

Characterizing the degradation of alginate hydrogel for use in multilumen scaffolds for spinal cord repair

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Abstract: Alginate was studied as a degradable nerve guidance scaffold material *in vitro* and *in vivo*. *In vitro* degradation rates were determined using rheology to measure the change in shear modulus vs time. The shear modulus decreased from 155 kPa to 5 kPa within 2 days; however, alginate samples maintained their superficial geometry for over 28 days. The degradation behavior was supported by materials characterization data showing alginate consisted of high internal surface area (400 m²/g), which likely facilitated the release of cross-linking cations resulting in the rapid decrease in shear modulus. To assess the degradation rate *in vivo*, multilumen scaffolds were fabricated using a fiber

templating technique. The scaffolds were implanted in a 2-mm-long T3 full transection rodent spinal cord lesion model for 14 days. Although there was some evidence of axon guidance, in general, alginate scaffolds degraded before axons could grow over the 2-mm-long lesion. Enabling alginate-based scaffolds for nerve repair will likely require approaches to slow its degradation. © 2015 Wiley Periodicals, Inc. *J Biomed Mater Res Part A*: 104A: 611–619, 2016.

Key Words: alginate, hydrogel, spinal cord injury, nerve regeneration, rheology, degradation

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INTRODUCTION

Damage to the spinal cord is typically permanent. Spontaneous axon regeneration is limited due to insufficient trophic factor support, the absence of supportive axonal growth substrates in the lesion site, the presence of inhibitory proteins and glia surrounding the lesion, and a failure of injured neurons to fully activate a growth state.^{1,2} Moreover, once axons are transected, directional guidance to appropriate targets is lacking.³ One approach to restore native nerve tracts involves implantation of multilumen scaffolds that promote and guide axonal regeneration.^{4–6}

Agarose hydrogel scaffolds with strictly linear channels have been fabricated and scaled to cm-long scaffolds for spinal cord repair.^{4–6} However, these implants are non-degradable and inflammation is frequently observed at the scaffold-host tissue interface.⁵ The ideal scaffold should have the following characteristics: (1) be biocompatible;^{7,8} (2) have high channel volume and/or thin walls to maximize open space for axonal growth; (3) have linear channels to guide axons and maintain their organization in the spinal

cord;^{4,9} (4) have channel diameters between 25 μ m and 200 μ m;^{4,10} (5) degrade after nerve regeneration to avoid permanently occupying the spinal cord;⁹ and (6) be cost-effective.⁹

In this work, a calcium cross-linked alginate hydrogel was investigated as a guidance scaffold material for axonal regeneration owing to its biocompatibility with nerve tissue,^{11–14} wide range of elastic moduli (1 kPa to 320 kPa),^{15–17} and tunable degradation rate.^{15,16} Ionically cross-linked alginate degrades through the release of divalent cross-linking cations such as calcium, and their exchange with monovalent cations such as sodium.¹³

The *in vitro* degradation of alginate has been previously studied. For example, McKay et al. studied the change in the shear modulus of 0.25 wt % and 0.5 wt % alginate hydrogel after 2 days of *in vitro* degradation.¹⁷ Other studies have performed cell attachment studies on alginate for up to 10 weeks and suggested techniques such as adding NaCl to preserve material integrity.^{18,19} However, to date there have been no studies that correlate the change in the shear

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modulus of calcium cross-linked alginate in week-long experiments.

Previous *in vivo* work on alginate such as Prang et al. and Suzuku et al. studied the efficacy of covalently cross-linked multilumen alginate scaffolds and calcium cross-linked alginate hydrogel sponges for spinal cord repair, respectively.^{14,20} However, there have been no studies to evaluate the *in vivo* degradation of calcium cross-linked alginate hydrogel scaffolds for spinal cord repair. To determine if alginate hydrogel is a viable nerve guidance scaffold material, it is important to understand its degradation behavior *in vitro* and to test its efficacy *in vivo*.

This study entailed a two-pronged approach to characterize the degradation of calcium cross-linked alginate hydrogel. First, the *in vitro* degradation was measured using rheology, and by assessing the specimen for macroscopic deterioration. Second, *in vivo* degradation was characterized by fabricating and implanting multilumen alginate scaffolds in rodent T3 full transections to determine if adequate integrity is maintained to effectively guide axons over two weeks. To achieve shear moduli similar to that of previously reported 3 wt % agarose hydrogel scaffolds,^{4–6} a 3 wt % alginate hydrogel composition cross-linked with 100 mM calcium chloride (CaCl₂) was chosen, which is reported to have the same modulus as 3 wt % agarose.^{(unpublished data),16} It was observed that the shear modulus of the alginate hydrogel decreased by 97% within 3 days *in vitro*. In addition, *in vivo* tests demonstrated that while scaffolds may initially maintain channels to guide axons, the scaffold was not visible through optical and fluorescent imaging after two weeks of *in vivo* testing.

METHODS

High-purity (HP) alginate suitable for *in vivo* studies and the more commonly used lower-purity (LP) alginate were used (Provona UP MVG and Provona MVG, respectively. FMC Nova Matrix, Philadelphia, PA). Both alginate grades had >60% guluronate monomer. All other chemicals were purchased from Sigma (St. Louis, MO) unless otherwise mentioned.

