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Chitin-Calcium Alginate Composite Fibers for Wound Care Dressings Spun from Ionic Liquid Solution

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Chitin-calcium alginate composite fibers were prepared from a solution of high molecular weight chitin extracted from shrimp shells and alginic acid in the ionic liquid 1-ethyl-3-methylimidazolium acetate by dry-jet wet spinning into an aqueous bath saturated with $CaCO_3$. The fibers exhibited a significant proportion of the individual properties of both calcium alginate and chitin. Ultimate stress values were close to values obtained for calcium alginate fibers, and the absorption capacities measured were consistent with those reported for current wound care dressings. Wound healing studies (rat model, histological evaluation) indicated that chitin-calcium alginate covered wound sites underwent normal wound healing with re-epithelialization and that coverage of the dermal fibrosis with hyperplastic epidermis was consistently complete after only 7 days of treatment. Using a single patch per wound per animal during the entire study, all rat wounds achieved 95-99% closure by day 10 with complete wound closure by day 14.

Introduction

The wound care sector has recently focused on the development of biocompatible dressings composed of natural biopolymers that accelerate skin healing.[1-3] The use of chitin (a natural polymer composed of β (1-4) linked N-acetylglucosamine units,[2] Fig 1A) in wound care dates back to a 1970 study by Prudden et al., identifying it as a "potent pure chemical wound healing accelerator".[4] The healing behavior of chitin, and its deacetylated derivative, chitosan (Fig. 1B), is attributed to the presence of *N*-acetylglucosamine or *N*-glucosamine monomers in chitin and chitosan, respectively. These glucosamine derivatives stimulate the production of hyaluronan and glycosaminoglycans (GAGs), present in the extracellular matrix which are responsible for binding and regulation of different proteins by human cells human cells such as fibroblasts.[5]

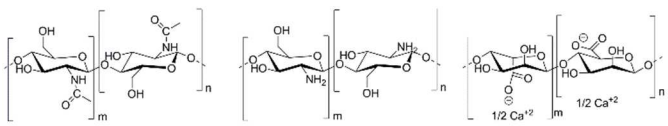

Fig. 1 Structures of chitin (a), chitosan (b), and calcium alginate (c).

When placed on a wound, chitin accelerates skin healing through regulation of inflammation and cell proliferation, and provides a matrix for tissue growth.[6,7] Chitin dressings have been prepared in the form of non-fibrous materials – membranes,[8]] hydrogels,[9] beads,[10] scaffolds,[11] and sponges, and have been found to be effective in regenerating wounded skin tissue[12] and re-epithelization. Numerous attempts have also been made to produce chitin fibers through spinning, by dissolving the polymer in an appropriate solvent system followed by extrusion into a coagulation bath. An extensive review by Ravikumar[13] covers multiple existing solvent systems for preparing chitin fibers, including fibers spun from *N,N*-dimethylformamide/LiCl,[14] trichloroacetic acid (TCA) with chloral hydrate or dichloromethane,[13] perfluorinated solvents,[15] superacidic solvents,[16] *N,N*-dimethylacetamide (DMAc)/LiCl, or *N*-methyl-2-pyrrolidone (NMP).[13] However, a problem remains in the removal of these solvents that can render them unsuitable for use in medical applications. For example, in the often used DMAc/LiCl system, Li^+ acts as a Lewis acid solvating the chitin amide group. It is unclear if this can be completely reversed through fiber washing.[13]

An often ignored aspect of this field, is that many reports claiming preparation of chitin wound care materials actually use chitosan,[17-22] or other water soluble chitin derivatives.[23] For example, in Unitika's chitin products patents, [24-,25,26,27,28] chitin was obtained by traditional chemical processing methods, i.e., treatment of crustacean shells with hydrochloric acid and

caustic soda at elevated pressure and temperatures. Such harsh conditions can result in the degradation of the chitin structure and deacetylation, changing the properties of the biopolymer,[29] and, in fact, after careful examination of the patents, one can conclude that the reported products are chitosan-based with a degree of deacetylation (DDA) of 50-70%.

Recognizing the importance of biopolymer extraction from raw biomass without degrading it and taking advantage of our previous work in biomass dissolution,[30] in 2010, we demonstrated that the ionic liquid (IL, now defined as salts which melt below 100 °C[31]) 1-ethyl-3-methylimidazolium acetate, [C_2mim][OAc], could be used to extract chitin by dissolving the biopolymer directly from raw crustacean shells, leading to the recovery of a high purity, high molecular weight (MW) chitin.[32-,33,34] We have also shown that due to the unique high MW of the extracted chitin compared to commercial chitin sources obtained by chemical degradation of the other shell components, it is possible to spin fibers directly from IL solution by dry-jet wet spinning[32] or electrospining.[35] The ability of certain ILs to dissolve (and co-dissolve) many biopolymers,[36-,37,38,39,40,41] also allows one to consider using solution techniques to prepare a variety of useful composite materials. For example, we have reported the preparation of biocomposite fibers through co-dissolution of microcrystalline cellulose (MCC) and alginic acid in the IL 1-butyl-3-methylimidazolium chloride ([C_4mim][Cl]).[42]

The hypothesis which led to the work described here, was that a single IL could co-dissolve chitin with calcium alginate (Fig. 1C) allowing spinning of composite fibers which would have utility for wound healing. We also predicted that by utilizing the high molecular weight chitin that ILs can extract directly from crustacean shells, we could spin fibers more readily at lower solution concentrations, and that these fibers would have increased strength.

