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Chitin-based tubes for tissue engineering in the nervous system

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Abstract

The purpose of this study was to investigate chitin and chitosan as potential materials for biodegradable nerve guides. Transparent chitin hydrogel tubes were synthesized, for the first time, from chitosan solutions using acylation chemistry and mold casting techniques. Alkaline hydrolysis of chitin tubes resulted in chitosan tubes, with the extent of hydrolysis controlling the resulting amine content. This, in turn, impacted compressive strength and cell adhesion. Chitosan tubes were mechanically stronger than their chitin origins, as measured by the transverse compressive test, where tubes having degrees of acetylation of 1%, 3%, 18% (i.e. chitosan) and 94% (i.e. chitin) supported loads at a 30% displacement of 40.6 ± 4.3, 25.3 ± 4.5, 10.6 ± 0.8, and 8.7 ± 0.4 g, respectively. However, the chitin processing methodology could be optimized for compressive strength, by either incorporating reinforcing coils in the tube wall, or air-drying the hydrogel tubes. Chitin and chitosan supported adhesion and differentiation of primary chick dorsal root ganglion neurons in vitro. Chitosan films showed significantly enhanced neurite outgrowth relative to chitin films, reflecting the dependence of nerve cell affinity on the amine content in the polysaccharide: neurites extended $1794.7\pm392.0\,\mu m/mm^2$ on chitosan films vs. $140.5\pm41.6\,\mu m/mm^2$ on chitin films after 2 days of culture. This implies that cell adhesion and neurite extension can be adjusted by amine content, which is important for tissue engineering in the nervous system. The methods for easy processing and modification of chitin and chitosan described herein, allow the mechanical properties and cytocompatibility to be controlled and provide a means for a broader investigation into their use in biomedical applications.

Keywords: Chitosan; Nerve guidance channels; Nerve regeneration; Hollow fiber membranes

1. Introduction

The biopolymer chitin is one of the most abundant organic resources on earth, and is widely found in the exoskeletons of arthropods, the shells of crustaceans, and the cuticles of insects [1]. The polysaccharide consists of *N*-acetyl-D-glucosamine monomeric units, which are a major component of extracellular matrix macromolecules in the human body. Chitin is a promising polymer for biomedical applications because of its biocompatibility, biodegradability and structural similarity to the glycosaminoglycans [2]. A number of clinical studies using chitin have been reported [3], including its use as a wound-dressing material [4–6], as a treatment of chronic gastroduodenitis [7], and as an endodontic treatment [8]. Recently, results have been reported from a clinical trial where chitin membranes were used to treat patients with deep burns, orthopedic injury, trauma and ulcer conditions [9].

Despite clinical applications of chitin, it is still described as a "biomaterial in waiting," mainly due to its insolubility in common solvents, which prevents facile processing [10].

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Chitosan, the deacetylated form of chitin, is more readily soluble and is being investigated for numerous biomedical applications [11,12]; however, chitin degrades faster than chitosan [13], is more compatible with blood [14], and activates fewer macrophages [15].

Chitosan matrices have previously been shown to have low mechanical strength under physiological conditions [16], which has limited their use as nerve guidance channels for clinical applications [17,18]. Chitin gels, on the other hand, which can be prepared by selective *N*-acetylation of chitosan amine groups [19], are known to be mechanically strong [20], which suggests that they may be able to overcome the insufficient strength described for chitosan based nerve guides. To our knowledge, chitin gels have not been investigated as nerve guidance channels to date.

We have found that by using simple acylation chemistry, together with mold casting techniques, chitin gel tubes can be fabricated having favourable mechanical properties for use as nerve guides. In this paper, we report on the synthesis of these novel chitin gel tubes, their mechanical properties and their cell compatibility, which was tested in vitro with dorsal root ganglion (DRG) neurons. In addition, we report on the synthesis of chitosan tubes, with controlled amine content, by the deacetylation of chitin tubes under well-defined conditions, which allowed both the mechanical strength and cell compatibility to be tuned.

2. Materials and methods

2.1. Materials

Chitosan, with a molar mass of about 400,000 g/mol, was received from Fluka (Oakville, Canada) and used as received. The degree of acetylation was determined to be 11% by ^{1}H-NMR spectroscopy (see Section 2.2.). Acetic anhydride was supplied by ACP Chemicals (Montreal, Canada). Deuterium oxide (D_2O) was received from Cambridge Isotope Laboratories (Andover, USA), and deuterium chloride (DCl, 35% in D_2O) from CDN Isotopes (Pointe-Claire, Canada). Poly(D,L-lactide-*co*-glycolide) (PLGA, 85/15, I.V. 1.11 dL/g) was received from Birmingham Polymers (Birmingham, USA). All other chemicals and solvents were purchased from Sigma-Aldrich (Oakville, Canada) and used as received unless otherwise noted. Water was distilled and deionized using Millipore Milli-RO 10 Plus and Milli-Q UF Plus (Bedford, USA) at 18 MΩ resistance. Water for cell culture was sterile-filtered through a 0.2 μm Millipore filter (Bedford, USA).

2.2. Analysis of the degree of acetylation

The degree of acetylation of chitin and chitosan samples were determined using ^{1}H-NMR spectroscopy according to a modified published procedure [21,22]. Briefly, chitin samples were dissolved in a mixture of 25% DCl in D_2O and chitosan samples in a mixture of 0.25% DCl in D_2O. The concentration of polymer in the solution was approximately 0.5% (w/v) in all cases. The ^{1}H-NMR spectra were recorded on a Gemini 300 spectrometer (Varian). The degree of acetylation was calculated by comparing the integrated area under the peaks associated with H2–H6 and that with the methyl group, as previously described [21,22].

