100 mM). (B) The syneresis decreases with increasing alginate concentration ($p < 10^{-15}$). Results for 200 mPa s alginate (0.9, 1.25, and 1.5 wt%) reacted with $CaCO_3$ (20, 50, and 100 mM). (C) The syneresis increases with increasing molecular weight ($p < 10^{-15}$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9, 208.6, and 386.7 cP and reacted with 50 mM $CaCO_3$. All results are reported as the average syneresis level ± standard error, $n = 4$.

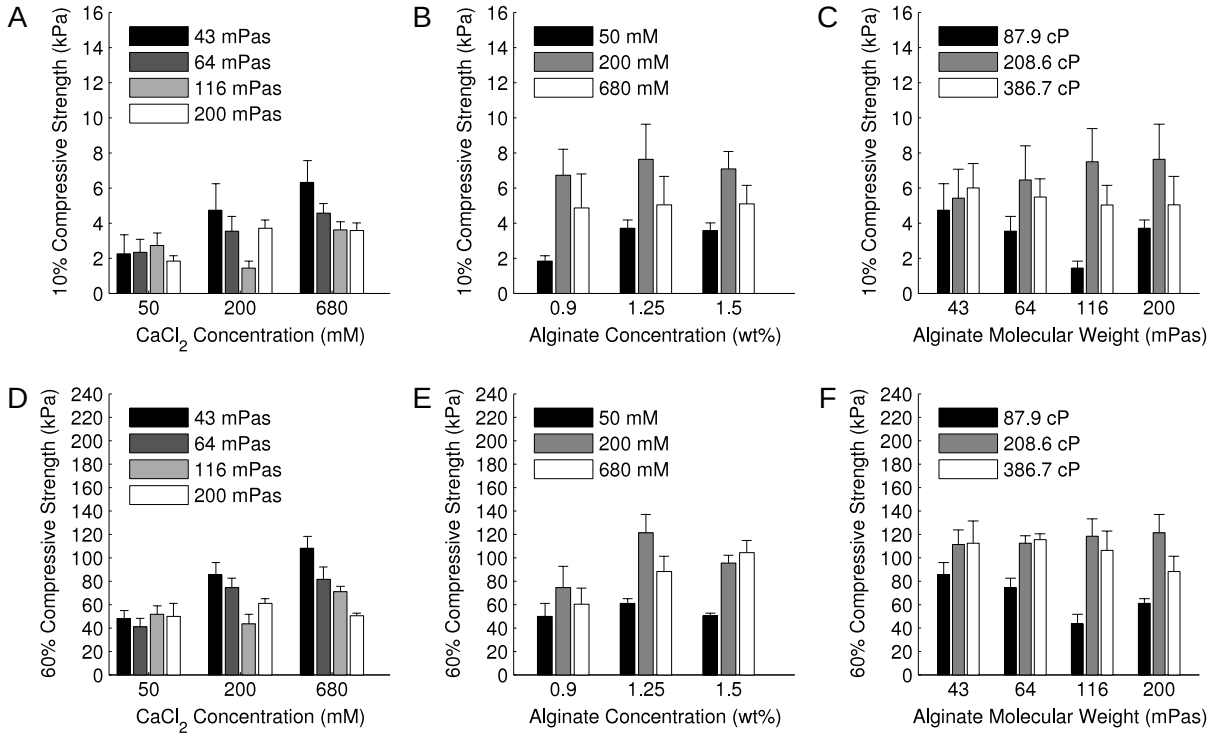

Fig. 6 – The compressive strength of diffusion-gelled alginate is dependent on the $CaCl_2$ concentration and the alginate concentration. (A)–(C) Results are reported as the average compressive strengths at 10% compression ± standard error, $n = 4$. (A) The gel strength is dependent on the $CaCl_2$ concentration ($p < 10^{-4}$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9 cP and reacted with $CaCl_2$ (50, 200, and 680 mM). (B) The compressive strength is dependent on the alginate concentration ($p < 0.01$). Results for 200 mPa s alginate (0.9, 1.25, and 1.5 wt%) reacted with $CaCO_3$ (50, 200, and 680 mM). (C) The alginate molecular weight has no significant effect on the compressive strength ($p = 0.89$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9, 208.6, and 386.7 cP and reacted with 200 mM $CaCl_2$. (D)–(F) Results are reported as the average compressive strengths at 60% compression ± standard error, $n = 4$. (D) The gel strength is dependent on the $CaCl_2$ concentration ($p < 10^{-15}$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9 cP and reacted with $CaCl_2$ (50, 200, and 680 mM). (E) The compressive strength is dependent on the alginate concentration ($p < 10^{-5}$). Results for 200 mPa s alginate (0.9, 1.25, and 1.5 wt%) reacted with $CaCl_2$ (50, 200, and 680 mM). (F) The alginate molecular weight has no significant effect on the compressive strength ($p = 0.066$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9, 208.6, and 386.7 cP and reacted with 200 mM $CaCl_2$.