The choice of calcium alginate arose from the widespread use of salts of alginic acid, polymeric 1,4-linked β-D-mannuronic acid and α-L-guluronic acid,[43] a natural polysaccharide available from brown seaweed, in wound care[19,44] as alginate[44] or alginate-cellulose composite fibers.[45,46] Alginates are absorbent, preventing both accumulation of wound exudate[47-,48,49] or wound dehydration,[50] stimulate human fibroblasts,[51,52] are cell proliferators,[51,53-,54,55,56,57,58] and hemostats which can be used for bleeding wounds,[59-,60,61,62] and be removed without much trauma due to their gel forming properties.

Only a few combinations of chitin with alginate have been described in the literature, including membranes prepared via casting solutions of chitin and sodium alginate in NaOH/urea[63] and hydrogels containing chitin and alginate (with 7 wt% chitosan and fucoidan) prepared via solid state mixing of biopolymers followed by alginate swelling in water/ethanol solution on a sterile support.[64] There are additional examples of chitosan blends with alginate[19] although these are often referred to as 'chitin' blends. To the best of our knowledge there are no examples of chitin-calcium alginate fibers suitable for weaving into patches and bandages (the most common type of dressings), most likely due to chitin's insolubility in most organic solvents and water.

Here we discuss our efforts to utilize the ability of the IL [C_2mim][OAc] to directly co-dissolve biopolymers to prepare chitin-calcium alginate fibers specifically for wound care. This work includes the evaluation of the fiber physical properties, their biocompatibility, and their wound closure efficacy.

Experimental

Chemicals and Materials

All materials were used as supplied unless otherwise noted. The ionic liquid 1-ethyl-3-methylimidazolium acetate ([C_2mim][OAc], purity >95%) was purchased from IoLiTec, Inc. (Tuscaloosa, AL). Alginic acid (MW ~240 kDa, 61% L-mannuronic acid, 39% D-guluronic acid), sodium alginate (alginic acid sodium salt), calcium carbonate ($CaCO_3$) and anhydrous calcium chloride ($CaCl_2$) were purchased from Sigma-Aldrich (Milwaukee, WI). Deionized (DI) water was obtained from a commercial deionizer (Culligan, Northbrook, IL, USA) with specific resistivity of 16.82 MΩ·cm at 25 °C.

Dried shrimp shells were received from the Gulf Coast Agricultural and Seafood Cooperative in Bayou La Batre, AL where the shrimp shells were dried at a specialized facility by pressing with a screw press to eliminate the majority of the water followed by heating at up to 160 °C in a fluidized bed dryer until the material had a final moisture content of less than 5 wt%. The dried material was pulverized with a hammer mill to particles 0.635 cm diameter and smaller. This dried, pulverized material was shipped to The University of Alabama. Before each laboratory experiment the shrimp shells were additionally ground using an electric lab mill (Model M20 S3, Ika®, Wilmington, NC). The ground shells were then separated by particle size using a set of four brass sieves with wire mesh (Ika Labortechnik, Wilmington, NC) decreasing in size (1000 μm, 500 μm, 250 μm, and 125 μm) into a collecting pan. Particle sizing was carried out in small aliquots of ca. 1-2 g, until a sufficient amount of ground shrimp shells with desired particle sizes (<125 μm) was obtained. For all the experiments described below, the ground shrimp shells were dried overnight in an oven at 80 °C (Precision Econotherm Laboratory Oven, Winchester, VA).

Extraction and Regeneration of Chitin

Chitin was extracted from dry, ground shrimp shells in 20 batches. In total, ca. 6 g of chitin was extracted from ca. 25 g of shrimp shells using 1200 g of [C_2mim][OAc], via the slightly modified procedure described in reference.[32] The procedure for one batch is given below.

Oven-dried ground shrimp shells (1.2740 grams) and [C_2mim][OAc] (62.3028 grams) were placed into a 250 mL Erlenmeyer flask, and heated via microwave irradiation (household microwave Emerson MW8999SB, Emerson Radio Corp., Moonachie, NJ) using 2 s pulses (to avoid overheating of the mixture). Between each pulse, the Erlenmeyer flask was

removed from microwave, the mixture manually stirred with a glass stirring rod to ensure uniform dispersion of shrimp shell powder in the IL, and the flask returned to the microwave. The mixture was heated for a total of 3-4 min (90-120 pulses). Extraction was monitored by the analysis of solution aliquots using an optical microscope (Reichert Stereo Star Zoom 580, Depew, NY), although complete dissolution was never achieved due to the presence of insoluble $CaCO_3$. After microwave irradiation for 3-4 minutes, the hot solution was transferred into eight 50 mL centrifuge tubes, and any undissolved residue was separated through centrifugation (Dynac Becton Dickinson Centrifuge model 42010, Sparks, MD) while the solution was still warm and therefore free flowing.

After centrifugation, the supernatant was carefully decanted from undissolved residue into ca. 400 mL DI water in a 600 mL beaker, and left to stir overnight. After 12 h the stirring was stopped and the white floc was allowed to settle for 2-4 h. About 350 ml of the aqueous solution was carefully decanted using a disposable plastic pipette while ca. 100 mL of residual aqueous solution with flocculated chitin remained in the beaker. The remaining solution and floc were transferred into two 50 mL centrifuge tubes, centrifuged and the aqueous phase decanted. Subsequently, ca. 45 mL of fresh DI water was added to each of the centrifuge tubes, and the resultant suspension sonicated (Branson Sonicator 5510-DTH, Danbury, CT) for 20 min.