Alginate was dissolved in reverse osmosis (RO) water (3 wt %) and cross-linked in a 100 mM CaCl₂ solution to form a hydrogel.

Fabrication of alginate hydrogel

To make homogeneous alginate hydrogels, a 1-dimensional (1D) cross-linking technique was developed to improve uniformity (Fig. 1). A glass microfiber filter article (Whatman grade GF/A) was placed between two 2024 alloy aluminum rings (7.6 cm inner diameter, 8.9 cm outer diameter. McMaster, Aurora, OH). The tubes and filter paper were adhered together using double-sided tape forming a reservoir to contain the alginate precursor during the cross-linking process. Alginate solution was poured on top of the filter paper, but was too viscous to permeate through it. The reservoir assembly was placed in a 100 mM CaCl₂ solution (Alfa Aesar, Ward Hill, MA) to allow the calcium ions to permeate

through the filter paper and into the alginate solution. A 6.4-mm diameter hole was made in the bottom ring, approximately 3 mm below the filter paper, to release air bubbles. After 24 h of immersion to cross-link the alginate solution, cylinders were cut from the alginate hydrogel using a custom punch consisting of 0.8 mm wall thickness, titanium 6 aluminum-4 vanadium alloy (McMaster) tube, sharpened to a knife edge (11.4 mm inner diameter). Alginate hydrogel faces were cut with a razor blade to produce 5 mm-tall samples with flat and parallel opposing surfaces.

To compare cross-linking techniques, the conventional “radial” cross-linking method was also used to fabricate alginate cylinders. Briefly, plastic mesh (1 mm opening size, McMaster) was shaped into cylindrical tubes about 25 mm in diameter. The tubes were immersed in alginate solution, where the longitudinal axis was oriented vertically, and immediately transferred to and kept in a 100 mM CaCl₂ solution, after which the hydrogels remained for 24 h. The alginate hydrogels were cut perpendicular to the longitudinal axis using a razor blade to produce cylinders about 5 mm tall and 11.4 mm in diameter.

Measuring shear modulus using rheometry

Parallel 2024 aluminum plates (11.4 mm diameter) were custom-made and inserted in an Ares rheometer (TA instruments, Schaumburg, IL) for shear modulus testing. Sand paper (1200 grit) was adhered to the parallel plates using double-sided tape to avoid sample slippage during testing (as suggested by Meyvis et al.²¹). First, the initial contact point, and thus the height of the sample, was measured upon the initial detection of normal force. Since the shear modulus of hydrogels changes with the frequency of the shear strain,^{21,23,24} the frequency was fixed at 1 Hz for all rheology experiments. To optimize the shear strain rate, a range of 0.01% to 50% shear strain was applied and the shear modulus was recorded. Since the shear modulus of alginate continuously decreased at dynamic shear strains higher than 0.5%, a dynamic shear strain of 0.5% was chosen for all experiments. In general, the procedure by Meyvis et al. was followed to measure the shear moduli.²¹ Briefly, the shear modulus was recorded after 50 s of applied dynamic shear strain with a frequency of 1 Hz. Each cylinder was then compressed for a distance of 25–100 μm (0.5–2.0% compression strain), and the modulus was measured. Subsequent compression was applied until the shear modulus did not change; full contact with the sample was established and the shear modulus was recorded ($n = 5$). In addition, to confirm 1D cross linking was uniform, the shear modulus measurements were conducted on both faces of a cylindrical specimen to ensure the modulus was the same on each face.

Assessing the *in vitro* degradation rate of alginate cylinders

The HP and LP alginate cylinders, made using the 1D and radial cross-linking methods ($n = 5$), were stored in 37°C 1xDPBS with a volume equivalent to five times the volume of each cylinder. The solution was exchanged daily. Images