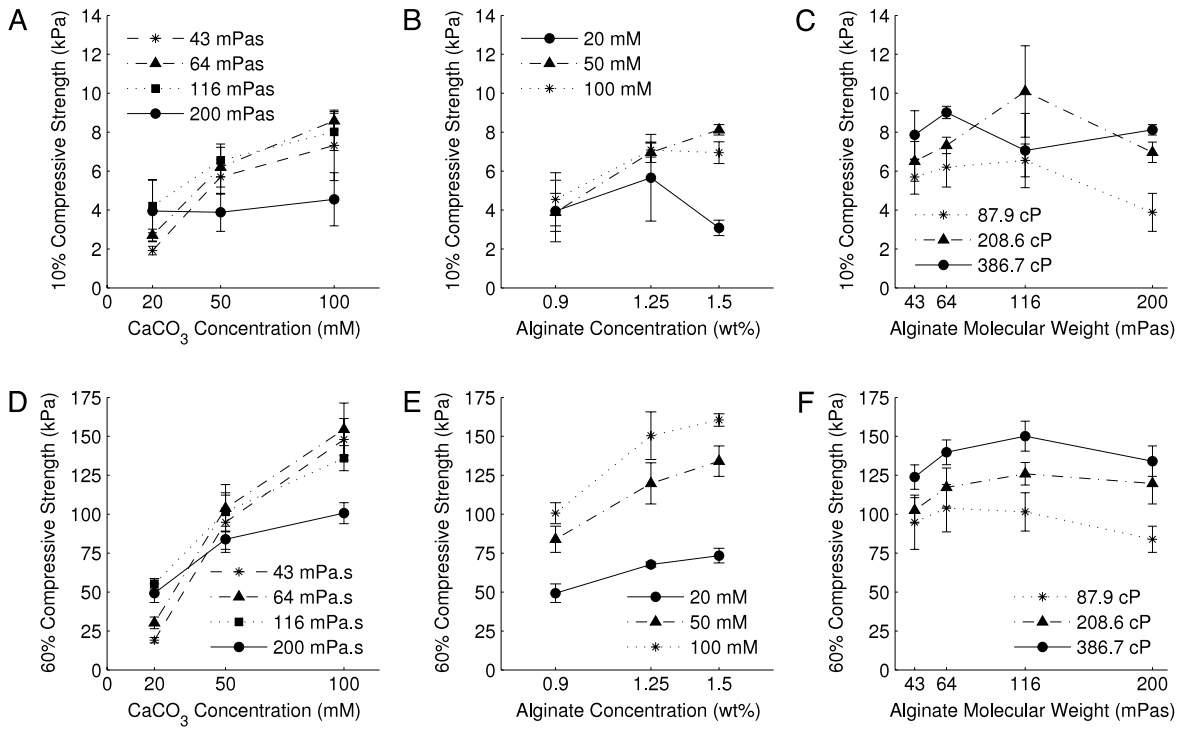

Fig. 7 – The compressive strength of *in situ*-gelled alginate is dependent on the $CaCO_3$ concentration and the alginate concentration. (A)–(C) Results are reported as the average compressive strengths at 10% compression ± standard error, $n = 4$. (A) The gel strength increases with increasing $CaCO_3$ concentration ($p < 10^{-12}$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9 cP and reacted with $CaCO_3$ (20, 50, and 100 mM). (B) The compressive strength increases with increasing alginate concentration ($p < 0.05$). Results for 200 mPa s alginate (0.9, 1.25, and 1.5 wt%) reacted with $CaCO_3$ (20, 50, and 100 mM). (C) The alginate molecular weight has no significant effect on the compressive strength ($p = 0.39$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9, 208.6, and 386.7 cP and reacted with 50 mM $CaCO_3$. (D)–(F) Results are reported as the average compressive strengths at 60% compression ± standard error, $n = 4$. (D) The gel strength increases with increasing $CaCO_3$ concentration ($p < 10^{-15}$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9 cP and reacted with $CaCO_3$ (20, 50, and 100 mM). (E) The compressive strength increases with increasing alginate concentration ($p < 10^{-4}$). Results for 200 mPa s alginate (0.9, 1.25, and 1.5 wt%) reacted with $CaCO_3$ (20, 50, and 100 mM). (F) The alginate molecular weight has no significant effect on compressive strength ($p = 0.052$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9, 208.6, and 386.7 cP and reacted with 50 mM $CaCO_3$.

at both compression levels. Additionally, a significant interaction between the alginate concentration and the $CaCl_2$ concentration ($p < 10^{-4}$) was also found at the 60% compression level. The average compressive strength and standard error for all diffusion-gelled combinations at both 10% and 60% compression are reported in Table 1.

The compressive strength of *in situ*-gelled alginate increased with increasing concentrations of $CaCO_3$ and alginate. The gel strength was dependent on the $CaCO_3$ concentration ($p < 10^{-12}$, Fig. 7(A) and (D)) and the alginate concentration ($p < 0.05$, Fig. 7(B) and (E)), but the molecular weight did not have a statistically significant effect ($p = 0.39$, Fig. 7(C) and (F)) for both compression levels. A significant interaction was found between the alginate molecular weight and the $CaCO_3$ concentration ($p < 0.01$) for both compression levels. Additionally, a significant interaction between the alginate molecular weight and the alginate concentration ($p < 10^{-6}$) was also found at the 60% compression level. The average compressive strength and standard error for all *in situ*-gelled combinations at both 10% and 60% compression are reported in Table 2.