Centrifugation, aqueous phase decantation, fresh DI water addition, and sonication steps were consecutively repeated 10 more times. After 11 total washes, the chitin was placed on a glass Petri dish in an 80 °C oven overnight, collected into a 20 mL screw cap vial, and stored. In the run reported here, 313 mg of chitin was obtained.

For all the experiments described below, the regenerated chitin was re-dried overnight in the oven at 80 °C, ground to a fine powder using a mortar and pestle, and sieved to below 125 μm particle size.

Preparation of Fibers

Chitin Fibers. Pure chitin fibers were prepared using a slightly modified protocol described in reference 32. Specifically, dry chitin powder (particle size < 125 μm, 0.178 g) was placed into a 20 mL screw top vial equipped with a stir bar, and 10 g of $[C_2mim][OAc]$ was added (1.75 wt% chitin loading). The vial was then placed into an oil bath (100 °C, 760 rpm) and heated with magnetic stirring for about 2 h or until complete dissolution of the chitin was achieved. Dissolution was monitored by the analysis of solution aliquots placed between two glass slides using an optical microscope (Reichert Stereo Star Zoom 580, Depew, NY). The vial was then taken out of the heating bath, the stir bar removed, and the solution placed into the centrifuge for 10 min in order to separate any undissolved residue, although typically there was none. After centrifugation, the solution was warmed in the oven (80 °C) for about 5 min to reduce its viscosity and transferred to a 12 mL plastic syringe. The solution was then degassed in the oven from 10 min to 1 h to remove any bubbles before being cooled to room temperature.

DI water (ca 10-12 L) was placed into a 0.6 m stainless steel coagulation bath. The syringe was then placed in a syringe pump (New Era Pump Systems, Inc. NE-1010, Farmingdale, NY) so that the syringe tip was located ca. 2 mm above the water surface. Fibers were extruded into the water bath using the previously reported apparatus (ESI†, Fig. S1).[65] The fiber was led through the first two steps of the godets, and then wound onto the take-up spool manually. An extrusion rate of 1.5 mL/min was used. The voltage settings for the godets were 0.10 V and 2.3 V. Plastic tweezers were used to guide the fiber around the godets. The resulting spool of fibers was placed into a 500 mL beaker filled with 400 mL of DI water that was subsequently changed several times to remove the IL, usually 3-4 water changes. Finally, the fibers were left in DI water (400 mL) overnight and then allowed to dry for 24 h in air before characterization.

The length of the fibers typically varied from 0.3 m to 1 m long, although often a continuous fiber (ca. 10 m long from 60 mL solution) was produced during the experiment. The pure chitin fibers retained ca. 1.9% water after being dried overnight in the oven at 80 °C. In such a procedure, typically 0.124 g of dry fibers was obtained.

Chitin-calcium alginate fibers. The composite fibers were prepared in a similar fashion. Below, the best conditions are provided in detail for a typical fiber preparation, while other biopolymer loadings (wt%), ratios, and the corresponding results obtained are summarized in the ESI†, Table S1. The dissolution of chitin (particle size < 125 μm, 0.178 g) in $[C_2mim][OAc]$ (10 g) was conducted in the same manner as for pure chitin fibers. Once the dissolution of chitin was complete, alginic acid (0.0593 g) was added and the solution heated in an oil bath for an additional 10 min. The vial was then taken out of the heating bath, the stir bar removed, and the solution centrifuged, transferred to a 12 mL plastic syringe, degassed in the oven and cooled to room temperature, in the same manner as described earlier for pure chitin fibers.

A saturated $CaCO_3$ coagulation bath was prepared by dissolving 0.13 g $CaCO_3$ in 10 L of DI water and the solution put into a 0.6 m stainless steel coagulation bath. The syringe was then placed in a syringe pump (New Era Pump Systems, Inc. NE-1010, Farmingdale, NY) so that the syringe tip was located ca. 2 mm above the coagulation bath surface. Fibers were extruded into the saturated $CaCO_3$ bath using the same apparatus as described for pure chitin fibers (ESI†, Fig. S1),[65] under the same spinning conditions (extrusion rate of 1.5 mL/min, godets voltage settings of 0.10 V and 2.3 V, two steps of the godets, manual winding onto the take-up spool). Again, the length of the fibers varied from 0.3 m to 1 m long, although often a continuous fiber (ca. 10m long from 60 mL solution) was produced during the experiment. The resulting spool of fibers was placed into a 500 mL beaker filled with 400 mL of the saturated solution of $CaCO_3$ for 5-10 min, before being placed into 400 mL of DI water that was subsequently changed

several times to remove the IL, usually 3-4 water changes. Finally, the fibers were soaked in DI water (400 mL) overnight and then allowed to dry for 24 h in air before characterization. The chitin-calcium alginate fibers retained ca. 0.9% water after being dried overnight in the oven at 80 °C. In such a procedure, typically 0.154 g of dry fibers was obtained.

Viscosity Measurements

Approximately 2 mL of the biopolymer solutions were placed in the sample chamber of a Cambridge Viscosity Viscometer, VISCOlab 3000 (Medford, MA). A piston corresponding to 500-10,000 cP was selected to obtain the measurement. The viscosity was recorded in the 40-70 °C range with 5 °C intervals. The software pre-installed on the viscometer, eliminates operator error by measuring the sample multiple times until the standard deviation over 20 measurements is less than 0.2% before displaying a viscosity value. Duplicates were recorded for each sample, and average values provided.

