Chitosan implants in the rat spinal cord: Biocompatibility and biodegradation

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Received 18 November 2010; revised 18 January 2011; accepted 24 January 2011
Published online 4 April 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.33070

Abstract: Biomaterials are becoming increasingly popular for use in spinal cord repair, but few studies have investigated their long-term biocompatibility in central nervous system tissue. In this study, chitosan was compared with two commercial materials, degradable polyglycolide (vicryl and polyglactin 910) and nondegradable expanded poly(tetrafluoroethylene) (Gore-Tex and ePTFE), in terms of host tissue response and biodegradation in the rat spinal cord in two different spinal cord implantation models. In an uninjured model, implants were placed in the spinal cord intrathecal space for up to 6 months. At 1 month, vicryl implants elicited an elevated macrophage/microglia response compared to chitosan and Gore-Tex, which subsided in all groups by 6 months. Fibrous encapsulation was observed for all three materials. At 6 months, the *in vivo* degradation of vicryl was complete, while Gore-Tex showed no signs of degradation, as assessed by mass loss and SEM. Chitosan implants showed evidence of chain degradation at 6 months as demonstrated by differential hematoxylin and eosin staining; however, this did not result in mass loss. In the second model, implants were placed directly into the spinal cord for up to 12 months. This resulted in increased immune and inflammatory responses but did not alter degradation profiles. The same trends observed for the materials in the intrathecal space were mirrored in the spinal cord tissue. These results demonstrate that chitosan is a relatively inert biomaterial that does not elicit a chronic immune response and is suitable for long-term applications for repair of the spinal cord. © 2011 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 97A: 395–404, 2011.

Key Words: chitosan, polyglycolide, poly(tetrafluoroethylene), vicryl, Gore-Tex, biodegradation, biocompatibility, spinal cord

INTRODUCTION

Spinal cord injury (SCI) presents a significant challenge for regeneration due to major losses to native cellular and extracellular matrix architecture. In particular, the formation of cysts, cavities, or gaps in the spinal cord at the injury site results in the lack of a physical substrate for regeneration. Biomaterials are increasingly popular as a potential strategy for the treatment of SCI in that they can serve to replace the extracellular matrix at the site of injury. A wide range of biomaterials, both of natural and synthetic origin, are being investigated for potential applications in the spinal cord.[1–4] These materials can support endogenous tissue regeneration,[5,6] promote directed axonal growth,[7,8] enhance cell transplant survival and engraftment,[9,10] deliver drugs locally,[11–13] and seal damaged dura mater.[14] It is important to ensure that these materials are safe and well characterized.

One of the key criteria of biomaterial design is biocompatibility. Biomaterials designed for spinal cord repair should provoke minimal chronic inflammation and immune responses when implanted in the body.[15,16] These responses depend not only on the inherent properties of the material itself but can also be affected by the form in which the material is presented, for example, implant shape,[17] size,[18] and porosity.[19] Degradable materials, in particular, are important to monitor over time because the degradation products can elicit different inflammatory responses than those of the parent material.

Chitosan is derived from the deacetylation of chitin, the primary polysaccharide component of crustacean shells. It is an attractive material because its degradation rate can be tuned based on its degree of deacetylation (DD), where fully deacetylated (DD = 100%) chitosan is nondegradable[20,21] and partially deacetylated (DD = 70%) is fully degradable.[21,22] Chitosan is a versatile material currently in clinical use in wound dressings, primarily for its hemostatic property.[23] We have previously reported that chitosan channels (DD = 90%) promote extensive tissue bridge formation following spinal cord transection in rats.[24,25] In these reports, chitosan channels remained structurally intact for 6 months

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Contract grant sponsors: Canadian Institute of Health Research; Natural Sciences and Engineering Research Council of Canada; Ontario Neurotrauma Foundation; and Canadian Paraplegic Association, Ontario Branch

in vivo and showed no evidence of degradation. In other tissues, as well as our own *in vitro* screening, chitosan is degradable when DDs are less than 85–90%.[20,21,26]

In this study, chitosan samples of DD=78% and 85% were investigated in terms of the *in vivo* foreign body response and degradation profile in the spinal cord and compared to two well-established commercial biomaterials—degradable polyglycolide (vicryl and polyglactin 910), which is used clinically as absorbable sutures and meshes, and nondegradable expanded poly(tetrafluoroethylene) (ePTFE and Gore-Tex™), which is used clinically to minimize tissue adhesion; 78% DD chitosan is the lowest DD limit for synthesized channels to have acceptable mechanical properties for application in the spinal cord.

The host tissue response and degradation profile of three biomaterials were compared in two complementary studies. In the first set of experiments, chitosan (DD = 85%), vicryl, and Gore-Tex were separately implanted in the intrathecal space, between the spinal cord and dura mater and characterized over a 6-month period. Implants were characterized for degradation by mass loss and SEM and for biocompatibility by fibrous capsule formation, activated macrophage response, and reactive astrocyte response. In the second set of experiments, the same materials were tested, with the addition of DD = 78% chitosan, in the intramedullary space, directly in the spinal cord tissue parenchyma, over a 12-month period.

MATERIALS AND METHODS

Material processing

For chitosan sheet implants, chitosan channels were first processed as previously described.[27] Chitosan chloride (Protosan UP CL213; NovaMatrix, Drammen, Norway) was dissolved as a 1% (w/v) solution in water and precipitated with 4% NaOH solution, washed and lyophilized. The dried chitosan was made into a 3% (w/v) solution in 2% acetic acid, followed by a 50/50 (v/v) dilution in ethanol and stored at 4°C.