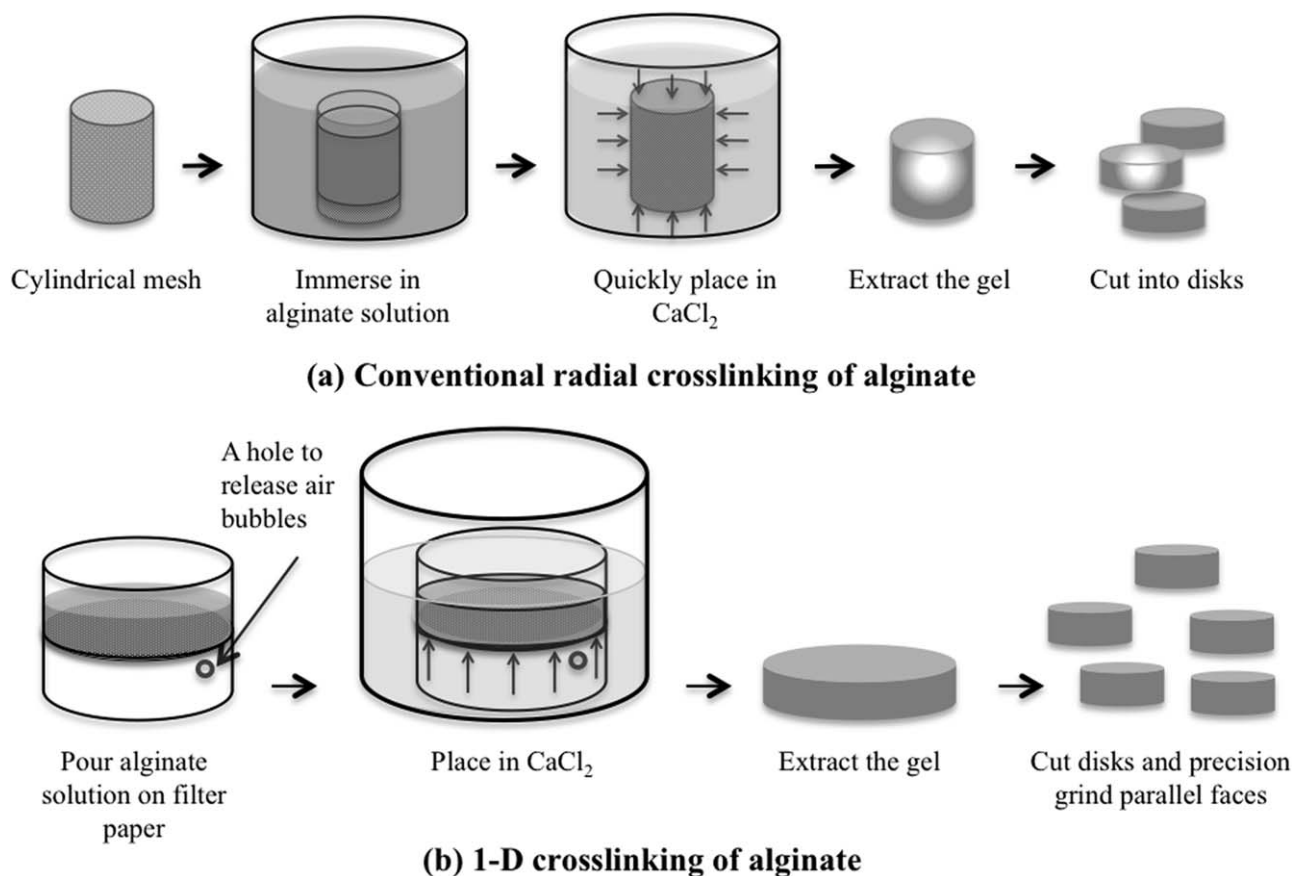


FIGURE 1. (a) Conventional radial and (b) 1D cross-linking of alginate. Unlike radial cross-linking, 1D crosslinking produces uniform disks for up to 15 mm tall samples.

of the alginate cylinders were taken to qualitatively assess swelling and degradation. To characterize the swelling of alginate, the diameters of the cylinders were measured using electronic calipers (Mitutoyo, Aurora, IL). The heights of the HP alginate cylinders were measured daily until day 6 using the initial contact point detected by the rheometer. The diameter and height measurements were used to determine the volume and hence track the degree of swelling. The shear moduli of the cylinders were measured daily until the modulus decreased to <3% of the original value.

Pore characterization

Cross-linked HP alginate was supercritically dried to preserve the pore structure. The procedure was the same as previously reported by Lynam et al.²⁵ The hydrogel was immersed in 100% ethanol (Decon Labs, Inc., King of Prussia, PA) for 3 days and the ethanol was changed every day. The samples ($n = 3$) were then washed with liquid CO_2 over 2 days followed by supercritical extraction. Scanning electron microscopy (SEM) (JOEL 7500F, Peabody, MA) was used to characterize the porosity. Brunauer–Emmett–Teller (BET) (Micromeritics ASAP 2020, Norcross, GA) was used to measure surface area and pore size distribution as described elsewhere.²⁵ Briefly, water was removed under vacuum (10^{-6} torr) at 80°C for 12 h before each nitrogen sorption test. Free space and surface area were measured

through the quantification of the adsorption/desorption behavior using ultra high purity helium and nitrogen, respectively and were correlated using the Barrett–Joyner–Halenda (BJH) desorption method.

Scaffold fabrication

HP alginate scaffolds were fabricated using a fiber templating technique.^{4,26} Briefly, multicomponent fiber bundles (MCFB) of hexagonally packed polystyrene (PS) fibers with $167\ \mu\text{m}$ diameter separated by a poly(methyl methacrylate) (PMMA) matrix with $67\ \mu\text{m}$ spacing were used (Paradigm Optics, Vancouver, WA). After templating, alginate scaffolds contracted longitudinally and expanded laterally when immersed in physiological solutions, likely caused by an osmotic effect. To account for these dimensional changes, the MCFBs were made 5% longer in the longitudinal direction and 10% shorter in the lateral dimensions, resulting in 2 mm long and 1.8 mm by 1.8 mm wide scaffolds. The MCFBs were bonded to PS caps, and the PMMA matrix was removed using a 80:20 vol/vol propylene carbonate:acetonitrile solution. Templates were then soaked in 3 wt % HP alginate solution and centrifuged at 1000 revolutions per minute (rpm) (9 relative centrifugal force (rcf)) for 30 s to facilitate hydrogel permeation between the PS fibers. To ensure complete permeation, templates were kept in an alginate solution overnight at room temperature, and