2.3. Chitin gel tube fabrication

A 3% solution of chitosan in 2% aqueous acetic acid was diluted with an equal volume of ethanol and, after cooling to ~10 °C, mixed with a twofold molar excess of acetic anhydride. The reaction mixture was sonicated to remove air-bubbles and injected into a sealed cylindrical glass mold (inner diameter, ID, 4.0 mm), which contained a fixed central cylindrical glass core (outer diameter, OD, 1.7 mm). Gelation occurred within ~3 min and after 24 h, during which syneresis occurred, the chitin hydrogel tube was removed from the mold and first washed with, and then stored in, water.

Chitin tubes reinforced with polymer coils were fabricated by the same technique after mounting a PLGA coil onto the cylindrical glass core. The coils were custom-made by winding PLGA fibers (diameter 160 μm) that were extruded from a high-pressure piston extruder with a fixed orifice of 0.3 mm (SpinLine, DACA Instruments) at a barrel temperature of 150 °C, with a piston advancing rate of 0.8 mm/min and a winder speed of 2.5 m/min.

2.4. Air-drying of chitin tubes

Air-dried chitin tubes were fabricated by drying chitin hydrogel tubes on a cylindrical glass core (OD 1.7 mm), which was pre-coated with a thin layer of poly(ε-caprolactone) in order to facilitate subsequent tube removal. After drying for 3 h at room temperature (RT) and normal pressure, the tubes were re-immersed in water, removed from the glass core, and stored in distilled water.

2.5. Deacetylation of chitin tubes

Chitin tubes, prepared as described in Section 2.3, were mounted on a cylindrical glass core (OD 1.7 mm) and stored in a 40% aqueous solution of sodium hydroxide at 110 °C for 2 h. The tubes were then intensely washed with distilled water, and air-dried for 3 h at RT and normal pressure. After re-soaking in water, the tubes were removed from the glass core, and stored in water. To achieve greater levels of deacetylation, tubes were stored for 2 h in the alkaline solution as

described above, followed by washing with water. This cycle of hydrolysis/washing was repeated up to 2 times.

2.6. Scanning electron microscopy (SEM)

For SEM imaging, samples were cut to 5 mm, quenched in liquid nitrogen and freeze-dried. Dried scaffolds were attached with double-sided tape to microscopy sample studs and gold-coated for 60 s. The samples were then placed on a Hitachi S-2500 SEM stage for imaging. Operating conditions included a working distance of 15 mm and an accelerating voltage of 20 kV.

2.7. Tube permeability testing

Chitin and chitosan tubes of 10 mm length were each filled with 5 μl of a 2% aqueous solution of vitamin B12 (Sigma-Aldrich, Oakville, Canada), sealed, and stored individually in vials containing 1 ml of distilled water at 37 °C. The release medium was replaced at predetermined time points over a 210 min period. The amount of vitamin B12 released was determined by UV spectroscopy at 550 nm using an Ultraspec 4000 spectrometer (Pharmacia Biotech). The data is expressed as means ± standard deviations of the cumulative percentage release of vitamin B12 from three samples. Effective diffusion coefficients were determined from these samples using the unsteady-state diffusion model, as previously described [23]. Variance analysis using an ANOVA single factor test was used for the statistical analysis, with 95% confidence.

2.8. Mechanical testing

Transverse compression testing of the tubes was performed on a Biosyntech micromechanical tester in phosphate buffered solution (PBS, pH 7.4) at 37 °C. The length of all samples was 8 mm. The tube wall thickness was measured before testing. The crosshead speed was maintained at 0.6 mm/min. The compressive strengths of chitin/chitosan tubes were compared from the load-displacement curves of three measurements. Results are expressed as means ± standard deviations. Variance analysis using an ANOVA single factor test was used for the statistical analysis, with 95% confidence.

2.9. Dorsal root ganglion (DRG) cell culture

Chitin samples for cell culture studies were prepared by a modification of the procedure described in [19]. Briefly, a 3% solution of chitosan in 2% aqueous acetic acid was diluted with an equal volume of ethanol and mixed with a twofold molar excess of acetic anhydride. The reaction mixture was poured into a Petri dish to form the hydrogel. Chitin films were prepared by air-drying the gels for 24 h at RT. The chitin gel and film were 92% acetylated. Chitosan films, with 11% acetylation, were prepared by pouring the chitosan solution into Petri dishes, followed by drying. Both the gels and films were treated with a solution of ammonia in methanol/water [24], followed by intense washing with distilled water, and finally stored in PBS. All samples were sterilized by autoclaving (120 °C, 20 min) and then immersed in Eagle's minimal essential medium for 24 h prior to plating DRG neurons.

Lumbar DRG were dissected from E9 White Rock chicks (Brampton, Canada) according to an established protocol [25]. DRG neurons were plated at a density of 1×10^4 cells/cm^2 to facilitate quantitative analysis and cultured on the chitin/chitosan samples in a cell culture medium consisting of Eagle's minimal essential medium, 10% horse serum, 0.2 mM glutamine, 1% penicillin/streptomycin, and 50 ng/ml nerve growth factor (NGF, all supplied by Gibco, Burlington, Canada), and incubated (37 °C, 5% CO_2 and 100% humidity). After 48 h, 10 random, separate fields per well were recorded at 20× magnification using a phase contrast microscope (LM410, Zeiss) to calculate the average number of adherent cells, the number of cells bearing neurites greater than 1 cell diameter, and the total length of these neurites. Each experiment was conducted in triplicate. Results are expressed as means ± standard deviations. Variance analysis using an ANOVA Single Factor test was used for the statistical analysis, with 95% confidence.