Tubes were prepared in 15-cm long cylindrical glass moulds made by inserting an inner glass rod (OD = 4 mm) into a larger glass tube (ID = 7 mm). The inner rod was fixed in place at both ends by rubber septa. The chitosan solution was used to form chitin tubes by adding 18.2 μL acetic anhydride per 1 mL of chitosan solution, mixed for 30 s at 5000 rpm (SpeedMixer DAC 150 FVZ; Hauschild Engineering, Hamm, Germany), then injected into the moulds. After 24 h, the chitin tubes were removed from the outer mould and washed in distilled water for an additional 24 h. The chitin tubes were converted back into chitosan by hydrolysis in 40 wt % NaOH solution at 110°C, first for 2 h followed by an additional 15 or 25 min to achieve different degrees of deacetylation (78% and 85%, respectively, as determined by ^{1}H-NMR).[28] After another 24-h wash, the chitosan tubes were removed from the glass rods and air dried over stainless steel cylindrical cores (OD = 3.7 mm). Tubes were rehydrated in water, removed from the steel core and cut into 1 × 2 mm sheets. These sheets were air-dried and sterilized by gamma irradiation at 2.5 MRad and rehydrated in sterile saline prior to use.

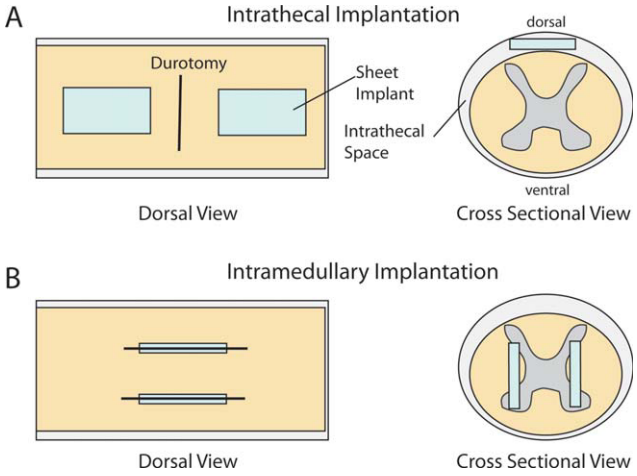

FIGURE 1. (A) Intrathecal implantion where sheets of chitosan, Gore-Tex, or vicryl were placed on the dorsal surface of the spinal cord on either side of the durotomy. (B) Intramedullary implantations are performed by longitudinal durotomy and myelotomy, followed by placement of the material into the spinal cord parenchyma. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Vicryl (polyglactin 910) woven mesh (Ethicon, Somerville, NJ) and Gore-Tex Preclude PDX dura substitute (Gore, Flagstaff, AZ) were received sterile and cut into 1 × 2 mm sheets prior to use.

In vivo implantation

All animal experiments were approved by the Animal Care Committee of the University Health Network. Adult female Sprague-Dawley rats (250–350 g, Charles River, St. Constant, QC) were anesthetized with 4% halothane and an oxygen/nitrous oxide (2:1) mixture, and then the halothane concentration was maintained at 2% during the operation. Following incision of the dorsal skin, a laminectomy was performed at the T8 vertebral level to expose dorsal dura overlying the spinal cord.

Intrathecal implantation. The dura was lifted and incised resulting in a 2-mm durotomy perpendicular to the midline. Each animal received two sheets (2 × 1 mm) of either chitosan (DD = 85%), Gore-Tex, or vicryl, and the sham control received only the durotomy. These sheets were inserted over the dorsal surface of the cord approximately 1 mm from the durotomy, one sheet rostral and one sheet caudal [Fig. 1(A)].

Intramedullary implantation. Longitudinal incisions were made into the dura and the underlying spinal cord 1 mm lateral to either side of the midline. 2 × 1 mm sheets of chitosan, vicryl, or Gore-Tex were inserted into the incision site [Fig. 1(B)]. For this experiment, we used two different formulations of chitosan (DD = 85% and 78%).

Following implantation in both models, the dural openings were overlaid with 20 μL of fibrin glue (Beriplast P;

CSL Behring, King of Prussia, PA) and the overlying muscle and skin were closed with vicryl sutures and metal clips, respectively. Rats were given buprinorphrine post-surgery, and every 8–12 h for the next 48 h. In the intrathecal implantation study, animals were kept for either 1 month or 6 months ($n = 4$ per group per timepoint). The animals receiving intramedullary implants were kept for 1, 6, or 12 months ($n = 3$ per group per timepoint).

Tissue preparation

At the specified times after implantation, rats were transcardially perfused with neutral buffered formalin as previously described,[29] and the entire segment of the spinal cord adjacent to or containing the implanted materials was removed. A 1-cm portion of the spinal cord encompassing the implantation site (i.e., the caudal sheet in the intrathecal model and both sheets in the intramedullary model) was harvested and post-fixed for up to 1 week in formalin followed by paraffin embedding.

For the intrathecally implanted animals, the rostral implanted sheet was removed, washed in saline, and dried for explant analysis as indicated below. Sections of the spinal cord were cut at 8-μm thickness and mounted on Superfrosted Plus slides (Fisher Scientific, Markham, ON). The spinal cords were sectioned parasagittally or as cross-sections for the intrathecal and intramedullary studies, respectively.

Explant analysis

For the intrathecal experiment, the rostral sheet samples were removed at 1 and 6 months and analyzed as follows. For chitosan and Gore-Tex, the fibrous capsules could be easily separated from the implant and removed. Due to the interwoven nature of vicryl, it was not possible to separate the fibrous capsule. The explanted materials were washed and air dried. Mass measurements were taken and compared with those before implantation. Samples were then gold-sputter coated and imaged by scanning electron microscopy (Hitachi S2500) at an acceleration voltage of 20 kV.

Staining of paraffin-embedded tissue

Every sixth section was stained with Luxol fast blue and hematoxylin and eosin (LFB/H&E) or Masson's trichrome. LFB/H&E was used to examine general tissue architecture including fibrous encapsulation and Masson's trichrome was used to stain collagen including the extent of fibrous encapsulation.