The variance in compression strength at 10% and 60% compression was greater for diffusion-gelled alginate. The normalized variance for the 36 $CaCO_3$-reacted alginate reaction conditions (0.367 ± 0.067%) was significantly less than that calculated for $CaCl_2$-reacted alginate (0.504 ± 0.031%) at 10 % compression ($p < 0.01$). Similarly, at the 60% compression level the normalized $CaCO_3$-reacted alginate variance (0.163 ± 0.006%) was significantly less than that for $CaCl_2$-reacted alginate (0.282 ± 0.015%, $p < 10^{-5}$).

The dependences of the compressive modulus of diffusion-gelled alginate on the $CaCl_2$ concentration, the alginate concentration, and the alginate molecular weight are seen in Fig. 8. The modulus was dependent on the $CaCl_2$ concentration ($p < 10^{-4}$, Fig. 8(A)) and the alginate concentration ($p < 0.005$, Fig. 8(B)), but the alginate molecular weight had no statistically significant effect ($p = 0.82$, Fig. 8(C)). A significant interaction between the alginate molecular weight and the $CaCl_2$ concentration ($p < 0.05$) was also determined from the collected data. The average compressive modulus and standard error for all diffusion-gelled combinations are reported in Table 1.

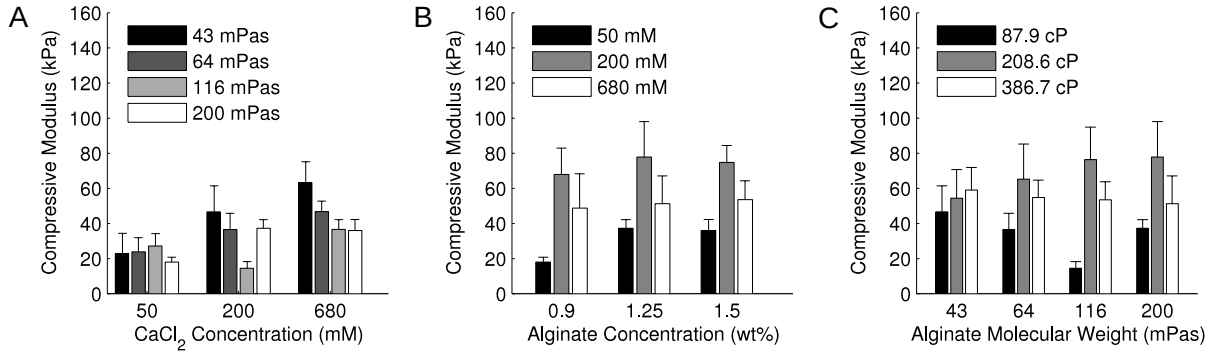

Fig. 8 – The compressive modulus of diffusion-gelled alginate is dependent on the $CaCl_2$ concentration and the alginate concentration. (A) The compressive modulus is dependent on the $CaCl_2$ concentration ($p < 10^{-4}$). All alginates (43, 64, 116, and 200 mPa s) were viscosity matched at 87.9 cP and reacted with $CaCl_2$ (50, 200, and 680 mM). (B) The compressive modulus is dependent on the alginate concentration ($p < 0.005$). Results for 200 mPa s alginate (0.9, 1.25, and 1.5 wt%) reacted with $CaCl_2$ (50, 200, and 680 mM). (C) The alginate molecular weight has no significant effect on the compressive modulus ($p = 0.82$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9, 208.6, and 386.7 cP and reacted with 200 mM $CaCl_2$. All results are reported as the average compressive modulus ± standard error, $n = 4$.