3. Results and discussion

3.1. Chitin gel tube synthesis

Chitin hydrogel tubes were synthesized by *N*-acetylation of chitosan using a twofold excess of acetic anhydride and a cylindrical mold containing a cylindrical core. The resulting tubes showed syneresis within 24 h after fabrication, leading to decreased lengths and wall thicknesses. However, the cylindrical core prevented any reduction of the inner diameter of the tube due to syneresis. This technique allowed precise control of the resulting chitin tube dimensions.

Chitin gel tubes were 94% acetylated, as determined by ^{1}H NMR spectroscopy, and were transparent, as shown in Fig. 1a. The diameters of the tubes were varied by simply changing the dimensions of the mold and core used, and the tube wall thickness was adjusted by the amount of chitosan solution added to the mold.

To enhance the mechanical properties of chitin tubes, they were designed as composite structures, with coils embedded within the wall, as shown in Fig. 1b, where the identical process to create chitin tubes was used, with only the addition of PLGA coils around the inner core.

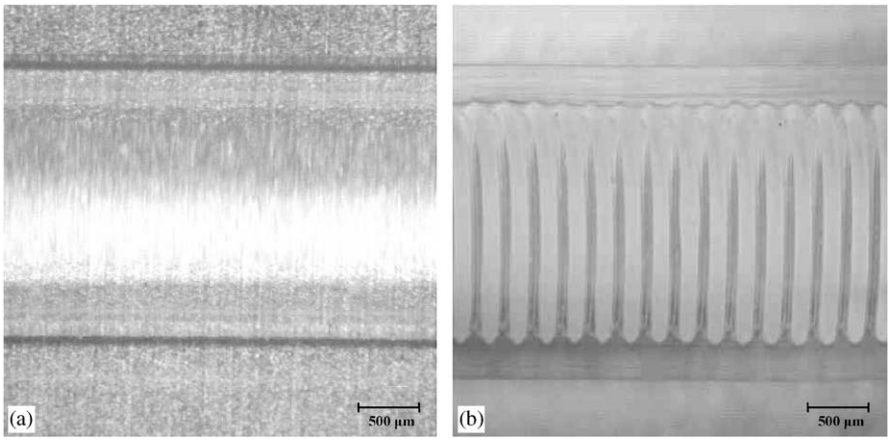

Fig. 1. Optical microscope longitudinal view of (a) a chitin hydrogel tube and (b) a chitin gel tube reinforced with a PLGA coils embedded in the wall.

3.2. Air-dried chitin tubes

The highly porous, honeycomb-like morphology of chitin hydrogels collapse irreversibly during air-drying (Fig. 2) [26], leading to dense chitin membranes with unique mechanical and permeability properties [27]. Using this knowledge, we prepared chitin membrane tubes by drying gel tubes around a cylindrical core of defined diameter. The air-dried tubes are transparent and have significantly reduced wall thicknesses relative to their hydrogel origins. After re-immersion in water, dried tubes show a slight re-swelling, which allows for their easy removal from the core. The final wall thickness approaches ~30% of that of the original hydrogel tube (Fig. 3). Drying the tubes allows for the removal of compounds used or formed in the acetylation reaction, such as ethanol and acetic acid. Additionally, dried tubes can be sterilized with ethylene oxide, and as a result can be stored prior to their use without their properties changing.

3.3. Hydrolysis of chitin tubes to form chitosan tubes

The alkaline hydrolysis and deacetylation of chitin gel tubes synthesized as described above resulted in transparent chitosan tubes. Chitin hydrogel tubes were mounted on a glass core prior to hydrolysis in order to preserve the tubular structure and control the final inner diameter of the resulting chitosan tubes. The lengths and wall thicknesses of the tubes were reduced to about 65% and 75% of their original dimensions, respectively, after 2 h of alkaline treatment. It can be expected that the small hydroxide anion readily penetrates the chitin gel and that no gradient in the degree of acetylation will be formed between the outside and the inside of the tube wall. During our experiments we noticed that the tubes became very slippery and could be easily moved along the glass core right after immersion into the alkaline medium which indicates access to and wetting of the inner surface of the chitin tube by the alkaline solution and similar surface properties of the inner and outer tube surfaces.

The hydrolysis of chitin in alkaline medium has been shown to be incomplete, even after extended reaction times [28], with a plateau of about 20% acetylation being reached [28,29], as was also confirmed here. To decrease the degree of acetylation, we exposed chitin tubes to several cycles of hydrolysis and washing, as has been previously described [29]. The degree of acetylation of chitin gel tubes decreased from 94% to 18% after 2 h of immersion in a 40% sodium hydroxide solution at 110 °C. After washing with water and repeating this alkaline treatment, the degree of acetylation further decreased to 3%, and after another cycle to 1%. By controlling these conditions, chitosan tubes having a defined degree of acetylation were synthesized, allowing for the control of mechanical properties, biodegradation, and biocompatibility, which depend on the amount of amine and acetamide groups in the polysaccharide structure [13,24,29-30].