Immunohistochemical staining with the following antibodies was also performed as previously described:[29] mouse anti-glial fibrillary acidic protein (GFAP; 1;200, Chemicon, Temecula, CA) to visualize reactive astrocytes, and mouse anti-rat monocytes/macrophages antibody (ED-1; 1:200, Serotec, Raleigh, NC) to visualize activated macrophages.

Statistics

Comparisons involving one independent factor (e.g., material type) or two independent factors (e.g., time and material type) were analyzed using one-way and two-way ANOVA, respectively, followed by Bonferroni post-hoc test. *P*-values less than 0.05 were used as the criteria for statistical significance. Statistical analysis was performed using GraphPad Prism Software. All values are represented as mean ± standard deviation.

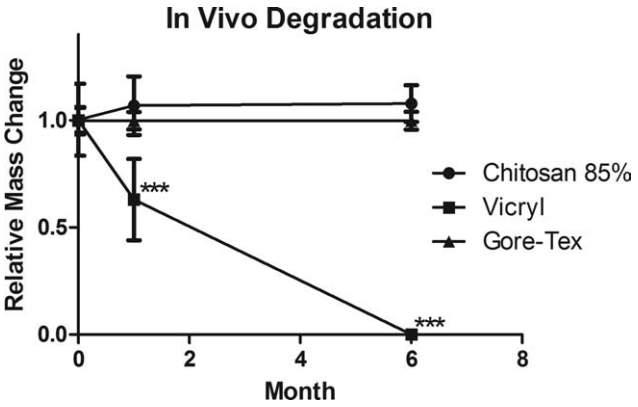

FIGURE 2. Measurement of weight change of implants removed after 1 and 6 months. Vicryl is the only material that exhibited degradation as assessed by weight loss ($n = 4$, mean ± standard deviation, ***$p < 0.001$).

RESULTS

Intrathecal implantation

Chitosan (DD = 85%), Gore-Tex, and vicryl were characterized for their biocompatibility and degradation properties in the intrathecal space of healthy adult rats. Degradation was assessed by explanting the material after sacrifice and measuring mass loss and imaging structural integrity with SEM. Figure 2 shows the dry mass measurements of the explanted materials over the course of the study. Vicryl was significantly degraded at 1 month and completely degraded at 6 months. Neither Gore-Tex nor chitosan (DD = 85%) showed any mass loss over the 6-month period. SEM images shown in Figure 3 are consistent with these findings, as neither chitosan nor Gore-Tex show evidence of degradation at either 1 or 6 months. Both dorsally and ventrally facing surfaces appeared identical to their respective preimplantation controls, with no signs of cellular infiltration or material breakdown. Conversely, vicryl showed significant breakdown of structure under SEM at 1 month and it could not be identified at 6 months, indicating complete degradation.

Histological characterization of the implants and underlying spinal cord tissue was also performed. Figure 4 shows sections stained with H&E/LFB at 1 month [Fig. 4(A–C)] and 6 months [Fig. 4(D–F)]. It should be noted that chitosan becomes very brittle upon fixation, resulting in fracturing of chitosan into shards as an artifact of sectioning, as seen in Figure 4(A). At 1 month, relatively thin fibrous encapsulation of chitosan and Gore-Tex implants was observed, whereas cells had thoroughly infiltrated the vicryl meshwork. The average fibrous capsule thickness was measured on the dural side of the implant and plotted in Figure 5. Vicryl had a significantly higher encapsulation thickness

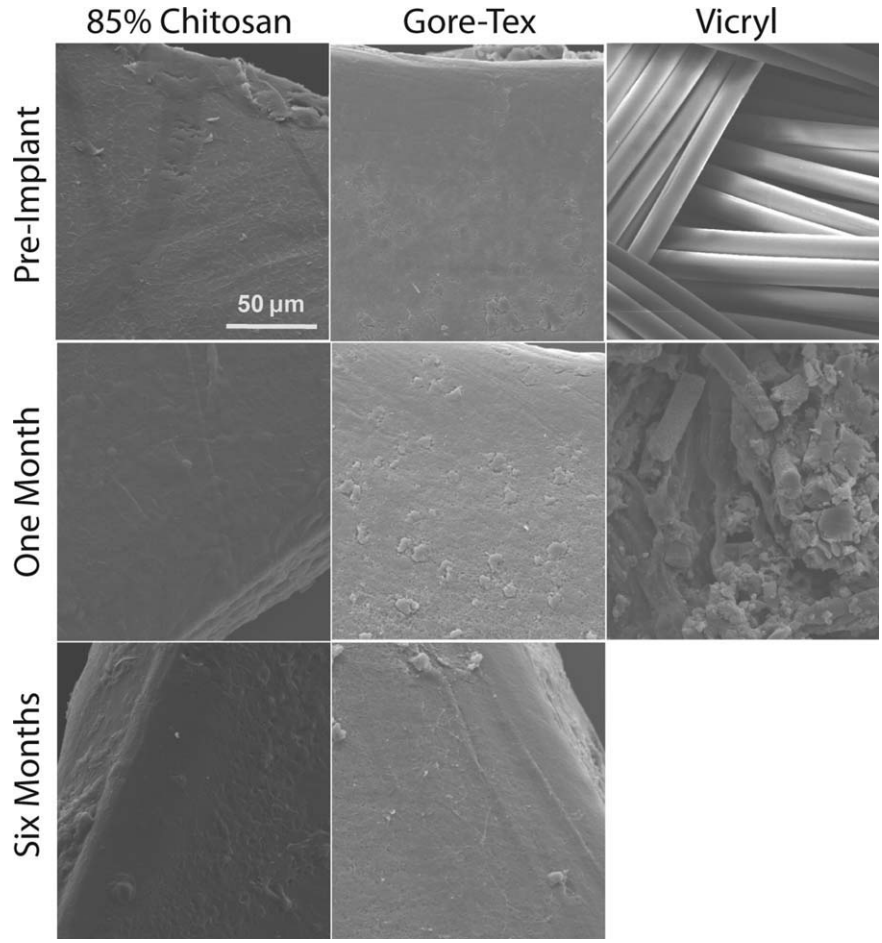

FIGURE 3. Scanning electron micrographs of intrathecal chitosan (DD = 85%), Gore-Tex, and vicryl sheets prior to implant and 1 and 6 months after implant. Chitosan and Gore-Tex show no significant signs of degradation. There was no vicryl sheet remaining at 6 months. All images are shown at the same magnification.

compared to both chitosan at 1 month ($p < 0.05$) and Gore-Tex at both timepoints ($p < 0.01$). No significant differences were seen between chitosan and Gore-Tex. The fibrous capsule thickness did not change significantly between 1 and 6 months of implantation.