Diameter Measurement

The average diameters were determined through measurement at five positions along the length of 5 different fibers, using calipers (General ULTRATech® digital caliper, rated accuracy ±0.02 mm or ±0.001 inch, New York City, NY). Measurements were made in inches and converted to mm.
Linear densities (mass per unit length) of the fibers were determined using ASTM Standard D1577 Option B – Single fiber weighting.[66] First, the length of a single fiber was measured to the nearest 0.1 mm using a digital caliper and the weight of each fiber was determined to the nearest 0.0001 mg (Sartorius AC 211 P balance, Elk Grove, IL). The linear density of each fiber was then calculated in denier units according to the eqn. 1:

$$D = \frac{9000\,W}{L} \quad (1)$$

where D is the average fiber linear density in denier; W is the mass of fiber in mg, and L is the length of the fiber in mm. The average denier was determined through measurement of ten specimens at random.

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra of the fibers were recorded on either whole or ground fibers using a Bruker Alpha FT-IR instrument, Bruker Optics Inc. (Billerica, MA) featuring an attenuated total reflection (ATR) sampler equipped with a diamond crystal. Spectra were obtained in the range of 600–4000 cm-1.

Thermogravimetric Analysis (TGA)

TGA analyses were performed on a Mettler-Toledo TGA/DSC 1 (Columbus, OH). The instrument's internal temperature was calibrated by observing the melting point of Au, Zn, and In standards (melting points 1064 °C, 419.5 °C, and 155.6 °C, respectively). The samples were analyzed in 70 µL alumina pans using dried air as a purge gas. Samples between 2-5 mg were heated from room temperature to 800 °C and measured in the dynamic heating regime, using a constant heating ramp of 5 °C min-1 with a 30 min isotherm at 75 °C. Decomposition temperatures are reported as onset (Tonset), 5 wt% mass loss (T5% dec) and 50 wt% mass loss (T50% dec), respectively.

Optical Microscopy

A Motic BA 200 Microscope (Carlsbad, CA) equipped with an XLI 2.0 camera (XL Imaging, Houston, TX) was used for visualization of the fibers at 40X, 100X, and 400X magnifications. XLI-Cap image analysis software (provided with the camera) was employed for saving and processing the image data.

Scanning Electron Microscopy (SEM)

Dried fibers were cut using a razor blade into 2-3 mm pieces and directly mounted onto carbon tape. The samples were then sputter-coated with a gold-palladium alloy, using an Anatech Hummer 6.6 sputter coater (Union City, CA). The coated samples were then placed into the sample chamber where they were brought under high vacuum for microscopy. Microscopy was performed on a Hitachi S-2500 Scanning Electron Microscope (Tokyo, Japan) at 200-4000X magnification.

Transmission Electron Microscopy (TEM)

TEM was taken on a Hitachi H-7650 (Tokyo, Japan). The fibers were cut with a razor and embedded into Spurr resin (Spurr embedding media, Ted Pella Inc., Redding, CA) using the heat polymerization technique. Embedding resin was added to a fiber sample in a vial, and the vial was kept in a vacuum desiccator for 48 h to allow the resin to completely infiltrate the fiber. The Spurr-infiltrated fibers were placed into conical BEEM® embedding capsules (Ted Pella Inc.) and heated at 65 °C in an oven (Cat 200 Serial 0966 Laboratory Oven, Chicago Surgical & Electrical Co., Melrose Park, IL) for 48 h to complete the polymerization process. Once the BEEM® capsules were taken out of the oven, the samples were trimmed with LKB 1 microtome (Leica Microsystems, Rockville, MD) and then cut into thin sections (90 nm) using a Leica Ultracut UC6 ultramicrotome (Leica Microsystems, Rockville, MD) with a diamond knife. Samples were then placed on grids (300 mesh) and visualized with TEM.

Tensile Properties (Ultimate Stress and Failure Strain)

Ultimate stress values were measured on a MTS Q-Test 25 instrument (Eden Prairie, MN) equipped with a specially designed pneumatic grip. The pneumatic grip used a load cell of 22.4 N capacity, cross head speed of 1.27 mm min-1, and a gauge length of 15.24 cm. Measurements were conducted according to the ASTM D3822-07 Standard Test Method for Tensile Properties of Single Textile Fibers.[67] Test fibers of at least 30 cm long without any visual flaws were cut from individual spools, and at least five samples were tested for each type of fiber. Fibers with about the same diameters (ESI†, Table S5) were selected and the average diameter was

determined from measurements of the diameter at five different sections of the fibers. Each fiber was fixed in the grips, and a constant load (22.4 N) was applied until the fiber broke. The test data in terms of stress and strain and load-elongation curves were obtained using a data acquisition and processing system Test Works QT Version 2.03 (Eden Prairie, MN).