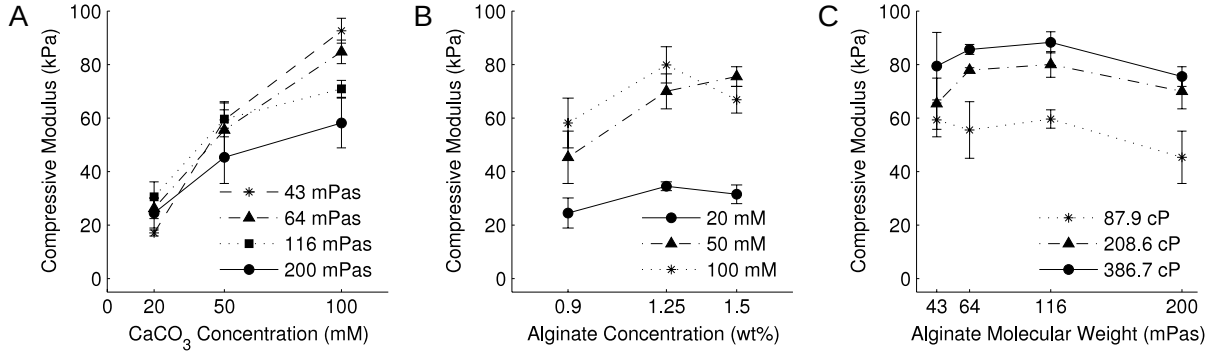

Fig. 9 – The compressive modulus of *in situ*-gelled alginate is dependent on the $CaCO_3$ concentration and the alginate concentration. (A) The compressive modulus increases with increasing $CaCO_3$ concentration ($p < 10^{-15}$). All alginates (43, 64, 116, and 200 mPa s) were viscosity matched at 87.9 cP and reacted with $CaCO_3$ (20, 50, and 100 mM). (B) The compressive modulus increases with increasing alginate concentration ($p < 0.001$). Results for 200 mPa s alginate (0.9, 1.25, and 1.5 wt%) reacted with $CaCO_3$ (20, 50, and 100 mM). (C) The alginate molecular weight has no significant effect on the compressive modulus ($p = 0.09$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9, 208.6, and 386.7 cP and reacted with 50 mM $CaCO_3$. All results are reported as the average compressive modulus ± standard error, $n = 4$.

Similarly, the compressive modulus of *in situ*-gelled alginate was dependent on the $CaCO_3$ concentration ($p < 10^{-15}$, Fig. 9(A)) and the alginate concentration ($p < 10^{-4}$, Fig. 9(B)); however, the alginate molecular weight had no statistically significant effect ($p = 0.095$, Fig. 9(C)). Significant interactions between the alginate molecular weight and the $CaCO_3$ concentration ($p < 10^{-4}$) and the $CaCO_3$ concentration and the alginate concentration ($p < 0.05$) were also found. Additionally, the variance in the compression modulus was significantly greater for $CaCl_2$-reacted alginate (0.509 ± 0.035%) than for $CaCO_3$-reacted alginate (0.22 ± 0.017%, $p < 10^{-8}$). The average compressive modulus and standard error for all *in situ*-gelled combinations are reported in Table 2.

3.6. *Viscoelasticity: complex modulus and loss angle*

The complex modulus and loss angle tested at 60% compression varied with the experimental parameters for diffusion-gelled alginate hydrogels. The frequency dependences of both G^* and δ are seen in Fig. 10(A). Although the $CaCl_2$ concentration had no statistically significant effect on G^* ($p = 0.41$, Fig. 10(B)), G^* was dependent on the alginate concentration ($p < 10^{-14}$, Fig. 10(C)), and the alginate molecular weight ($p < 10^{-15}$, Fig. 10(D)). A significant interaction between the alginate molecular weight and the alginate concentration ($p < 0.05$) and the alginate concentration and the $CaCl_2$ concentration ($p < 0.01$) was also found. There was significant dependence of δ on the $CaCl_2$ concentration ($p < 0.05$, Fig. 11(A)), the alginate concentration ($p < 0.005$, Fig. 11(B)), and the alginate molecular weight ($p < 10^{-5}$, Fig. 11(C)). The average G^* and δ and respective standard error for both measurands and all tested diffusion-gelled combination are reported in Table 1.

Slightly different results for G^* and δ were determined for *in situ*-gelled alginate. The complex modulus was dependent on the $CaCO_3$ concentration ($p < 10^{-15}$, Fig. 12(A)) and the alginate concentration ($p < 0.001$, Fig. 12(B)), but not on the alginate molecular weight ($p = 0.07$, Fig. 12(C)). Significant