Notably, a shift occurs in the H&E/LFB staining of chitosan over time, with the chitosan being more eosinophilic (pink) at 1 month and transitioning to more basophilic (blue) at 6 months [Fig. 4(A,D)]. This shift in H&E staining pattern has been previously observed during chitosan degradation[30] and its significance is discussed more thoroughly below.

The inflammatory response against these materials is shown in Figure 6. Activated (i.e., phagocytic) macrophages and/or microglia were characterized by their round morphology and positive staining for ED1.[31] ED1 staining was highest with vicryl at 1 month, with evidence of foreign giant body cells. Chitosan and Gore-Tex both elicited minimal phagocytic activity at 1 month. At 6 months, ED1 activity at the implant site subsided in all cases (data not shown), indicating that there was no chronic inflammatory response associated with any of these materials. No ED1 positive cells were observed in the sham control animals at either timepoint.

GFAP staining was also conducted to visualize reactive astrocytes in the adjacent spinal cord. Reactive astrocytes were present at both 1 month (Fig. 7) and 6 months and were mainly localized to the pial surface underlying the implants. Moderate reactivity was seen in all groups and was slightly elevated in vicryl groups.

Intramedullary implantation

The intramedullary implantation model (i.e., direct insertion into the spinal cord) was used to determine whether the more robust inflammatory and immune response associated with the tissue injury would alter the inflammatory reaction or degradation profile of the materials. The lack of observed physical degradation of 85% chitosan after intrathecal implantation prompted the addition of a second formulation of chitosan (DD = 78%), which we hypothesized would be more susceptible to degradation.

Implants inserted intramedullary were histologically characterized at 1, 6, and 12 months by H&E/LFB [Fig. 8(A–L)]. Vicryl degraded completely by 6 months, while both

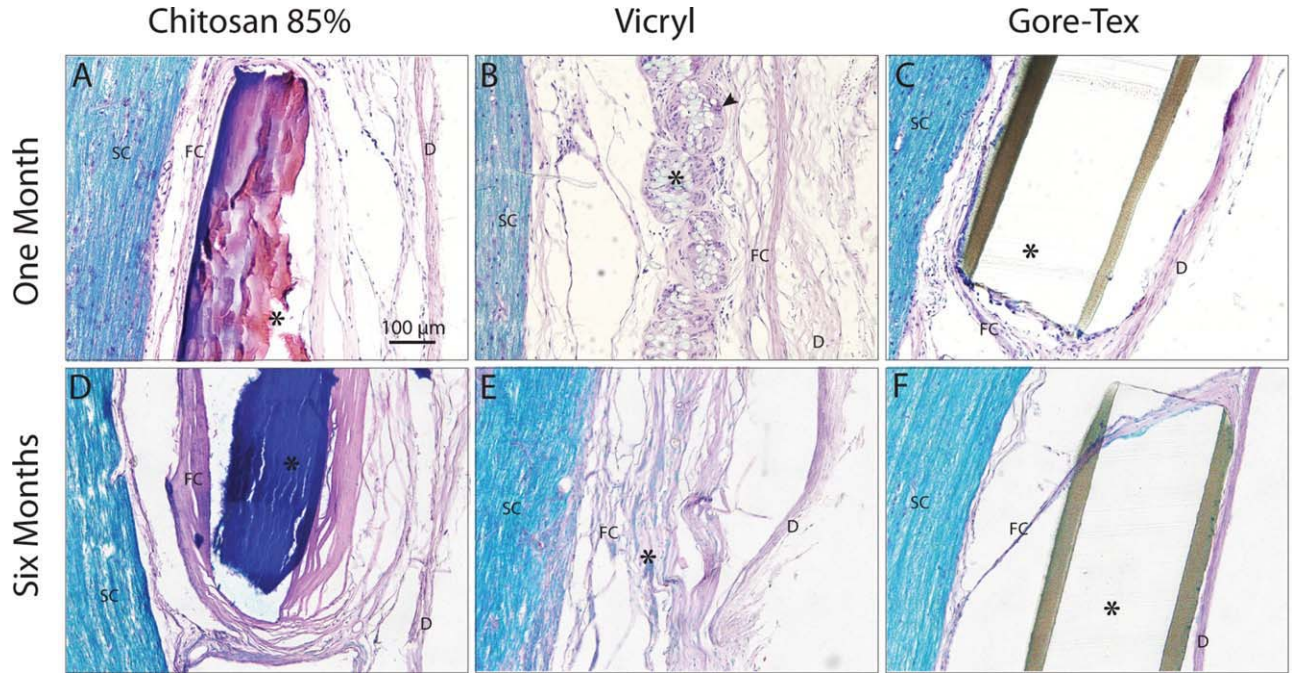

FIGURE 4. Representative images of intrathecal implants. LFB/H&E staining of chitosan, vicryl, and Gore-Tex implants at (A–C) 1 month and (D–F) 6 months. Fibrous capsules formed around all three materials and persist up to 6 months. Chitosan stains mainly with eosin (pink) at 1 month and hematoxylin (blue) at 6 months. At 6 months, vicryl is degraded whereas chitosan and Gore-Tex implants show no discernable evidence of mass loss. Symbols: SC, spinal cord; FC, fibrous capsule; *, implant; D, dura. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

chitosans (DD = 78% and DD = 85%) and Gore-Tex remained intact and unaltered over 12 months. The increased inflammatory and immune responses elicited by the trauma induced by intramedullary insertion did not result in accelerated degradation of chitosan. Likewise, lowering the degree of deacetylation to 78% did not affect the degradation of chitosan implants over 12 months as the implants appeared to be intact. It is again notable that a change in staining was seen with both chitosan formulations as evidenced in the color shift in the H&E/LFB staining from pink to blue over time (Fig. 8).