3.4. Tube permeability

Permeability across the tube wall is important for the transport of nutrients and waste products in vivo. To gain a greater perspective on permeability and the impact of acetylation and processing (i.e. drying), we investigated the release profile of vitamin B12, a 1355 g/mol molecule, that had been loaded into sealed tubes and released into distilled water at 37 °C. As shown in Fig. 4, we found no statistically significant differences between wet chitin (94% acetylated) and wet chitosan (1% acetylated) tubes in terms of vitamin B12 release. Interestingly, the release of vitamin B12 from air-dried chitin tubes (with a dense wall morphology) was significantly faster than that of chitin gel tubes (with a honeycomb-like morphology) (cf. Fig. 2), which may be attributed to the decreased wall thickness of the dried

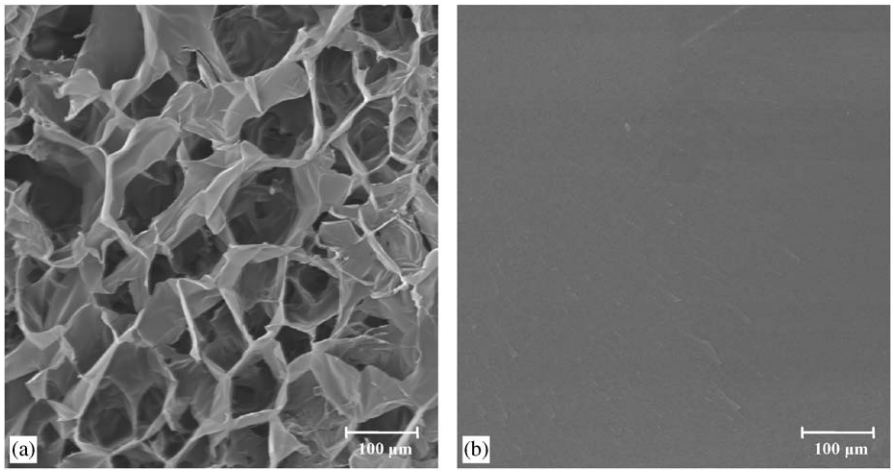

Fig. 2. SEM micrographs of representative chitin gels (a) before and (b) after air-drying. The honeycomb morphology of wet samples (a) collapsed with drying (b).

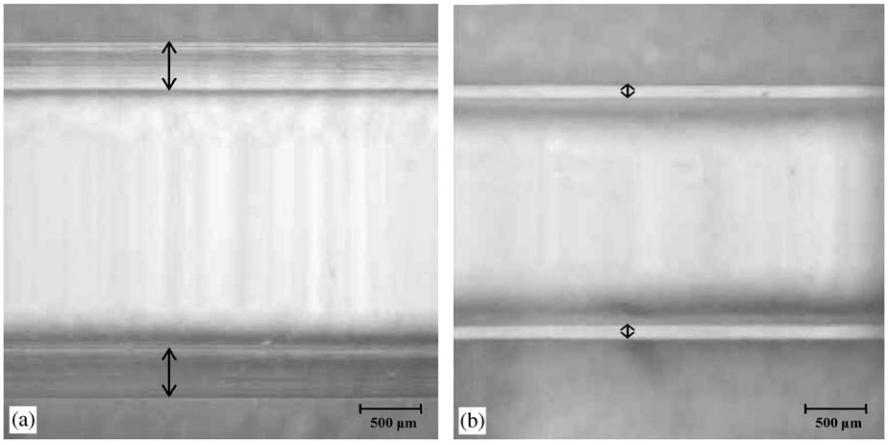

Fig. 3. Optical microscope longitudinal view of chitin tubes (a) before and (b) after air-drying with re-immersion in water. Arrows delineate the wall thickness of the tubes.

tubes. In attempt to reconcile these differences, we determined the diffusion coefficients of vitamin B12 by using the unsteady-state diffusion model [23], thereby considering different tube dimensions and wall thicknesses. The effective diffusion coefficients of vitamin B12 were found to decrease from $1.4 \pm 0.4 \times 10^{-6}$ to $8.5 \pm 0.6 \times 10^{-7}\,cm^2/s$ for wet and dried chitin tubes, and from $6.8 \pm 0.9 \times 10^{-7}$ to $1.5 \pm 0.1 \times 10^{-7}\,cm^2/s$ for wet and dried chitosan tubes. These results suggest that air-drying as well as hydrolysis, both leading to reduced wall thicknesses and denser wall morphologies, decrease diffusive transport through the tube wall. Overall, the diffusion coefficients are close to that of vitamin B12 in water ($3.9 \times 10^{-6}\,cm^2/s$ [23]).