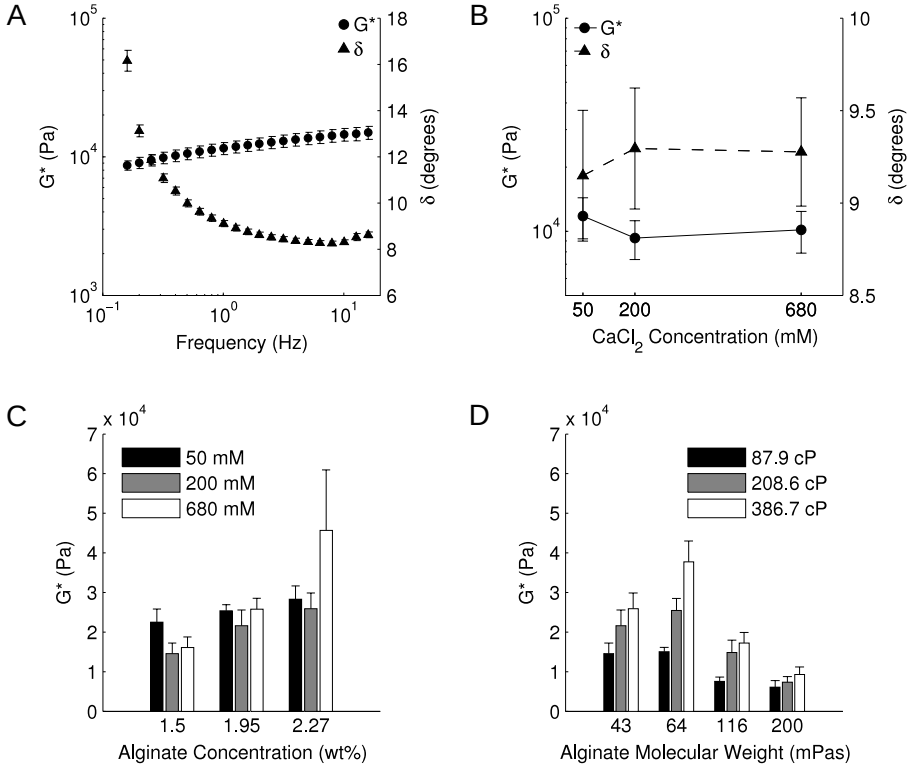

Fig. 10 – The complex modulus of diffusion-gelled alginate is dependent on the alginate concentration and the alginate molecular weight. (A) The complex modulus and the loss angle are both dependent on the frequency. This is representative frequency sweep data for 1.5 wt% 43 mPa s alginate reacted with 680 mM $CaCl_2$. (B) The complex modulus is not dependent on the $CaCl_2$ concentration ($p = 0.41$). Results for both G^* and δ for 1.5 wt% 200 mPa s alginate reacted with $CaCl_2$ (50, 200, and 680 mM). (C) The complex modulus is dependent on the alginate concentration ($p < 10^{-14}$). Results for 43 mPa s alginate (0.9, 1.25, and 1.5 wt%) reacted with $CaCO_3$ (20, 50, and 100 mM). (D) The complex modulus is dependent on the alginate molecular weight ($p < 10^{-15}$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9, 208.6, and 386.7 cP and reacted with 200 mM $CaCl_2$. All results are reported as the average complex modulus ± standard error at 1 Hz, $n = 5$.

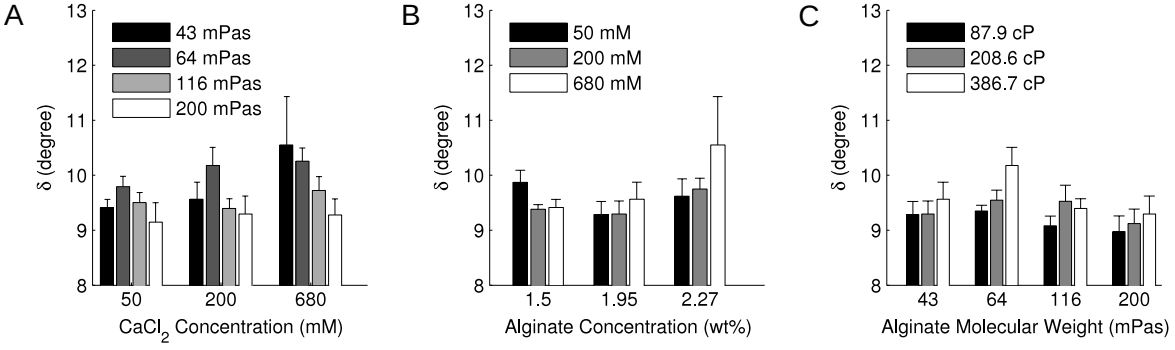

Fig. 11 – The loss angle of diffusion-gelled alginate is dependent on the $CaCl_2$ concentration, the alginate concentration, and the alginate molecular weight. (A) The loss angle is dependent on the $CaCl_2$ concentration ($p < 0.05$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 386.7 cP and reacted with $CaCl_2$ (50, 200, and 680 mM). (B) The loss angle is dependent on the alginate concentration ($p < 0.005$). Results for 43 mPa s alginate (1.5, 1.95, and 2.27 wt%) reacted with $CaCl_2$ (50, 200, and 680 mM). (C) The loss angle is dependent on the alginate molecular weight ($p < 10^{-5}$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9, 208.6, and 386.7 cP and reacted with 200 mM $CaCl_2$. All results are reported as the average loss angle ± standard error at 1 Hz, $n = 5$.

interactions between the alginate molecular weight and the $CaCO_3$ concentration ($p < 0.01$), the alginate molecular weight and the alginate concentration ($p < 0.05$), and the $CaCO_3$ concentration and the alginate molecular weight ($p < 0.05$)