Fibrous capsules were formed around the different materials and were composed mainly of fibroblasts and collagen, as assessed by Masson's trichrome stain [Fig. 8(M–P)]. Measurement of the capsule thickness showed that chitosan (DD = 78%) elicited a greater fibroblast response compared to chitosan (DD = 85%) and Gore-Tex ($p < 0.05$), as measured by average capsule thickness (Fig. 9). Vicryl was omitted from quantification due to its marked degradation.

ED1 staining (Fig. 10) peaks at 1 month but ED1 positive cells did not penetrate the fibrous capsule of either chitosan 85%, 78%, or Gore-Tex. However, many ED1 positive cells were seen closely associated with vicryl [Fig. 10(C)]. At 6 months, ED1 reactivity subsided in the surrounding spinal cord parenchyma, indicating no chronic inflammatory response generated by any of the tested materials. GFAP staining showed reactive astrocytosis surrounding the fibrous capsules of each implant at 1 month (Fig. 11) and this persisted at 12 months (data not shown).

DISCUSSION

The biocompatibility of chitosan was compared with two commercially available materials: vicryl, an absorbable synthetic polymer mesh made primarily from poly(glycolic acid), and Gore-Tex, a flexible but inert ePTFE membrane. The materials were tested both in uninjured and injured spinal cord settings.

The results of the intrathecal implantation study for both vicryl and Gore-Tex were consistent with similar

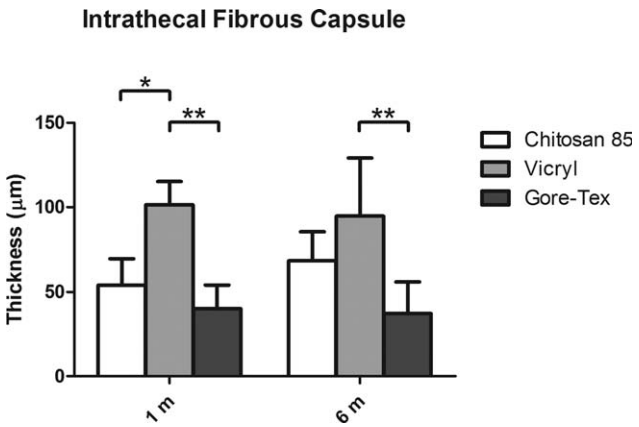

FIGURE 5. Comparison of fibrous capsule thickness between chitosan, vicryl, and Gore-Tex implants at 1 and 6 months. Vicryl elicited a stronger fibroblastic response versus chitosan at 1 month and Gore-Tex at 1 and 6 months. No significant differences were seen between chitosan and Gore-Tex. ($n = 4$, mean ± standard deviation, $*p < 0.05$, $**p < 0.01$).

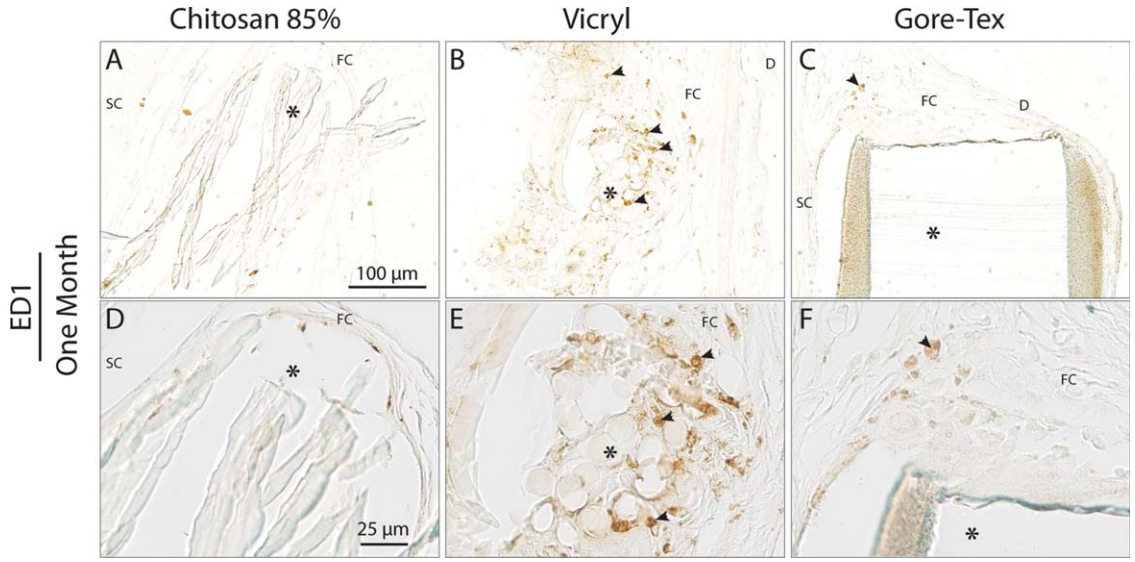

FIGURE 6. Representative images of ED1 staining at 1 month. Very few activated macrophages (arrowheads) interacting with chitosan or Gore-Tex, but high activation against vicryl. It should be noted that chitosan becomes brittle upon fixation and sectioning, resulting in a fractured appearance. Symbols: SC, spinal cord; FC, fibrous capsule; *, implant; D, dura. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

studies in which these two materials were investigated as dural substitutes[32–34] and showed a minimal inflammatory response, the formation of a thin membrane with Gore-Tex, and a slightly elevated inflammatory response to vicryl. Chitosan (DD = 85%) performed similar to Gore-Tex, without signs of chronic inflammatory or immune response. The results with chitosan show that when it is presented as a pure composition, nonporous sheet, it is an acceptable material in spinal cord tissue. Although positive GFAP staining was observed on the pial surface of the underlying spinal cord, much of this was attributed to the sustained pressure on the spinal cord caused by the implants and not necessarily a reaction to the chemical properties of the material itself. Indeed, there was some indentation of the spinal cord caused by both Gore-Tex and chitosan implants.