3.5. Compression testing

The transverse compressive strengths of chitin tubes were measured by applying a displacement perpendicu-

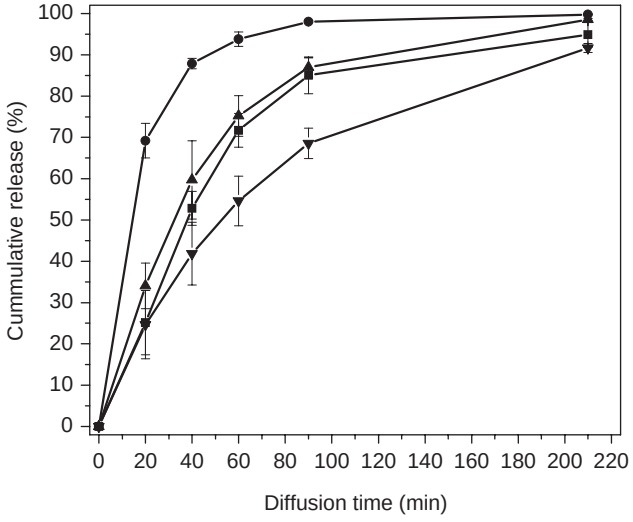

Fig. 4. Release of vitamin B12 from: chitin tubes (94% acetylated) (■) before and (●) after air-drying compared to chitosan tubes (1% acetylated) (▲) before and (▼) after air-drying.

lar to the longitudinal axis, and analysing the resulting load. Results from the load–displacement analyses are shown in Fig. 5. There was no statistically significant difference between air-dried tubes (wall thickness 125 μm) and their hydrogel origins (wall thickness 400 μm). However, the mechanical properties of tubes can be further adjusted by altering their wall thicknesses by varying mold dimensions and amounts of chitosan used for tube fabrication. For example, air-dried chitin tubes (wall thickness 250 μm) were significantly stronger than both air-dried chitin tubes (wall thickness 125 μm) and chitin gel tubes (wall thickness 400 μm). Thus, air-dried tubes can be synthesized to be mechanically strong yet have relatively thin walls.

While chitin tubes initially have high compressive strengths, they will lose their mechanical integrity in the course of degradation. Several strategies might allow for maintaining the mechanical strength and preventing the collapse of tubes during the period of nerve regeneration and maturation. For example, the incorporation of polymer coils into the tube wall (Fig. 1b) can stabilize the tubular structure and may prevent early collapse of the degrading chitin tube. As shown in Fig. 5, chitin gel tubes reinforced with PLGA coils had the greatest compressive strength measured. The tube strength appeared to plateau above 30% displacement suggesting plastic deformation of the polymer coil.

Another promising strategy is the modification of chitin tubes to chitosan tubes, by alkaline hydrolysis. As shown in Fig. 6, the compressive strength of these tubes increased with decreased degree of acetylation from 94% to 18% to 3% to 1% (or increased amine content). Statistically significant differences were found between

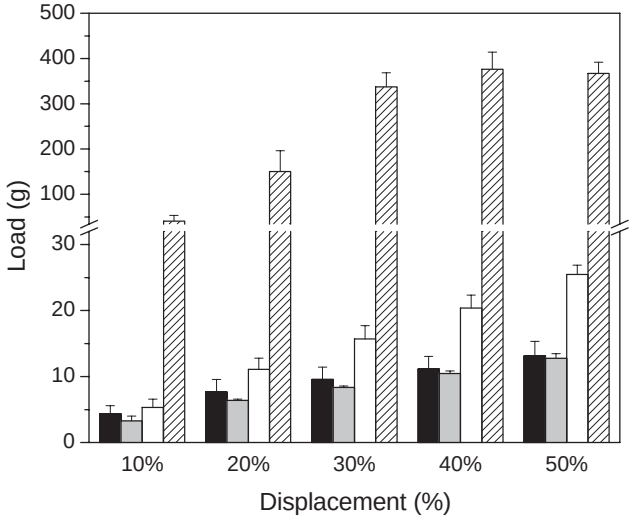

Fig. 5. Transverse compressive test of chitin tubes with wall thicknesses of: (■) 400 μm for chitin gel tubes and (▭) 125 μm and (▭) 250 μm for chitin air-dried tubes, compared to that of the coil-reinforced chitin gel tube (▨) wall thickness 400 μm. (Data shown as means + standard deviations, $n = 3$.)

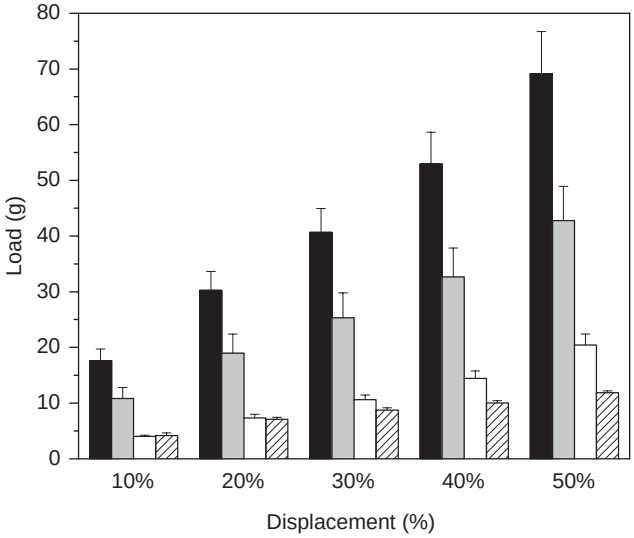

Fig. 6. Transverse compressive test of chitosan tubes having different degrees of acetylation: (■) 1%; (▭) 3%; (▭) 18%, compared to that of the chitin gel tube (▨) 94%. (Data shown as means + standard deviations, $n = 3$.)

all tubes at displacements of 30% and higher. These data agree with a study on wet chitosan films, where the tensile strength increased with decreased degree of acetylation possibly due to increased crystallization [29]. These chitosan tubes, with improved mechanical strength, may allow us to overcome the problems previously described for chitosan nerve guides [18,31–34], where an early loss of mechanical integrity and strength was observed in vivo. A very low degree of acetylation seems to be crucial to achieving both high mechanical strength and slow degradation [13] of chitosan nerve guides, which is pre-requisite for their long-term stability in vivo.