Water Uptake

The absorption capacity of the fibers was measured according to the standard protocol from the British Pharmacopoeia monograph for Alginate Dressings and Packings.[68] The experiments were performed at room temperature during a 24 h time period. A 2.5 cm dry piece of fiber was weighed and then immersed in a 20 mL screw top vial filled with 20 mL of DI water. After 24 h, the fiber was taken out of solution, placed between two Kimberly-Clark® KimWipes™ (Neenah, WI) tissues to remove excess water, and then weighed. The test was conducted using three samples of each fiber, and the average values are reported. The degree of swelling was calculated according to eqn. 2:

$$\text{Absorption Capacity}\left(\frac{\text{g}}{\text{g}}\right) = \frac{\text{Mass wet} - \text{Mass dry}}{\text{Mass dry}} \quad (2)$$

Intracutaneous Testing for Biocompatibility

The potential for the chitin and chitin-calcium alginate fibers to generate dermal irritation was evaluated using a rabbit intracutaneous method based on the ISO 10993-10 guidance document.[69] All animal work was performed at WuXi AppTec, St. Paul, MN, which is an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility. All animal procedures were approved by WuXi AppTec's IACUC committee, and all housing and animal care was performed according to National Institute of Health (NIH) and AAALAC International guidelines and according to WuXi AppTec Standard Operating Procedures. Animals were individually housed with access to food and water ad libitum.

Extractions of each material (either chitin or chitin-calcium alginate fibers) were prepared by placing the fibers in 0.9% normal saline solution (NS) or cottonseed oil (CSO), to provide both polar (NS) and non-polar (CSO) extraction solutions, at a ratio of 2 g of material to 20 mL of extraction vehicle. The mixtures and corresponding control blanks were incubated at 50 ± 2 °C for 72 ± 2 h with agitation during the course of the extraction period. At the end of the extraction period, observations of the extract appearance were recorded, the vessels were shaken well, and the liquid aseptically decanted into a sterile glass vessel, then maintained at room temperature and used within 24 h of preparation.

Three New Zealand white rabbits (Bakkom Rabbitry, Red Wing, MN), ca. 11 weeks old and 2.3-2.8 kg, were acclimated for a minimum of 5 days under the same conditions as the actual test. The animals' backs were shaved and 0.2 mL of each extract (or the vehicle controls for comparison) was injected intracutaneously along the dorsal surface of each rabbit, with five sites of the test or control material extracts on one side of the spine, and five sites of the vehicle control on the contralateral side. Animals were observed daily for general signs of health. Each injection site was scored at 24, 48, and 72 ± 2 h post-injection for macroscopic evidence of erythema and edema (0 - 4 = none, very slight, slight, moderate, or severe, respectively). The erythema and edema scores for each extract were then totaled and averaged, and the average score of the test or control material was subtracted from the average score of the corresponding vehicle control. A material is considered an irritant if the difference in average score between the test and control material is greater than 1.0.[69]

Rat Model of Wound Healing

Wound dressings were created from the chitin and chitin-calcium alginate fibers by cutting the chitin or chitin-calcium alginate fibers into 1 cm pieces and placing onto the adherent surface of 3M (St. Paul, MN) Tegaderm Transparent Dressing (2 3/8" x 2 3/4" #1624W) to create a patch approximately 1 cm2.

Twenty Sprague Dawley females rats (Charles River Laboratories, Kingston, NY) weighing 277-352 g at the time of wound creation were assigned to two study groups of ten animals each. Rats were anesthetized using 0.5-4% isoflurane, and their backs were shaved and prepared for aseptic surgery. Two 8 mm in diameter full dermal thickness wounds were aseptically created bilaterally, one either side of the spine using a sterile, disposable dermal punch apparatus. Wound measurements and photographs were obtained of the wounds immediately after wound creation. Each of the two wounds received the same treatment (i.e., chitin or chitin-calcium alginate). Care was taken to be sure that the wound was dry prior to applying the test and control articles. The wounds were then wrapped with a non-adhesive and non-absorbent secondary dressing (Petflex®, Andover Healthcare, Inc., Salisbury, MA) to keep the test or control material in place and to prevent incidental wound irritation (e.g., scratching).

At 3, 7, and 10 days after wound creation, the secondary wrap was removed from the animals to allow visual inspection and measurement of the wound area and dressing. Whenever possible, the wound dressing itself (fibers + Tegaderm) was kept in place over the wound for photographs and measurements. If the fibers obstructed the view of the wound edges, the dressing was carefully pulled back from the wound for measurement and photography, and then carefully placed back on the wound. In one case where the dressing was no longer in place (a chitin-group animal on Day 6), the material was replaced with a fresh piece of fiber + Tegaderm. Measurements were taken from open wound edge to open wound edge, in the cranial-caudal direction, or "clock method" of 12:00 – 6:00, for length (mm). The width (mm) of the wound was taken from open wound edge to open wound edge at the longest point in the side-to-side direction or "clock method" 3:00 – 9:00.

Animals were euthanized on Day 14 by CO_2 inhalation effect. Wound measurements and photographs were obtained as described previously. The wounds were then excised with at

least 0.5 cm margin of normal tissue surrounding the lateral margins of the wound and with a sufficient depth margin not to disrupt the wound bed. Excised tissue was placed in 10% aqueous formalin for fixation. Tissue sections were obtained through the center of each wound, mounted onto glass slides, and stained with hematoxylin and eosin. A WuXi AppTec veterinary pathologist analyzed the sections for signs of irritation and wound healing (i.e., re-epithelialization, epithelial gap, wound area). A representative digital photomicrograph was obtained of histology sections from each group at each time point.

Results and Discussion

An immediate roadblock to our work arose when it was determined that neither of the commercial salts of alginic acid we studied, sodium alginate or calcium alginate, were soluble in [C_2mim][OAc] to an appreciable degree even when heated in a 100 °C oil bath for several days. To overcome this, we investigated the use of chitin-alginic acid solutions and in situ conversion of the alginic acid to calcium alginate during the spinning process. Alginic acid is soluble up to 20 wt% in [C_2mim][OAc] compared to the 2.75 wt% solubility of the high MW extracted chitin.