In the 6-month intrathecal study, chitosan did not show any physical signs of degradation. Unlike vicryl, which degrades due to hydrolysis, the degradation of chitosan requires enzymatic catalysis. Lysozyme catalyzes the hydrolysis of glycosidic bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine,[35] the latter of which is the acetylated component of chitosan. It has been suggested that lysozyme requires at least three consecutive acetylated monomer units to recognize the cleavage site,[36] consistent with the slower degradation rates of highly deacetylated chitosans.

Lysozyme concentrations vary largely, from $\sim$ 1 mg/mL in tears[37] to $\sim$ 10 µg/mL in serum[38] and $\sim$ 1 µg/mL or lower in cerebrospinal fluid.[39] The lysozyme level in normal rat spinal cord is very low but is upregulated in the spinal cord after injury, mainly localized to microglia and macrophages.[40] Accordingly, a second study was performed to investigate whether activating the immune response with a mild injury would accelerate the degradation process of chitosan. For this study, a second formulation of chitosan was also tested, with a DD of 78%, and the duration of the implantation was extended to 12 months. Chitosan with greater *D*-acetylglucosamine content has also been shown to stimulate a stronger macrophage response,[41] which should also accelerate the degradation response.

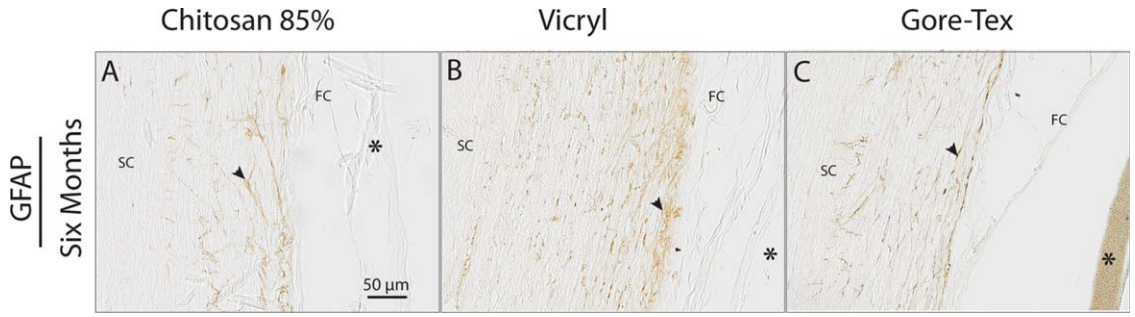

FIGURE 7. Representative GFAP staining of intrathecal implants at 6 months. GFAP reactivity (arrowheads) on the dorsal surface of the cord is seen adjacent to each implant. Symbols: SC, spinal cord; FC, fibrous capsule, *, implant. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