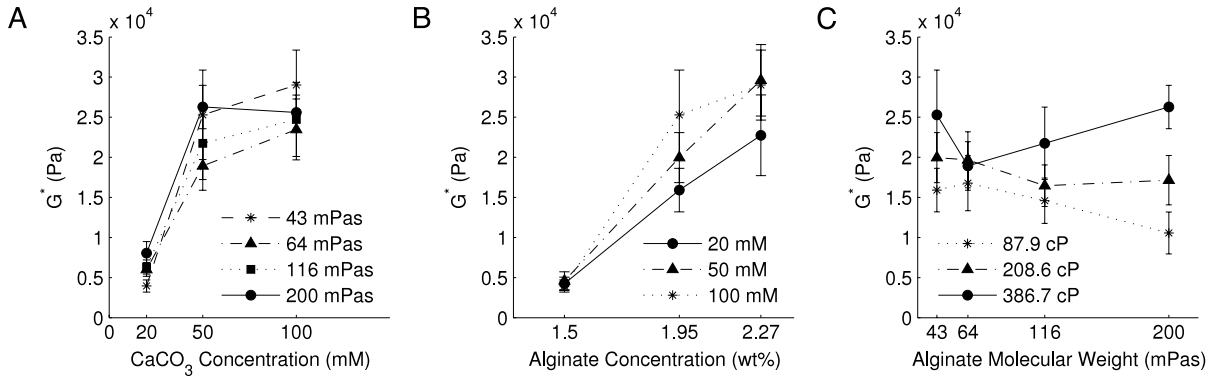

Fig. 12 – The complex modulus of *in situ*-gelled alginate is dependent on the $CaCO_3$ concentration and the alginate concentration. (A) The complex modulus increases with increasing $CaCO_3$ concentration ($p < 10^{-15}$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 386.7 cP and reacted with $CaCO_3$ (20, 50, and 100 mM). (B) The complex modulus increases with increasing alginate concentration ($p < 0.001$). Results for 43 mPa s alginate (1.5, 1.95, and 2.27 wt%) reacted with $CaCO_3$ (20, 50, and 100 mM). (C) The complex modulus was not significantly dependent on the alginate molecular weight ($p = 0.07$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9, 208.6, and 386.7 cP and reacted with 50 mM $CaCO_3$. All results are reported as the average complex modulus ± standard error at 1 Hz, $n = 5$.

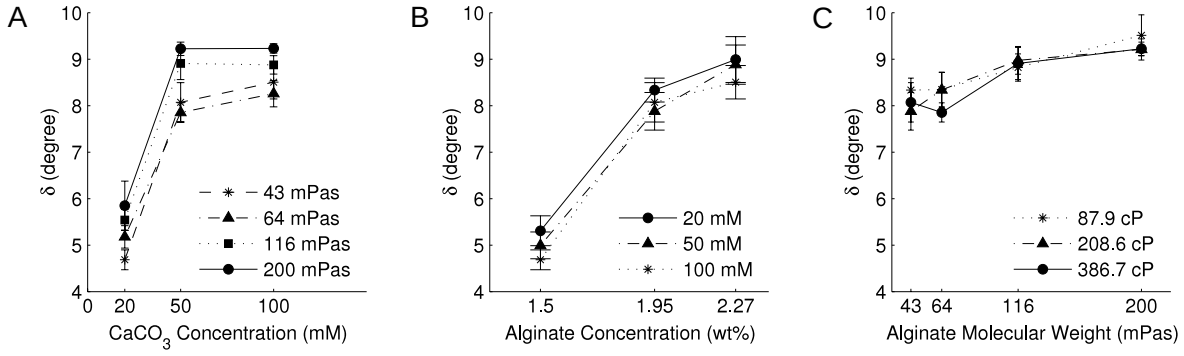

Fig. 13 – The loss angle of *in situ*-gelled alginate is dependent on the $CaCO_3$ concentration, the alginate concentration, and the alginate molecular weight. (A) The loss angle increases with increasing $CaCO_3$ concentration ($p < 10^{-15}$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 386.7 cP and reacted with $CaCO_3$ (20, 50, and 100 mM). (B) The loss angle increases with increasing alginate concentration ($p < 0.001$). Results for 43 mPa s alginate (1.5, 1.95, and 2.27 wt%) reacted with $CaCO_3$ (20, 50, and 100 mM). (C) The loss angle increases with increasing alginate molecular weight ($p < 10^{-6}$). Results for all alginates (43, 64, 116, and 200 mPa s) viscosity matched at 87.9, 208.6, and 386.7 cP and reacted with 50 mM $CaCO_3$. All results are reported as the average loss angle ± standard error at 1 Hz, $n = 5$.

were found for G^* at the 60% level. The loss angle was dependent on the $CaCO_3$ concentration ($p < 10^{-15}$, Fig. 13(A)), the alginate concentration ($p < 0.001$, Fig. 13(B)), and the alginate molecular weight ($p < 10^{-11}$, Fig. 13(C)). The average G^* and δ and respective standard error for both measurands and all tested *in situ*-gelled combination are reported in Table 2.

4. Discussion

To further investigate alginate as a potential dural sealant or dura mater replacement, the effects and interactions of alginate molecular weight, alginate concentration, and $CaCl_2$ or $CaCO_3$ concentration were identified. Specifically, the effects of these experimental parameters on the gel rate, syneresis, gel strength, compressive modulus, complex modulus, and loss angle were determined for gels in equilibrium.

The results aid in the identification of an ideal combination of alginate and $CaCl_2$ that produce a gel with ideal properties for small applications, such as a dural sealant. Based on the data collected in this study, we would suggest the use of 1.95 wt% 43 mPa s alginate with 200 mM $CaCl_2$ for approximation of minor dural defects with or without additional sutures. This combination results in a gel that forms in 8.23 ± 1.74 min, has a syneresis level of 52.25 ± 1.03%, a compressive modulus of 54.37 ± 16.32 kPa, a complex modulus of 21.62 ± 3.97 kPa, and a loss angle of 9.30 ± 0.24 degrees.