The first critical factors in achieving desired composite fiber performance, are the concentration and relative ratio of the two biopolymers needed to achieve the desired viscosity of the spinning dope. We therefore investigated the effect of both the relative ratio of the polymers and their concentrations in the IL on our ability to pull continuous fibers. Using a fiber spinning apparatus (ESI†, Fig. S1) and process we have previously described,[65] the various IL solutions were extruded through a syringe into a coagulating bath containing DI water, fed around two godets, and spooled. The results were evaluated in terms of a) viscosity of the spinning solution, b) ability or inability to pull fibers, and c) characteristics of these fibers (weak or strong, flexible or brittle). The conditions used for these tests and the results are summarized in the ESI†, Table S1.

It was apparent that the relative ratio of the biopolymers was critical for fiber pulling. While chitin provides good mechanical properties, alginic acid is structurally weak and fibers with less than 50 wt% of chitin relative to alginic acid could not be spun. Fibers with chitin content of 50 to 70 wt% were successfully formed, but were quite weak, often breaking on pulling or while attempted to be spooled. Contrarily, when the amount of chitin was too high (> ca. 80%), more brittle and not very flexible fibers were formed. The best fibers were obtained with a 3:1 chitin to alginic acid ratio.

The overall concentration of biopolymers in the IL (biopolymer loading) is also critical since this affects the viscosity of the resultant solution. When the viscosity was too low, the solution formed droplets rather than a continuous fiber, and when the viscosity was too high, the fibers broke during the pulling process. A spinning solution viscosity of ca. 850-900 cP (ESI†, Fig. S2) was needed to pull the best fibers, and concentrations of chitin and alginic acid of 1.75 wt% and 0.583 wt% with respect to the IL were found to result in the optimal viscosity giving strong, stiff, composite fibers with the best strength characteristics.

Having proven that chitin-alginic acid fibers could be spun using the appropriate conditions, we investigated how best to convert the alginic acid to calcium alginate while retaining the ability to form a composite fiber. Typically, sodium alginate is converted to calcium alginate via dripping sodium alginate into a solution of aqueous $CaCl_2$,[70] where the cross-linking of alginate into 3D network by calcium ions takes place. However, since we were only able to use alginic acid and not sodium alginate, we required a basic calcium salt. Since Na_2CO_3 has been used to convert bulk alginic acid to sodium alginate,[71] we chose to spin the composite fibers into a saturated $CaCO_3$(aq) coagulating bath to convert alginic acid directly into calcium alginate as shown in Scheme 1.

Scheme 1. Conversion of alginic acid into calcium alginate.

In a typical procedure, 12 mL of a [C_2mim][OAc] solution containing 1.75 wt% chitin and 0.583 wt% alginic acid was dry jet wet spun[65] into a 0.6 m coagulating bath containing saturated $CaCO_3$ solution. The length of the fibers varied from 0.3 to 1 m long, although often a continuous fiber (ca. 10 m long from 60 mL solution) was produced during the experiment. The resulting spool of fibers was placed into a 500 mL beaker filled with 400 mL saturated solution of $CaCO_3$ for 5-10 min, before being placed into 400 mL of DI water that was subsequently changed several times to remove the IL, usually 3-4 water changes. Finally, the fibers were soaked in DI water (400 mL) overnight and then allowed to dry for 24 h in air before characterization. In such a procedure typically 0.154 g of dry fibers were obtained.

Fiber Appearance and Morphology

When spools of freshly prepared chitin and chitin-calcium alginate fibers were placed into DI water side-by-side, it appeared the chitin fibers appeared rather translucent, while the chitin-calcium alginate fibers were of white milky color. However, no substantial visual differences were found between fibers of pure chitin and chitin–calcium alginate when dried; both types of fibers were beige to brown in color. The average diameters (from measurements of 5 fibers at 5 different locations each) for each type of fiber after drying were determined to be 0.080(10) and 0.091(7) mm for chitin and chitin-calcium alginate fibers, respectively (ESI† Table S5). Optical microscopy (ESI†, Table S2) suggested that the fibers did not contain entrapped air (no voids or dark areas were observed) and that the surfaces of the fibers were uniform and homogeneous. Optical microscopy (ESI†, Table S2) of the

fibers also indicated that the fibers were continuous with aligned fiber orientation (visually determined). SEM micrographs of the fibers (Fig. 2A, B; ESI†, Table S3) demonstrated that the surfaces of the fibers exhibited a homogeneous morphology with no obvious splits, supporting the optical microscopy results and indicating blend homogeneity between both biopolymers. This was confirmed by the absence of higher-density/dark regions around the edges in TEM micrographs (Fig. 2C, D; ESI† Table S4).