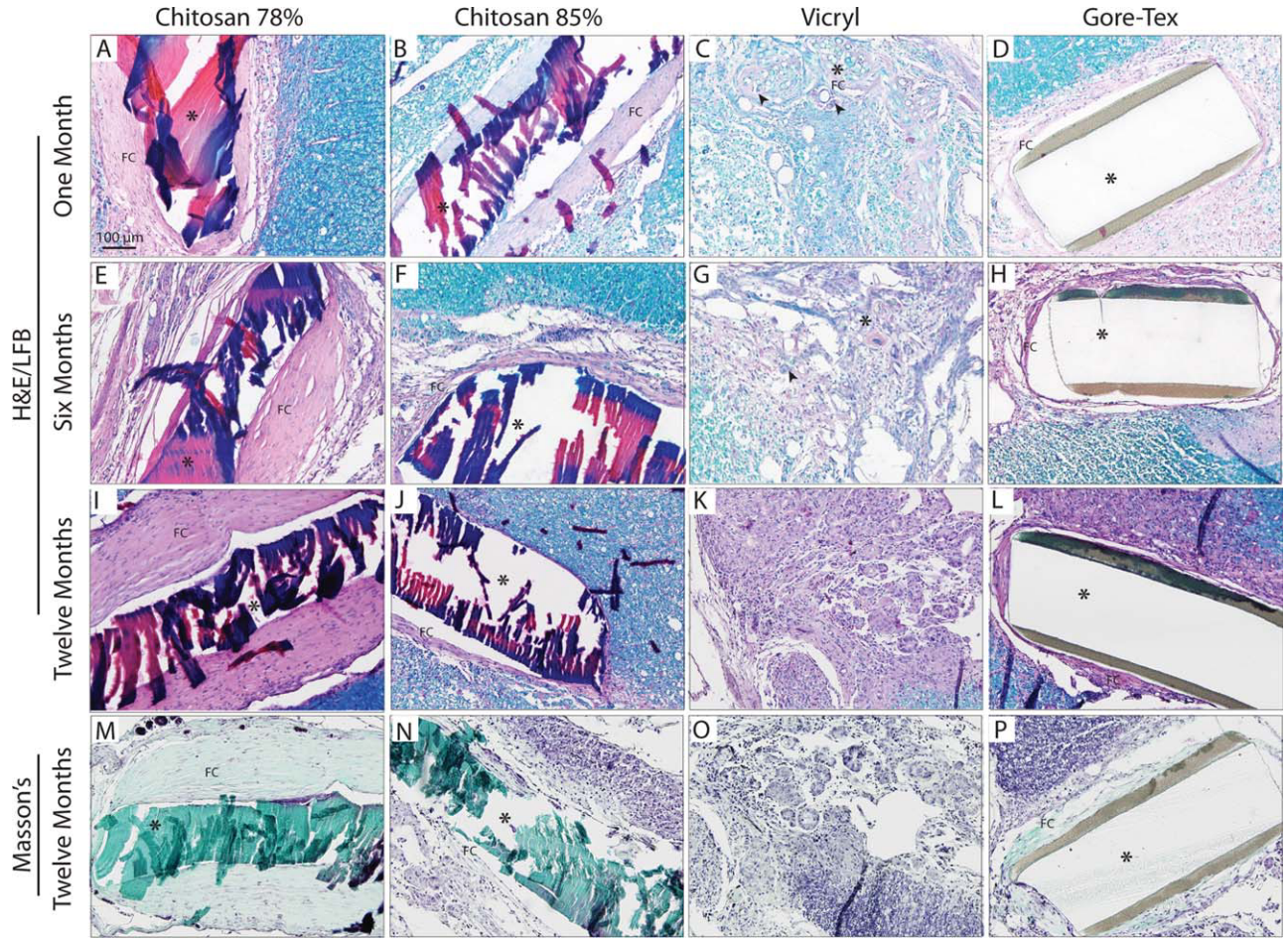

FIGURE 8. Representative images of intramedullary implants with H&E/LFB and Masson's trichrome staining; (A–D), 1 month; (E–H), 6 months, and (I–L) 12 month images with H&E/LFB, showing fibroblast infiltration and fibrous encapsulation of implants. No signs of direct cellular engagement are observed with chitosan or Gore-Tex, whereas foreign body giant cells are seen engulfing portions of vicryl (C and G arrowheads). Both 78% DD and 85% DD chitosan exhibit greater hematoxylin staining (blue) over time. (M–P) Masson's staining of the implant sites at 1 and 12 months show fibrous capsules to consist mainly of collagen (green). It should be noted that chitosan becomes brittle upon fixation, resulting in its fractured appearance upon sectioning. Symbols: FC, fibrous capsule; *, implant. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Intramedullary implantations of chitosan (DD = 85% and 78%), vicryl, and Gore-Tex resulted in fibrous encapsulation, and notably, the 78% DD chitosan resulted in a two-fold increase in capsule thickness compared to the 85% chitosan. This may be attributed to the different surface chemistries, where 85% chitosan carries a more positive charge due to increased amine content. One of the first and most influential events in the acute response to the material is protein adsorption. Differences in the adsorption of immunoglobulins, complement system proteins, and adhesion molecules can dictate the strength or selective recruitment of leukocytes, and, consequently, the severity of the ensuing inflammatory events. It has been reported that there are differences in protein binding to chitosan depending on the degree of deacetylation. In particular, lowering the degree of deacetylation leads to stronger activation of the complement system,[42] which would explain the more robust inflammatory response to 78% chitosan compared with 85% chitosan.

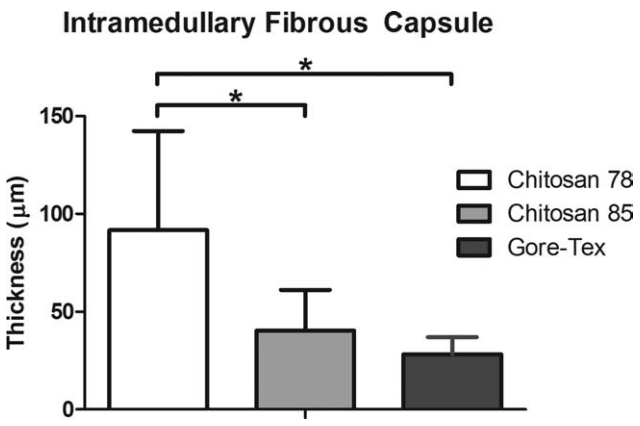

FIGURE 9. Comparison of fibrous capsule thickness between chitosan (DD = 78%), chitosan (DD = 85%), and Gore-Tex after intramedullary implantation. Chitosan (78%) had a significantly thicker fibrous capsule than either chitosan (85%) or Gore-Tex, which were not significantly different from each other. Data are pooled from 1, 6, and 12 month timepoints. ($n = 6$, mean ± standard deviation, $*p < 0.05$).