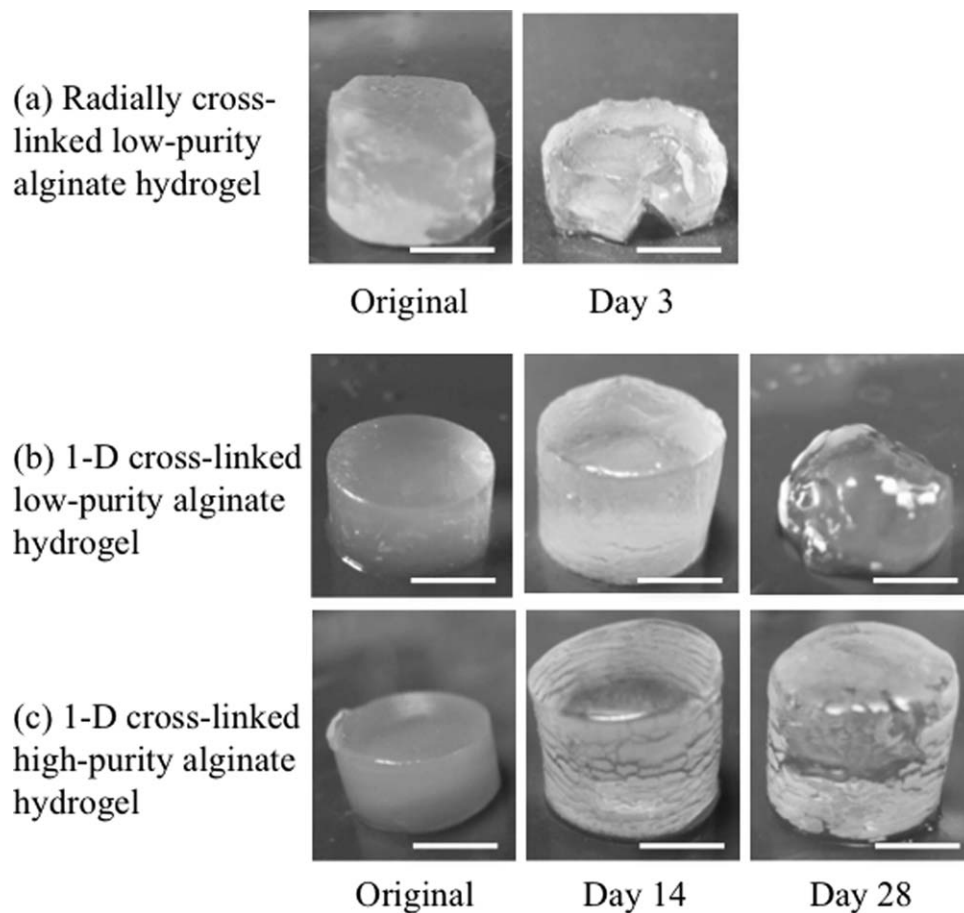


FIGURE 2. Visual changes of 11.4 mm-diameter (a) radially cross-linked low-purity alginate and 1D cross-linked (b) low-purity and (c) high-purity alginate during *in vitro* degradation. Unlike radially cross-linked hydrogels, 1D cross-linked alginate does not fragment and exhibits uniform degradation. Scale bars are 5 mm.

centrifuged at 1000 rpm (9 rcf) for another 30 s before cross-linking in 100 mM CaCl₂ for 4 h. Excess alginate hydrogel along the template periphery was trimmed off using a razor blade. The alginate-permeated templates were immersed in 37°C cyclohexane under agitation for 2 days to selectively etch away the PS fibers. The alginate scaffolds with linear channels were then washed with 100 mM CaCl₂ to remove any remaining residue.

Alginate scaffolds were placed in 1 mL of 1xDPBS at 37°C and the solution was replaced every 24 h. Optical images of the scaffolds were taken on days 1, 3, 7, 14, 21, and 28 to ensure the channels and the bulk geometry of the scaffolds were maintained *in vitro*.

Agarose (3 wt %) hydrogel was selected as a control scaffold material, because it is known to be nondegradable and its efficacy for spinal cord repair has been studied.⁴⁻⁶ Agarose scaffolds were prepared similar to alginate scaffolds following the protocol first described by Stokols *et al.*⁴ Briefly, fiber templates were cut to 2.0 × 1.8 × 1.8 mm, bonded to PS caps and reinforced with PS lateral supports as explained above. Agarose (3 wt %) was heated to 70°C to produce a molten agarose solution. The fiber template was placed in the molten agarose and centrifuged at 1000 rpm (9 rcf) for 45 s to remove air bubbles and

promote fiber wetting. Upon cooling to room temperature and gelling, the fiber templates were extracted from agarose and any extra agarose was trimmed from the template using a razor blade. The fiber template permeated with agarose construct was placed in tetrahydrofuran (Alfa Aesar, Ward Hill, MA), at 37°C for 3 days under agitation while exchanging the solution every day. The scaffolds were then washed in RO water.