3.6. DRG cell compatibility

The cell compatibility of chitin hydrogels and air-dried films was tested in vitro by analysing adhesion and neurite extension of primary chick DRG neurons, which, to our knowledge, has not been previously described. Cell adhesion to nerve guides is important for contact-mediated guided regeneration. Our in vitro experiments showed differences in DRG cell adhesion and neurite outgrowth on chitin gels and films, as shown in Fig. 7 by optical microscopy and in Fig. 8 by quantitative analysis.

The chitin hydrogel showed limited cell adhesion, with cell clusters formed on the gel surface, and little neurite outgrowth after 2 days of culture (Fig. 7a). Air-dried chitin films showed enhanced cell adhesion, but the cells also formed clusters, indicating greater affinity for each other than the surface. Moderate neurite outgrowth was observed on these films (Fig. 7b). Significantly more DRG neurons adhered to the

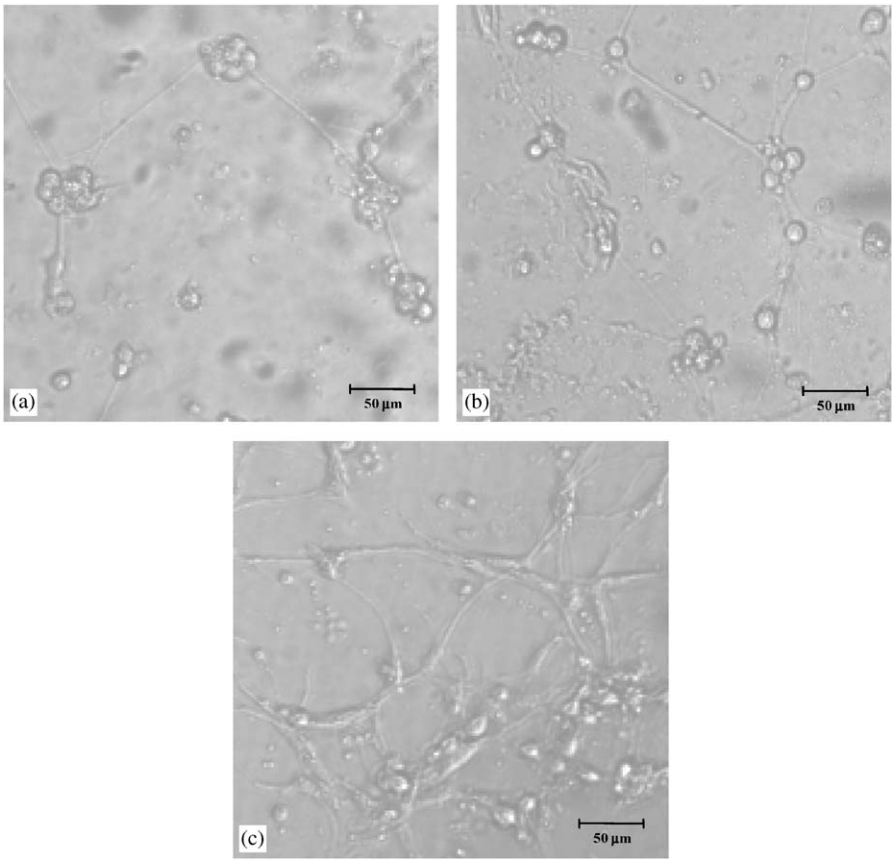

Fig. 7. Light micrograph images of DRG cell adhesion and neurite outgrowth after 2 days of culture on surfaces of: (a) chitin gel (92% acetylated), (b) chitin film (92% acetylated), and (c) chitosan film (11% acetylated) (initial cell seeding was 5×10^4 cells/cm^2).

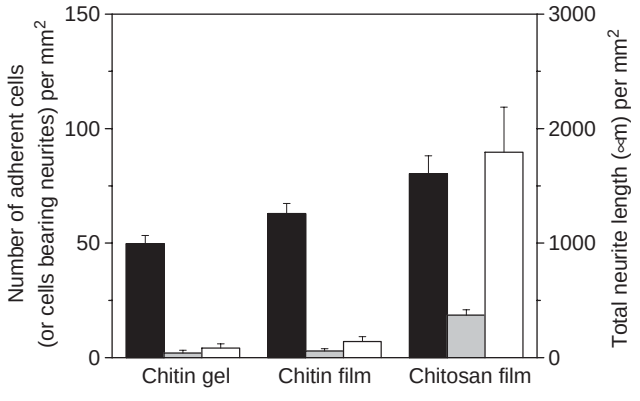

Fig. 8. Quantitative analysis of DRG cell adhesion and neurite outgrowth after 2 days of culture on surfaces of: chitin gel (92% acetylated), chitin film (92% acetylated), and chitosan film (11% acetylated) (initial cell seeding was 1×10^4 cells/cm^2): (■) total number of adherent cells; (▭) number of cells bearing neurites longer than 1 cell body length; (□) total neurite length (μm/mm^2). Statistically significant differences ($p < 0.05$) were found for the total number of adherent cells on all 3 materials, as well as in the number of cells bearing neurites and the total neurite length on chitosan in comparison to the chitin groups. (Data shown as means + standard deviations, $n = 3$.)

chitosan film surface than the other surfaces tested and fewer cell clusters formed (Fig. 7c).