These results also suggest that specific mixing conditions of alginate and $CaCO_3$ result in a material that is sufficient for closing large dural defects. Furthermore, internally cross-linked hydrogels should be preferentially pursued over diffusion cross-linked gels for use as a dural replacement due

to lower levels of syneresis, higher levels of homogeneity, and lower loss angle values. Based on the data collected in this study, we would suggest the use of 1.95 wt% 43 mPa s alginate with 100 mM $CaCO_3$ and 80 mM GDL. This combination results in a gel that forms in 19.28 ± 1.87 min, has a syneresis level of 3.55 ± 0.68%, a compressive modulus of 85.35 ± 7.5 kPa, a complex modulus of 11.88 ± 1.52 kPa, and a loss angle of 6.82 ± 0.20 degrees.

To date, a number of groups have studied or modeled the gelation rate of alginate under a variety of conditions and methods (Bienaime et al., 2003; Blandino et al., 1999; Kuo and Ma, 2001; Liu et al., 2003; Mikkelsen and Elgsaeter, 1995; Smidsrod and Haug, 1972; Stokke et al., 2000; Wang and Spencer, 1998; Wang et al., 1995, 1993, 1994). We employed a recently described and relatively simple method from the literature to quantify the rate of gel formation (Bienaime et al., 2003). Murexide, a calcium chelator, indicates cross-link formation based on color, and it indicates the rate of $CaCl_2$ penetration into the liquid alginate volume. An excess of $CaCl_2$ solution was used to (1) mimic *in vivo* application of a dural sealant, and (2) accommodate any small changes in gel rate that could be attributed to the interaction of calcium with murexide instead of the carboxyl groups on the alginate chains. Although 200 ml of alginate was cross-linked with 500 ml $CaCl_2$ to simulate surgical use, it is possible that less alginate and $CaCl_2$ volume would be indicated, depending on the length of the dural interface requiring approximation. Less volume would thereby decrease the cross-link time, further decreasing the time to create a seal between the approximated tissues. These rapid cross-link times are highly desirable in dural repair because they reestablish the barrier with the external environment quickly. Our results indicate that both the $CaCl_2$ concentration and the alginate concentration have a significant effect on the gel rate ($p < 10^{-4}$), which supports the findings of previous investigations (Blandino et al., 1999). We identified that the alginate molecular weight, as well as the interaction between the $CaCl_2$ concentration and the alginate concentration both contribute significantly to the gel time. This finding has not been previously reported, and current theoretical models for diffusion-based cross-linking of alginate do not accommodate these parameters (Blandino et al., 1999; Mikkelsen and Elgsaeter, 1995). Further investigation may result in experimentally derived models of the mathematical relationship between the alginate molecular weight, alginate concentration, $CaCl_2$ concentration, and the interaction of the $CaCl_2$ concentration and the alginate concentration.

In situ gelation of alginate can have a highly variable gel time (minutes to days) (Kuo and Ma, 2001); thus, the gel time is a potentially important limitation in the development of an alginate dural replacement patch. Previously, Shchipunov et al. employed rheology to investigate the relationship between GDL concentration, pH, and gel rate with alginate, and they developed a pseudophase diagram based on GDL and $CaCO_3$ concentration (Shchipunov et al., 2002). Based on this model, we were able to choose a range of $CaCO_3$ concentrations and an ideal GDL concentration, and measure gel times of 8–55 min for combinations of alginate and $CaCO_3$ with 80 mM GDL (Table 2). These results not only corroborate the findings of several research groups (Draget et al., 1991; Kuo and Ma, 2001; Shchipunov et al., 2002; Smidsrod and Haug, 1972), but additionally identify the effects due to alginate molecular weight and concentration. Furthermore, several of the gelation times for $CaCO_3$ are rapid enough to use in select dura mater repair applications. Although the gelation rate of alginate with $CaCO_3$ is slower than that of $CaCl_2$, the components can be mixed and then applied as a single viscous solution to a large dural defect. Site-specific application can be further improved by waiting for the alginate:$CaCO_3$-reacted mixture to reach a high, but still useable, viscosity (~400 cP) before filling the dural defect.

Hydrogel syneresis is an indicator of the level of gel shrinkage that could result in a malformed seal between the gel and the surrounding meningeal tissue. A defective seal could allow gaps to form between the dura mater and the alginate, allowing a pathway for pathogen entry and cerebrospinal fluid leakage. As discussed previously, increasing the calcium concentration expedites the cross-linking process and leads to a decreased gelling time and an increased cross-link density (Draget et al., 2001, 2003). However, it is this cross-link density that is responsible for the occurrence of syneresis, a slow time-dependent exudation of liquid from the hydrogel (Draget et al., 2001; Shchipunov et al., 2002). $CaCO_3$-reacted alginate demonstrated low levels of syneresis (<10%) for most of the tested combinations, indicating good long-term sealing potential as a dural replacement. $CaCl_2$, however, demonstrated high levels of syneresis (>25%) for all tested combinations, indicating low sealing potential. Based on these results, we would suggest the use of $CaCl_2$ below 200 mM or alternatively utilizing $CaCO_3$ to minimize syneresis levels.