Surgical procedures and *in vivo* characterization

Details regarding the animal handling and surgeries are described elsewhere.^{6,27} Briefly, adult female Fischer 344 rats (150–200 g) were used in this study according to the NIH guidelines for laboratory animal care and safety. A combination (2 mL/kg) of ketamine (25 mg/mL), xylazine (1.3 g/mL), and acepromazine (0.25 mg/mL) was used to anesthetize the animals. T2-T4 laminectomies were performed. Micro scissors were used to create 2 mm-long lesion cavities in the T3 spinal cord segments. The lesions were cleared using micro aspiration. Animals were divided into three experimental groups (*n* = 3 per group): group 1 received no treatment; group 2 received nondegradable agarose hydrogel scaffolds; and group 3 received alginate hydrogel scaffolds (alginate hydrogel scaffolds were soaked

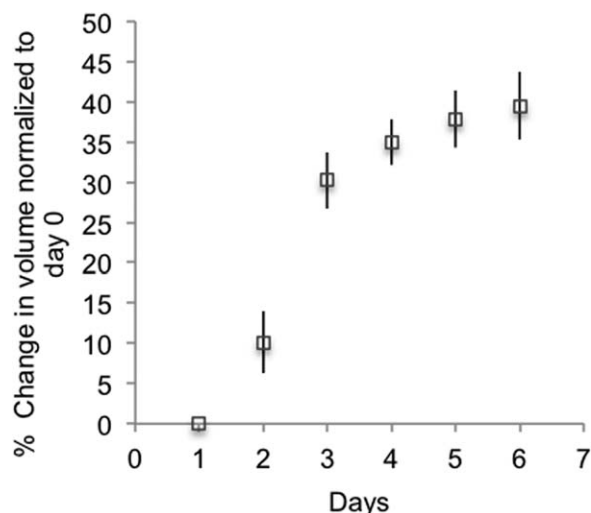


FIGURE 3. The change in the volume of HP alginate cylinders during degradation. The data are normalized to the volume of the samples from day 0. The cylinders swell to about 40% by day 6.

in 1 mL of phosphate buffer (pH 7.4) for 20 min prior to implantation).

To examine scaffold degradation *in vivo*, animals were sacrificed 2 weeks post-implantation and perfused with 4% paraformaldehyde (PFA) in 0.1M phosphate buffer at 4°C. Segments (2 cm-long) centered above the lesion sites were separated and post-fixed in 4% PFA overnight at 4°C, and then in 30% sucrose (w/v) in 0.1 M phosphate buffer at 4°C for another 48 h prior to cryosectioning. Longitudinal sections at a thickness of 30 μ m were collected on a cryostat. For immunolabeling, slides were suspended in proteinase K (1:20, antigen retrieval) (Millipore, Darmstadt, Germany) for 20 min followed by postfix with methanol for 5 min at room temperature. Slides were blocked using 5% goat serum in Tris-buffered saline (TBS) containing 0.25% Triton X-100 for 1 h. The slides were then incubated overnight with mouse-

anti-Neurofillament 200 for axons staining (Millipore) with a 1:500 dilution. The sections were washed with TBS and labeled with secondary antibody: goat-anti-chicken 647 (1:250, for 2.5 h in dark at room temperature) (Life Technologies, Carlsbad, CA). The slides were then washed with TBS, labeled with Fluoromount G (Southern Biotechnology Associates, Inc., Birmingham, Alabama) and imaged using a fluorescent microscope (Olympus BX53, Center Valley, PA).

RESULTS

In vitro degradation testing

Images of the conventional and 1D cross-linked gels after immersion in 1xDPBS are shown in Figure 2. The radially cross-linked alginate hydrogel cylinders fragmented within 3 days, thus it was not possible to make dimensional measurements as a function of time (Fig. 2). Conversely, the alginate hydrogel cylinders fabricated by the 1D crosslinking technique maintained their geometry making it possible to quantitatively assess the change in physical properties as a function of time (Fig. 2). Thus, only 1D cross-linked samples were considered for quantitative degradation characterization. Since only the high purity (HP) alginate would be implanted, only HP swelling was characterized. Negligible changes in the diameters of the samples were measured. The majority of the changes in height (and therefore swelling) occurred between days two and three, after which the volume increase subsided, e.g. between days three and six (Fig. 3).

To accurately characterize and quantify the degradation of alginate hydrogel cylinders, changes in shear moduli were measured using rheology. The shear moduli of HP and LP alginate hydrogel were 155 ± 21 kPa and 186 ± 20 kPa, respectively. The different moduli may have resulted from the different purities; regardless, the shear moduli of both HP and LP alginate hydrogel cylinders decreased by $\sim 97\%$ by day three (Fig. 4).

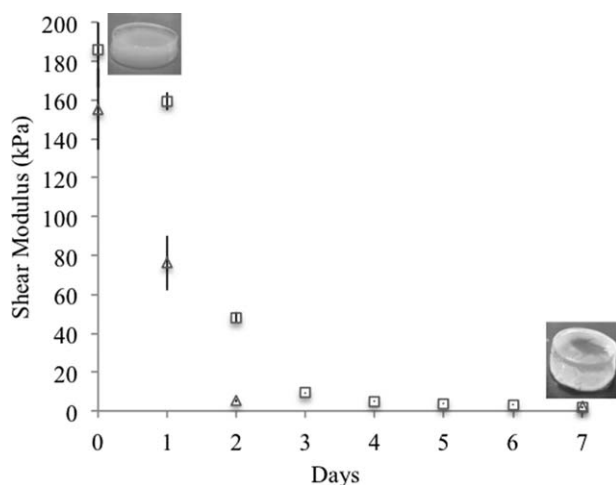


FIGURE 4. Rheology measurements for low-purity (□) and high-purity (Δ) alginate, including optical images of the high-purity samples (with a diameter of 11.4 mm) on days 0 and 7. While the shear modulus drops by 97% within 3 days, the superficial geometry is maintained.