Despite apparent differences by microscopy, there were no statistically significant differences between chitin gels and chitin films in terms of the numbers of cells bearing neurites and total neurite length. There were, however, significantly more cells bearing neurites and greater neurite lengths on chitosan films in comparison with the chitin samples (Fig. 8).

DRG cell adhesion and neurite extension on chitin/chitosan surfaces is affected by material characteristics, such as surface morphology, hydrophilicity and charge. Together, the similarity in results for chitin gels and films, and the greater cell adhesion and neurite outgrowth for chitosan films, suggest that the predominant factor determining the DRG cell compatibility with these polymers is charge—i.e. the amount and availability of amine groups—and not morphology. The charge density of chitosan increases with decreasing degree of acetylation, leading to greater cell adhesion. This agrees with published results using keratinocytes [24] and fibroblasts [24,30], suggesting that chitosan

strongly interacts with cells of different types due to the presence of cationic ammonium sites at physiological pH, that interact electrostatically with the negative charges of cell membrane surfaces [24].

Previous studies have shown that chitosan promotes survival and neurite outgrowth of nerve cells in vitro [35–40]. Our tests confirmed that both chitin and chitosan support DRG cell adhesion and neurite outgrowth. The higher the amine content (the lower the degree of acetylation), the higher the number of cells found on the material surface and the longer the neurites extending from the neurons. This implies that cell compatibility can be adjusted by amine content, which is important for tissue engineering in the nervous system.

4. Conclusions

Chitin hydrogel tubes were fabricated from chitosan solutions using acylation chemistry and mold casting techniques. This method allows for the preservation of the natural chemical composition of chitin, and no toxic crosslinking agent was necessary for the hydrogel preparation. Chitin tubes are transparent and mechanically strong. Their dimensions and wall thicknesses can be varied by simple modifications to the fabrication method. Air-dried chitin tubes were prepared with thin walls, yet maintained mechanical stability. Chitosan tubes with high compressive strength were fabricated by alkaline hydrolysis of chitin tubes. Tubes with different amine contents were prepared, thereby adjusting material properties.

Chitin and chitosan support nerve cell adhesion and neurite outgrowth, making these materials potential candidates for matrices in neural tissue engineering. These chitin/chitosan tubes will be tested for their regenerative capacity in vivo, where tube patency, as achieved here through processing or composite designs, has been shown to be critical to nerve regeneration. The chitin/chitosan tubes, films and gels can also be considered promising materials for other tissue engineering applications because of their biodegradability and biocompatibility. These strategies for the easy processing of chitin and chitosan will allow for a broader investigation into their use in biomedical applications in the future.

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References

[1] Muzzarelli RAA. Chitin. New York: Pergamon; 1977.
[2] Khor E. Chitin: fulfilling a biomaterials promise. Amsterdam: Elsevier; 2001.
[3] Shigemasa Y, Minami S. Applications of chitin and chitosan for biomaterials. Biotech Gen Eng Rev 1995;13:383–415.
[4] Balassa LL, Prudden JF. Applications of chitin and chitosan in wound-healing acceleration. In: Muzzarelli RAA, Pariser ER, editors. Proceedings of the first international conference on chitin/chitosan. Cambridge: MIT Sea Grant Program; 1978. p. 296–305.
[5] Kifune K. Clinical application of chitin artificial skin (Beschitin W). In: Brine CJ, Sandford PA, Zikakis JP, editors. Advances in chitin and chitosan. London: Elsevier; 1992. p. 9–15.
[6] Hirota Y, Tanioka S, Tanigawa T, Tanaka Y, Ojima R. Clinical applications of chitin and chitosan to human decubitus. In: Domard A, Jeuniaux C, Muzzarelli R, Roberts G, editors. Advances in chitin science 1. Lyon: Jacques Andre; 1996. p. 407–13.
[7] Gorovoj L, Seniouk O, Beketova G, Savichuk N, Amanbaeva G. Use of the chitin-containing preparation Mycoton in pediatric gastroenterology. In: Muzzarelli RAA, editor. Chitosan per os: from dietary supplement to drug carrier. Grottammare: Atec; 2000. p. 201–19.
[8] Ikeda T, Yanagiguchi K, Viloria IL, Hayashi Y. Relationship between lysozyme activity and clinical symptoms following the application of chitin/chitosan in endodontic treatment. In: Muzzarelli RAA, editor. Chitosan per os: from dietary supplement to drug carrier. Grottammare: Atec; 2000. p. 275–92.
[9] Khor E, Lim LY. Implantable applications of chitin and chitosan. Biomaterials 2003;24:2339–49.
[10] Khor E. Chitin: a biomaterial in waiting. Curr Op Solid State Mater Sci 2002;6:313–7.
[11] Kumar MNV. A review of chitin and chitosan applications. React Funct Polym 2000;46:1–27.
[12] Singh DK, Ray AR. Biomedical applications of chitin, chitosan, and their derivatives. J Macromol Sci 2000;C40:69–83.
[13] Tomihata K, Ikada Y. In vitro and in vivo degradation of films of chitin and its deacetylated derivatives. Biomaterials 1997;18:567–75.
[14] Lee KY, Ha WS, Park WH. Blood compatibility and biodegradability of partially *N*-acylated chitosan derivatives. Biomaterials 1995;16:1211–6.
[15] Nishimura K, Nishimura S, Nishi N, Saiki I, Tokura S, Azuma I. Immunological activity of chitin and its derivatives. Vaccine 1984;2:93–9.
[16] Madihally SV, Matthew HWT. Porous chitosan scaffolds for tissue engineering. Biomaterials 1999;20:1133–42.
[17] Yang F, Li X, Cheng M, Gong Y, Zhao N, Zhang X. Performance modification of chitosan membranes induced by gamma irradiation. J Biomater Appl 2002;16:215–26.
[18] Yamaguchi I, Itoh S, Suzuki M, Osaka A, Tanaka J. The chitosan prepared from crab tendons: II. The chitosan/apatite composites and their application to nerve regeneration. Biomaterials 2003;24:3285–92.
[19] Hirano S, Ohe Y, Ono H. Selective *N*-acylation of chitosan. Carbohydr Res 1976;47:315–20.
[20] Vachoud L, Domard A. Physicochemical properties of physical chitin hydrogels: modeling and relation with the mechanical properties. Biomacromolecules 2001;2:1294–300.
[21] Vachoud L, Zydowicz N, Domard A. Formation and characterisation of a physical chitin gel. Carbohydr Res 1997;302:169–77.
[22] Lavertu M, Xia Z, Serreqi AN, Berrada M, Rodrigues A, Wang D, Buschmann MD, Gupta A. A validated ^{1}H NMR method for the determination of the degree of deacetylation of chitosan. J Pharm Biomed Anal 2003;32:1149–58.