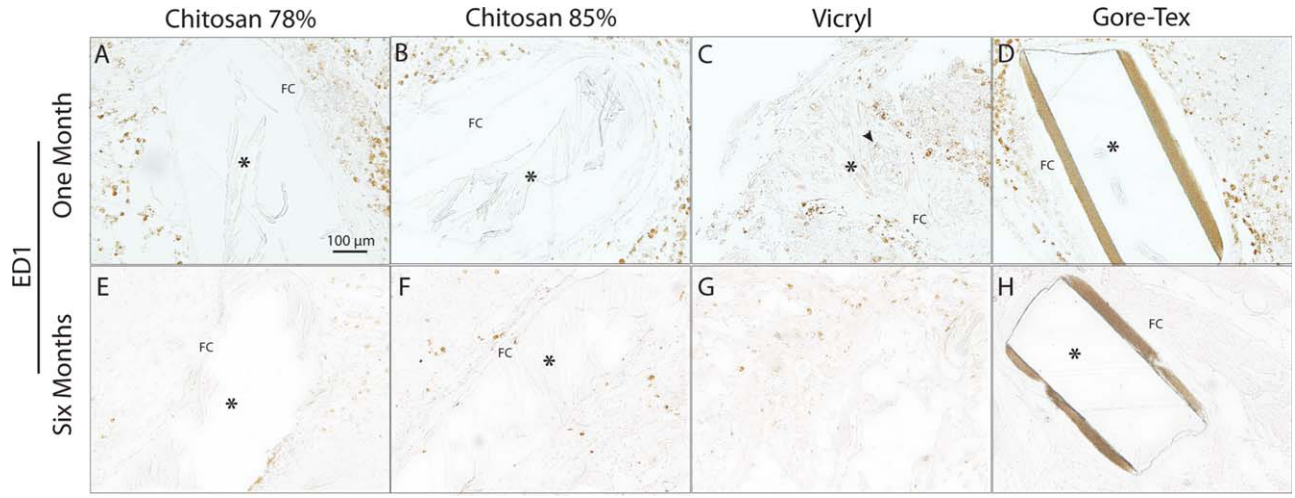

FIGURE 10. Representative images of ED1 staining of intramedullary implants at (A–D) 1 month and (E–H) 6 months. Macrophage activity is elevated at 1 month in the spinal cord parenchyma but subsides by 6 months. Direct macrophage interaction with the material is only observed in the case of vicryl (C, arrowhead). All images were taken at the same magnification. Symbols: FC, fibrous capsule; *, implant. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Another measure of the immune response to the intramedullary implanted materials was the activated microglia/macrophages response. ED1-positive cells were found at 1 month in all cases, largely a result of the injury to the spinal cord during implantation. However, only in the case of vicryl were they seen to interact directly with the material. ED1 staining subsided in the surrounding spinal cord tissue at 6 and 12 months, suggesting that there were no detrimental long-term or chronic effects induced by these materials. GFAP-positive reactive astrocytes were also present surrounding the fibrous capsules of each material and remained for the duration of the study. GFAP is considered a persistent signal, so this was not necessarily an indication of a chronic response to the materials. The majority of the GFAP reactivity was attributed to the injury caused by implantation and was not a direct effect of the materials themselves given that the implants are sequestered from the surrounding spinal cord tissue by the fibrous capsule.

Although both 78% and 85% chitosan implants were unaltered physically after one year *in vivo,* the shifts in the H&E/LFB staining from eosinophilic to basophilic suggest some degradation in the chitosan.[30] Hematoxylin staining, used mainly to identify cell nuclei, is the result of oxidized hematoxylin (hematein) combining with aluminum ions to form a hemalum dye-metal complex. This complex is then able to label nucleic acids via interaction with the aluminum.[43] Upon chitosan degradation by lysozyme, the hydrolyzed β1-4 glycosidic bond results in a free anomeric hydroxyl group on the cleaved residue. We hypothesize that this free anomeric hydroxyl group, along with the C2 amine group, is able to complex with aluminum-containing hemalum dye. Indeed, glucosamine residues have been shown to form complexes with other metal ions in this manner.[44]

The lack of physical degradation of chitosan implants suggest that although scission of the chitosan chains may be occurring, these chains are sufficiently long that they are insoluble and do not diffuse away. Chitosan degradation does not immediately result in mass loss but requires the degraded fragments to have a low enough molar mass to dissolve in the surrounding environment.[26] Our preparation of chitosan results in a very packed, nonporous structure.[45] Strong intermolecular hydrogen bonding between the chitosan chains results in higher crystallinity and can lead to reduced swelling[26] and solubility,[46] thereby limiting the

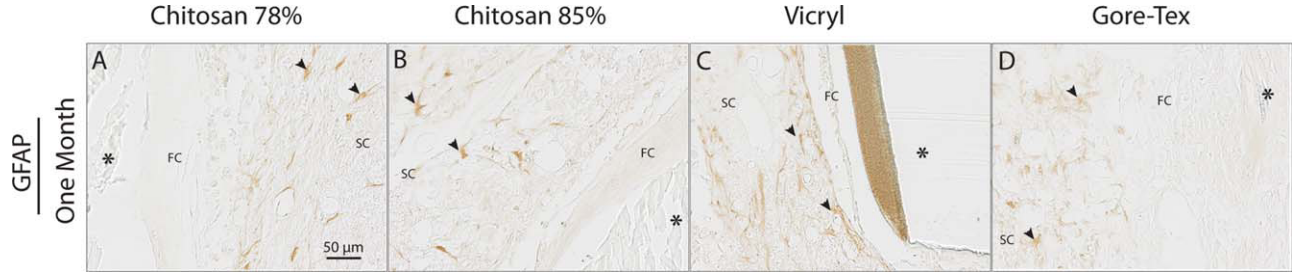

FIGURE 11. Representative images of GFAP staining of intramedullary implants at 1 month. Reactive astrocytes (arrowheads) were located in the spinal cord parenchyma, separated from the implanted materials by the fibrous capsule. All images were taken at the same magnification. Symbols: SC, spinal cord; FC, fibrous capsule; *, implant. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

access of lysozyme to the bulk material. This is evidenced here by a color shift in H&E staining initiating at the edges of the chitosan implants and migrating inwards over time [Fig. 8(A,B,E,F,I,J)]. Porosity[47] and crosslinking[48] have also previously been shown to affect the chitosan degradation rate.

In conclusion, chitosan has been shown to be a safe and relatively inert material in the spinal cord. It elicits a minor foreign body response similar to that of Gore-Tex, including thin fibrous encapsulation and early, yet nonpersisting, activation of microglia/macrophages. Changes in the charge profile of chitosan occur over time, suggesting degradation of polymer chains; however, this level of degradation does not result in mass loss or changes in the physical integrity of chitosan implants up to one year *in vivo*. Chitosan is a relatively inert biomaterial that does not elicit a chronic inflammatory or immune response, making it suitable for long-term spinal cord applications.

ACKNOWLEDGMENTS

We thank Rita van Bendegem for technical assistance with histology and immunohistochemistry and Roger Tam for helpful discussions.

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