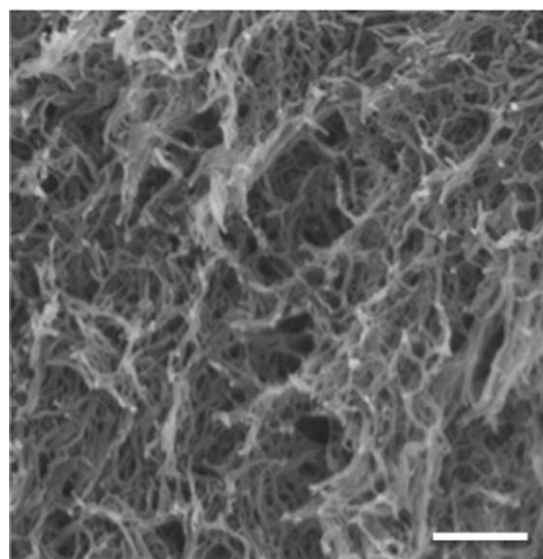


FIGURE 5. SEM image of supercritically dried 3 wt % alginate showing interconnected pores, which are in the tens of nanometer range. Scale bar is 200 nm.

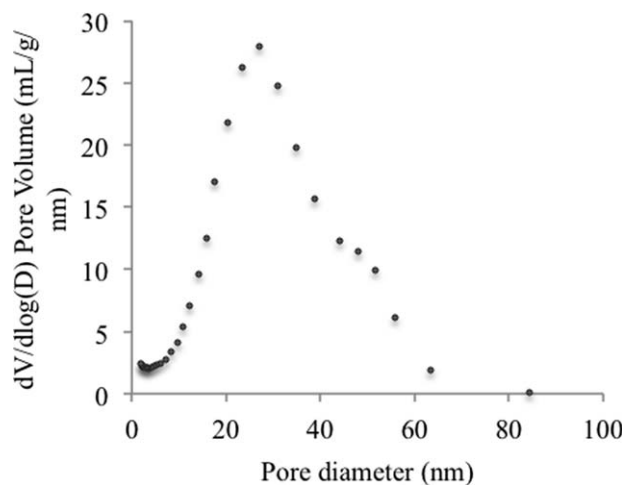


FIGURE 6. Nitrogen desorption data of supercritically dried alginate showing that the majority of nanopores are 25–35 nm in diameter.

Pore characterization

SEM (scanning electron microscopy) analysis showed 3 wt % alginate hydrogel consisted of cross-links in the 10–20 nm range and interconnected pores in the tens of nanometer range (Fig. 5). The BJH method on nitrogen desorp-

tion data on supercritically dried 3 wt % alginate hydrogel corroborates the SEM analysis, indicating that most pores fell within the 25–35 nm diameter range with a total surface area of $415.7 \pm 12.0 \text{ m}^2/\text{g}$ ($n = 3$) (Fig. 6). These data are consistent with similar studies on supercritically dried 3 wt % agarose and 2 wt % alginate hydrogels.^{25,28}

Fabrication of alginate scaffolds and *in vivo* testing

Images of a template and an alginate scaffold are shown in Figure 7. The average template fiber diameter and the spacing between fibers were $219.7 \pm 16.0 \text{ }\mu\text{m}$ and $95.7 \pm 9.1 \text{ }\mu\text{m}$, respectively. The alginate hydrogel scaffold matched the template pattern and had an average channel diameter and scaffold wall thickness of $197.5 \pm 25.4 \text{ }\mu\text{m}$ and $83.5 \pm 35.6 \text{ }\mu\text{m}$, respectively. Contraction during cross-linking may explain the slight reduction in dimensions compared to the templates. Differences between template-to-scaffold channel diameter and wall thickness was 10.1% and 14.6%, respectively. This was comparable to fiber templated agarose hydrogel scaffolds exhibiting 14.2% and 22.0% channel diameter and wall thickness template-to-scaffold shrinkage, respectively. Optical images (not shown) demonstrated that alginate scaffolds generally maintained their channels for at least 28 days.