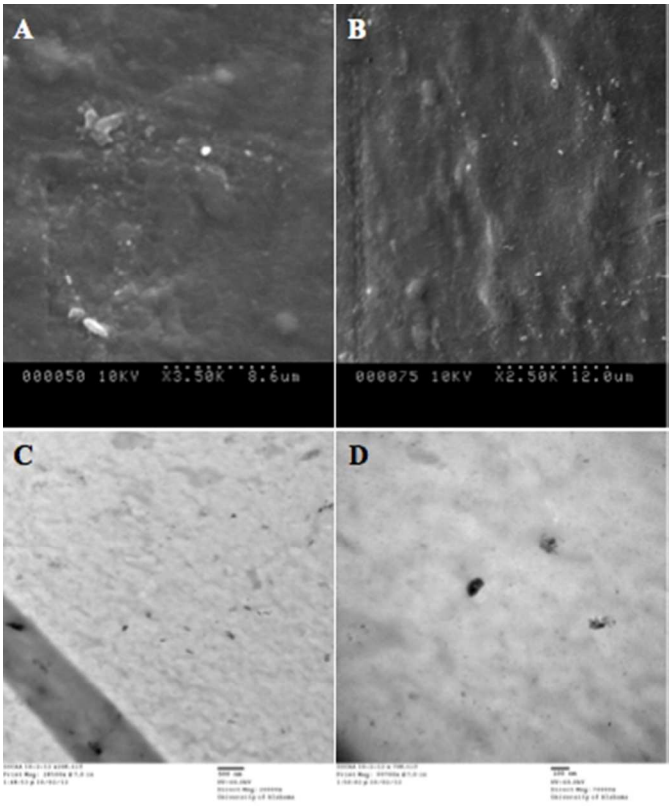

Fig.2 Selected SEM (A, B) and TEM (C, D) micrographs of chitin-calcium alginate fibers: A: 10 kV, x3.5K, 8.6 µm, B: 10 kV, x2.5K, 12.0 µm; C: 500 nm, HV 60.0 kV, Direct Mag: x20000; D: 100 nm, HV 60.0 kV, Direct Mag: x70000.

Chitin's structure is highly ordered, but if subjected to stress (i.e., pulling) the biopolymer's hydrogen bonded sheets slide, retaining the same ordered orientation. As a result, regions with greater electron density form, and the darker lines on the magnified TEM micrographs, represent these regions of force-induced sliding of hydrogen-bonded sheets of the chitin biopolymer.

ATR FT-IR Analysis

FT-IR spectra were obtained for both whole fibers and for fibers which were first ground to powder. The spectra of the chitin-calcium alginate fibers revealed a series of narrow absorption bands typical for chitin[72,73] (Fig. 3, black).

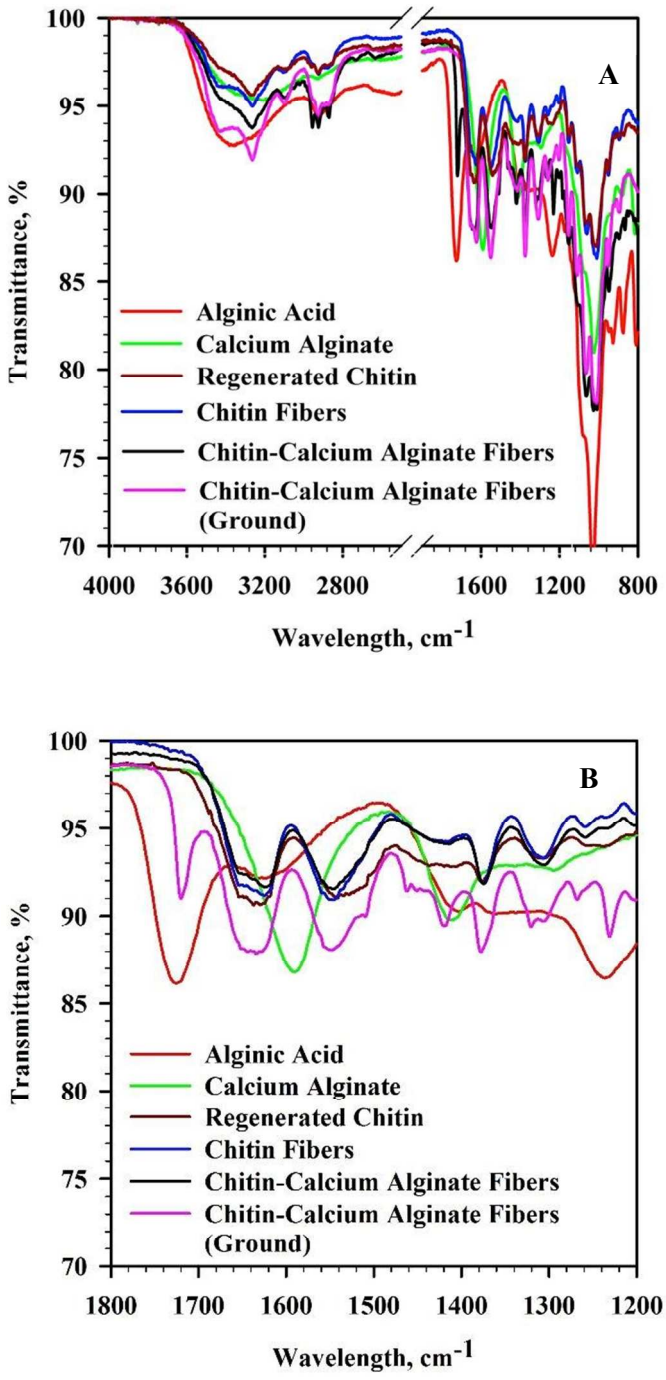

Fig.3 Full (A) and expanded (B) FT-IR spectra of commercial alginic acid (red), commercial calcium alginate (green), regenerated chitin powder (dark red), chitin fibers (blue), whole chitin-calcium alginate fibers (black), and ground chitin–calcium alginate fibers (pink).