[23] Luo Y, Dalton PD, Shoichet MS. Investigating the properties of novel poly(2-hydroxyethyl methacrylate-*co*-methyl methacrylate) hydrogel hollow fiber membranes. Chem Mater 2001;13: 4087–93.
[24] Chatelet C, Damour O, Domard A. Influence of the degree of acetylation on some biological properties of chitosan films. Biomaterials 2001;22:261–8.
[25] Nishi R. Autonomic and sensory neuron cultures. Methods Cell Biol 1996;51:249–63.
[26] Hirano S, Tobetto K, Noishiki Y. SEM ultrastructure studies of *N*-acyl- and *N*-benzylidene-chitosan and chitosan membranes. J Biomed Mater Res 1981;15:903–11.
[27] Hirano S, Tobetto K, Hasegawa M, Matsuda N. Permeability properties of gels and membranes derived from chitosan. J Biomed Mater Res 1980;14:477–86.
[28] Wu ACM, Bough WA. A study of variables in the chitosan manufacturing process in relation to molecular-weight distribution, chemical characteristics and waste-treatment effectiveness. In: Muzzarelli RAA, Pariser ER, editors. Proceedings of the first international conference on chitin/chitosan. Cambridge: MIT Sea Grant Program; 1978. p. 88–102.
[29] Mima S, Miya M, Iwamoto R, Yoshikawa S. Highly deacetylated chitosan and its properties. J Appl Polym Sci 1983;28:1909–17.
[30] Prasitsilp M, Jenwithisuk R, Kongsuwan K, Damrongchai N, Watts P. Cellular responses to chitosan in vitro: the importance of deacetylation. J Mater Sci Mater Med 2000;11:773–8.
[31] Santos PM, Winterowd JG, Allen GG, Bothwell MA. Nerve growth factor: increased angiogenesis without improved nerve regeneration. Otolaryngol Head Neck Surg 1991;105:12–25.
[32] Suzuki M, Itoh S, Yamaguchi I, Takakuda K, Kobayashi H, Shinomiya K, Tanaka J. Tendon chitosan tubes covalently coupled with synthesized laminin peptides facilitate nerve regneration in vivo. J Neurosci Res 2003;72:646–59.
[33] Itoh S, Suzuki M, Yamaguchi I, Takakuda K, Kobayashi H, Shinomiya K, Tanaka J. Development of a nerve scaffold using a tendon chitosan tube. Artif Org 2003;27:1079–88.
[34] Itoh S, Yamaguchi I, Suzuki M, Ichinose S, Takakuda K, Kobayashi H, Shinomiya K, Tanaka J. Hydroxyapatite-coated tendon chitosan tubes with adsorbed laminin peptides facilitate nerve regeneration in vivo. Brain Res 2003;993:111–23.
[35] Zielinski BA, Aebischer P. Chitosan as a matrix for mammalian cell encapsulation. Biomaterials 1991;15:1049–56.
[36] Dillon GP, Yu X, Sridharan A, Ranieri JP, Bellamkonda RV. The influence of physical structure and charge on neurite extension in a 3D hydrogel scaffold. J Biomater Sci Polym Edn 1998;9: 1049–69.
[37] Dillon GP, Yu X, Bellamkonda RV. The polarity and magnitude of ambient charge influences three-dimensional neurite extension from DRGs. J Biomed Mater Res 2000;51:510–9.
[38] Gong H, Zhong Y, Li J, Gong Y, Zhao N, Zhang X. Studies on nerve cell affinity of chitosan-derived materials. J Biomed Mater Res 2000;52:285–95.
[39] Cheng M, Cao W, Gao Y, Gong Y, Zhao N, Zhang X. Studies on nerve cell affinity of biodegradable modified chitosan films. J Biomater Sci Polym Edn 2003;14:1155–67.
[40] Cheng M, Deng J, Yang F, Gong Y, Zhao N, Zhang X. Study on physical properties and nerve cell affinity of composite films from chitosan and gelatin solutions. Biomaterials 2003;24:2871–80.