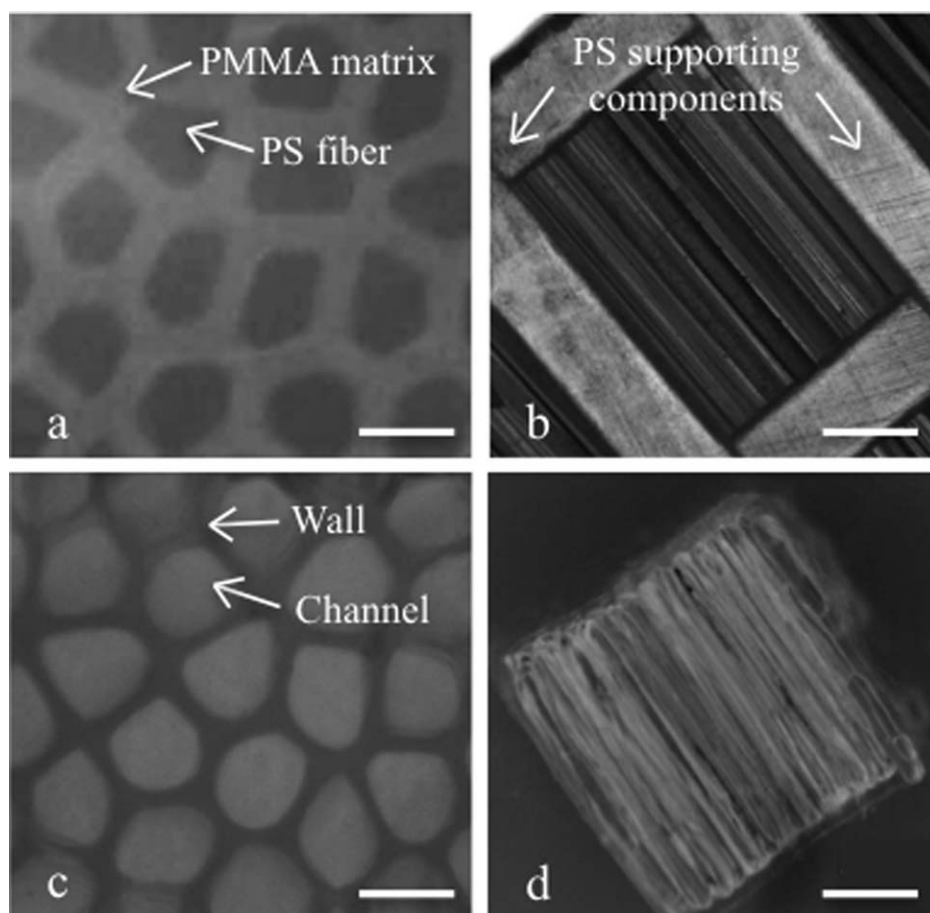


FIGURE 7. (a) Cross-section and (b) longitudinal view of a multicompnent fiber bundle template consisting of a PMMA matrix and PS fibers. The PS components (b) maintain PS fiber linearity after the PMMA matrix is etched. Templated alginate scaffold cross-section and side view (c) and (d), respectively. Scale bars in (a) and (c) are $400 \text{ }\mu\text{m}$ and in (b) and (d) are $500 \text{ }\mu\text{m}$.

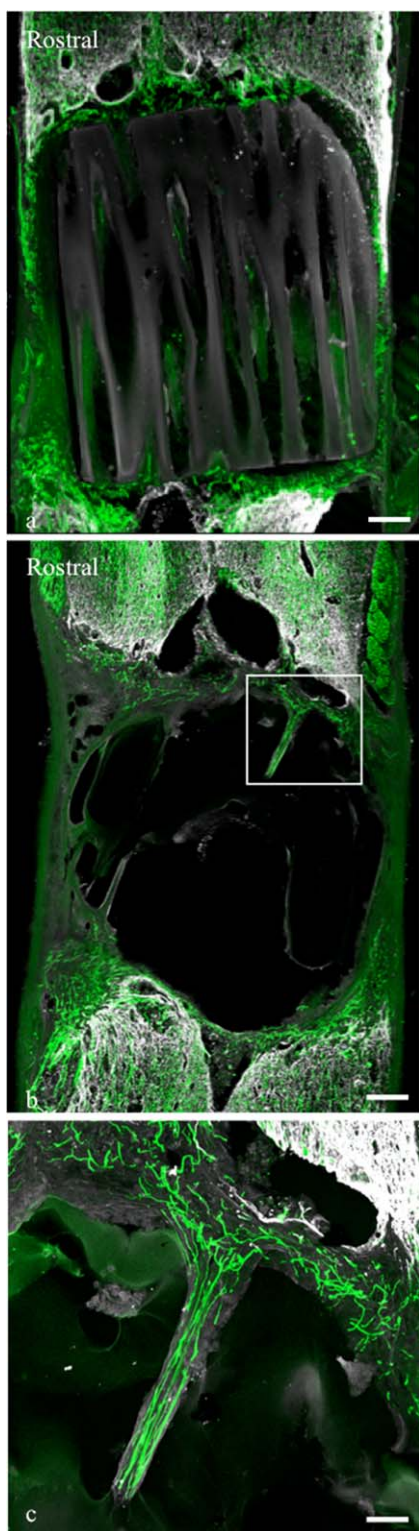


FIGURE 8. Representative images of neurofilament (green) labeling of a completely transected rat spinal cord 2 weeks post-implantation: (a) agarose scaffold and (b) alginate scaffold. The agarose hydrogel scaffold remained intact while the alginate scaffold mainly degraded. Some axonal growth into the agarose scaffold is observed. A higher magnification image (c) shows some axonal growth at the rostral end of the alginate scaffold. The scale bars in (a) and (b) are 200 μm and the scale bar in (c) is 50 μm .

Histology sectioning 2 weeks post-implantation showed that nondegradable agarose hydrogel scaffolds were intact and the channels were distinguishable [Fig. 8(a)]. Some linear axonal growth was observed throughout the agarose hydrogel scaffolds. However, the channels and the alginate hydrogel scaffolds were not, in general, distinguishable in the histology [Fig. 8(b)]. Nevertheless, there was some evidence of linear axonal regeneration in Figure 8(b,c), suggesting that some microchannels could maintain integrity long enough to guide axons up to ~ 0.5 mm [Fig. 8(c)].