Alginate gel behavior under compression is important in the development of a dura mater repair material. After application of the alginate, the skull is sealed and the gel is then subjected to a constant intracranial pressure (approximately 1 kPa) that compresses the gel between the brain and the skull. Immediately following surgery, intracranial pressure is increased due to trauma and edema. Our results indicate that the compressive strength of the gel far exceeds the pressures that the alginate would be subjected to *in vivo*. This suggests that alginate used alone or with sutures to approximate tissue or replace portions of the tissue would not be compressed to failure by intracranial pressure and would thereby be able to maintain a seal or fill a large defect. The compressive strength near the elastic limit of alginate, 60% compression, indicates that alginate can withstand pressures greater than 100 kPa. Even after significant neural trauma, the highest pressure a dural sealant would likely be exposed to is 3 kPa (Czosnyka et al., 2005). Alginate is thereby expected to withstand intracranial pressure under any physiological circumstance without failure.

The compressive modulus is an ideal measure for comparison of alginate with brain tissue. It is desirable to match the modulus of alginate to that of the underlying brain tissue to decrease the occurrence of trauma due to a mechanical mismatch. Brain tissue has been shown to have a compressive modulus of 20–100 kPa, depending on the tissue source and measurement technique (Sarron et al., 2000; Walsh and Schettini, 1976). Most of the alginate combinations tested fit in this range (Figs. 8 and 9, Tables 1 and 2), and the compressive modulus was not a major contributor to identifying the optimal combination of alginate and $CaCl_2$ or $CaCO_3$.

Hydrogel homogeneity is important in the development of a dural patch. The hydrogel should have the same response to compression throughout to avoid localized mechanical failure of the patch. Overall, the hydrogel response to compression is more consistent for alginate reacted with $CaCO_3$ than with $CaCl_2$. The normalized variance for 10% compressive strength, 60% compressive strength, and compressive modulus all indicate that $CaCO_3$-reacted alginate is significantly less variable. This suggests that $CaCO_3$ results in more homogeneous and uniform gels than $CaCl_2$, and is thereby more appropriate for use as a dural patch. These results corroborate an earlier study comparing alginate reacted with $CaSO_4$ and $CaCO_3$, which indicated that the slow gelation kinetics of $CaCO_3$ were responsible for increased hydrogel homogeneity (Kuo and Ma, 2001).

Due to the pulsation of the brain with heart rate and respiratory rate, the alginate will be subject to oscillatory strain. Thus, alginate's response to shear is an important design factor for a dural sealant as it is a good indicator of the fatigue resistance and how it will perform *in vivo*. Brain tissue typically exhibits a complex modulus ranging from 1 to 24 kPa (Donnelly and Medige, 1997). Based on our results, alginates have a complex modulus range of 6–186 kPa. Therefore, the oscillatory behavior of intracranial pressure should not adversely affect the fatigue resistance of alginate, and alginate is expected to remain stable *in vivo*. Additionally, the complex modulus of alginate can be tailored to match that of the brain, reducing mechanical mismatch at the alginate–dura interface.

The final gel parameter investigated, δ, addresses the elastic behavior of the gel due to internal cross-linkages. While it is clear that the alginate must exhibit low values of δ, the exact δ cannot be identified at this time. The hydrogel must be able to retain its shape and orientation to the tissue while accommodating some motion of the dura, yet the magnitude of such motion *in vivo* is not known. The ideal dural patch would exhibit elastic solid behavior (low loss angle values), enabling the hydrogel to maintain its original form. $CaCO_3$-reacted hydrogels resulted in more densely cross-linked gels (lower loss angle) than did those gels made with $CaCl_2$. This increased cross-link density potentially indicates a superior stability for internally gelled hydrogels versus diffusion-gelled hydrogels *in vivo*. Therefore, $CaCO_3$ is potentially more appropriate to use to cross-link alginate when used as a dural patch because of an increased need for long-term stability. However, the ideal value for δ is currently unknown. The gel should keep its original shape but still be able to move with the dura and underlying brain. Previous *in vitro* studies indicate that the loss angle will become more important when measuring the elastic behavior of the gels over time (LeRoux et al., 1999; Nunamaker et al., 2007; Wang et al., 1994). We expected that, after prolonged exposure to low concentrations of inert electrolytes *in vivo*, the calcium ions that are holding the hydrogel together may be displaced, resulting in a less densely cross-linked gel and ultimately a higher loss angle. However, by this extended time point we postulate that significant meningeal replacement would have occurred beneath the dural sealant. Further *in vivo* testing is necessary to answer these questions.

In summary, we have investigated gel formation and the material properties of alginate hydrogels created by diffusion and *in situ*-based gelling in the context of dura mater repair. We elucidated how these properties are influenced by the alginate molecular weight, alginate concentration, and $CaCl_2$ or $CaCO_3$ concentration. The data collected in this study suggest that the use of 1.95 wt% 43 mPa s alginate with 200 mM $CaCl_2$ is sufficient for approximating small dural defects for closure alone or in conjunction with suture. Alternatively, the use of 1.95 wt% 43 mPa s alginate with 100 mM $CaCO_3$ is sufficient for tissue replacement in large dural defects.

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