DISCUSSION

Alginate hydrogel was investigated as a degradable nerve scaffold for spinal cord repair owing to its reported biocompatibility and tunable degradation rate.^{11–13,15,16} After developing a technique to fabricate uniform cylinders, the degradation of alginate hydrogel was quantified *in vitro* by monitoring the change in its shear modulus over time. Scaffolds with linear channels were then fabricated and implanted in rodent spinal cords to test their efficacy for axonal guidance *in vivo*.

To measure the change in the shear modulus during degradation, first a technique for fabricating homogeneous alginate hydrogel cylinders was developed. Conventional radial cross-linking was initially attempted, but samples fragmented within 3 days likely due to the nonuniform permeation of calcium ions. To achieve uniform cross-linking, a novel 1D cross-linking technique was developed. The uniformity of samples with a height < 15 mm was confirmed since the same modulus was obtained when performing rheometry on the top and on the bottom of the cylinders (with the bottom of the cylinder being the side against the filter paper).

Once a technique was established to fabricate homogeneous alginate hydrogel cylinders, rheometry was used to characterize their degradation *in vitro*. Rheometry was selected because, unlike techniques such as uniaxial compression testing, swelling during loading (the “barreling effect”) is minimized.²⁹ The barreling effect can cause deformation and sometimes rupture, which prevents the retesting of the same sample as it degrades. In this study, rheology was used to measure the shear modulus to characterize the degradation rate *in vitro*. Applying a dynamic sinusoidal shear stress minimizes the barreling effect and yields information about both the shear and the viscous behavior by providing shear and loss moduli, respectively. The initial shear modulus on day zero was in agreement with the previously reported value,¹⁶ but had relatively lower standard deviations (generally below 10%) compared to previous work.^{16,22,23,30} The 1D cross-linked samples maintained their integrity to allow for mechanical testing beyond day 1, unlike samples obtained by radial cross-linking. These results confirmed the effectiveness of rheology for quantifying the degradation of hydrogels, which was previously suggested by Meyvis et al.²¹

Rheological analysis showed that the shear modulus of HP alginate hydrogel dramatically decreased from 155 kPa

to 5 kPa in 2 days, while no change in the superficial geometry was observed for over 28 days. The exchange of cross-linking calcium cations with monovalent sodium cations is the main cause of degradation of calcium cross-linked alginate hydrogel.^{13,28,31} The combination of high internal surface area ($415.7 \pm 12.0 \text{ m}^2/\text{g}$) and nano scale interconnected porosity (Fig. 2), likely facilitated calcium ion loss and swelling (Figs. 2 and 3), which decreased the shear modulus and later caused fragmentation.

The observed degradation behavior suggested that alginate hydrogel undergoes bulk degradation. Since alginate hydrogel maintained its superficial geometry *in vitro* for over 1 month, alginate was considered a promising candidate for degradable nerve guidance scaffolds. To test this hypothesis, multilumen alginate hydrogel and nondegradable agarose hydrogel (control) nerve guidance scaffolds were fabricated using a fiber templating technique.⁴ The fiber template construction resulted in alginate permeation from two opposing faces [Fig. 7(b) shows one of only two open template faces that the alginate solution could permeate]. Thus, upon immersion in the CaCl_2 solution, the cross-linking direction was one-dimensional. The degradation of alginate scaffolds was first studied *in vitro* and it was confirmed that the scaffolds generally maintained their channels and bulk geometry for at least 28 days. In light of the promising *in vitro* data both on alginate cylinders and scaffolds, the scaffolds were implanted in a T3 full transection rodent model to test their efficacy *in vivo*. The approach used to characterize degradation was based on a qualitative assessment of the lesion cavity two weeks post implantation (Fig. 8). In agreement with previous work,⁴⁻⁶ linear walls and channels are clearly discernable in the lesion containing the agarose scaffold. However, compared to the agarose scaffolds, there was no clear evidence of linear walls or channels in the lesion containing the alginate scaffold, aside from a few linear axons on the proximal and distal ends of the cavity; perhaps indicating that initially the alginate scaffold guided axons before it degraded away. Though the details of the degradation mechanism are difficult to determine, we believe the images in Figure 8 indicate that the alginate scaffold was not present/functional after two weeks post implantation.

CONCLUSIONS

Alginate hydrogel was evaluated as a nerve guidance scaffold material. The degradation rate of alginate was measured *in vitro* using rheology. A methodology was developed to fabricate uniformly cross-linked alginate hydrogel cylinders to measure the shear modulus as a function of time. It was determined that the shear modulus of alginate hydrogel decreased by 97% in 2 days, but no change in the superficial geometry of the hydrogel was observed in 28 days. *In vivo* studies demonstrated, qualitatively, that calcium cross-linked alginate hydrogel did not maintain adequate mechanical integrity to guide axons toward distal targets over a 2 mm T3 full transection spinal cord injury model. It is possible that if alginate hydrogel scaffold walls degraded

slower, linear guidance to the distal targets may have occurred. In future work, approaches to decrease the degradation rate of alginate should be pursued to make it a viable scaffold material for nerve regeneration scaffolds.

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