To confirm the conversion of alginic acid into calcium alginate, the carbonyl stretch was compared. This band in alginic acid is observed at about 1723 cm^{-1}, while in calcium alginate an asymmetric/antisymmetric stretch is observed at about 1589/1410 cm^{-1}.[74]

The IR spectrum of whole fibers of chitin-calcium alginate were nearly identical to the spectrum of pure chitin. The absence of an alginic acid carbonyl peak suggested conversion to calcium alginate at least on the surface of the fiber. Since the C=O peak of calcium alginate overlaps with chitin amide stretches at 1627 cm^{-1} (C=O) and 1558 cm^{-1} (C-N), no pronounced peaks of calcium alginate were detected either, perhaps due to the 3/1 excess of chitin in the fibers.

The fibers were also ground into a powder and reexamined. The FT-IR spectrum of the ground fiber (Fig. 3, pink) did reveal the presence of alginic acid indicating that conversion of alginic acid into calcium alginate was incomplete. This suggests that $CaCO_3$ was not able to diffuse inside the fibers during spinning to entirely neutralize the acid. The FT-IR data did provide evidence (at least to the level of detection) that the [C_2mim][OAc] was completely washed out of fibers where spectra of the whole and ground fibers revealed no peaks related to the presence of the IL (ESI†, Fig. S3).

Mechanical Properties

Before testing, both chitin and chitin-calcium alginate fibers were prescreened to select ones of uniform thickness and with no obvious flaws. Five test fibers at least 30 cm long and with average diameters of 0.0031(4) inches for chitin and 0.0036(5) inches for chitin-calcium alginate fibers were selected for testing. The results were compared to fibers of chitin spun from [C_2mim][OAc] solution under slightly different conditions and from a different chitin source,[32] and to literature values for chitin and alginate fibers prepared using various solvent systems (Table 1,[32,44,75-,76,77,78]). The data for ultimate stress and failure strain for chitin and chitin-calcium alginate fibers are summarized in the ESI†, Table S6.

The chitin-calcium alginate fibers exhibited an average ultimate stress of 216(5) MPa and 4.1(8)% elongation; slightly lower strength than those obtained for the pure chitin fibers (ultimate stress and elongation 256(57) MPa and 4.5(5)%, respectively). Similarly, the Young's modulus was slightly lower for the composite fibers (6.9 GPa) than for the pure chitin fibers (8.8 GPa). Values in the same range were obtained previously for chitin fibers spun from [C_2mim][OAc] solution but with a higher chitin loading and with chitin extracted from raw shrimp shells (ultimate stress = 237 MPa, Young's modulus = 10 GPa).[32]

Several other reports of chitin fibers, do report stronger fibers, but these studies are typically of multifilament fibers. For example, the use of DMAc-LiCl resulted in fibers with an ultimate stress of 490.3 MPa[75], while chitin fibers from TCA/CH2Cl2 had a strength of 382.5 MPa.[76] The only reported example of monofilament chitin fibers we found was a preparation from a solution of 40% trichloroacetic acid, 40% chloral hydrate, and 20% CH_2Cl_2.[77] Though the ultimate stress was quite high (617 MPa), these fibers were very thick (0.250 mm), almost 3 times thicker than the chitin-calcium alginate or chitin fibers reported here.

Table 1 Comparison of tensile properties (ultimate stress and failure strain) of chitin and chitin-calcium alginate fibers.

Type of Fiber	Solvent [a]	Conc. wt%	D, µm	Ultim. Stress, MPa	Fail. Strain %
Chitin-Calcium Alginate	[C_2mim][OAc]	Chitin 1.75 Alginate 0.58	0.091(7)	216(5)	4.1(8)
Chitin [b]	[C_2mim][OAc]	1.75	0.080(1)	257(56)	4.5(5)
Chitin	[C_2mim][OAc][32]	2.1	0.070(1)	237(26)	13.0
Chitin [c]	95% DMAc/5% LiCl[75]	5.0	0.005 [d]	490 [e]	-[e]
Chitin [c]	92% NMP/8% LiCl[76]	5.2	0.025 [d]	525 [f]	-[e]
Chitin [c]	Mixtures of TCA/DCM (various ratios) *Ex. 1A and* *Ex. 1D: 50%* TCA/50% DCM[77]	<10.0 2.9 2.9	 0.080 [g] 0.150 [g]	 222 [f] 382 [f]	 20.0 18.0
Chitin	40% TCA/40% CH/20% CH_2Cl_2[78]	2.3	0.250	617 [e]	13.1
Sodium alginate	4.6% LiOH/10% urea/4.5 % thiourea/80.9% water[44]	- [h]	0.100 [g]	193 [i]	8.3

[a]Compositions in wt%; abbreviations/chemical formulas: DMAc – N,N-dimethylacetamide; LiCl – lithium chloride; NMP - N-methyl-2-pyrrolidone; TCA – trichloroacetic acid; DCM - dichloromethane; CH – chloral hydrate; LiOH – lithium hydroxide; [b]Chitin fibers were prepared using same chitin source and same processing conditions as for chitin-calcium alginate fibers; [c]Multifilament fiber; [d]Diameter of each filament, wet; [e]Value was provided in kg/mm^2 and recalculated to MPa for comparison using the equation: *MPa = 9.807 x 1 kg/mm^2*; [f]Value was provided in g/den and recalculated to MPa for comparison using the equation: *1 MPa = 9 x (1 g/den) x d x 9.807*, where d is the specific gravity of the chitin, 1.4; [g]Diameter of the extruder holes, diameter of dry fiber not provided; [h]Value not provided; [i]Value was provided in cN/dtex and recalculated to MPa for comparison using the equation *1 cN/dtex = (100 x d) MPa*, where d is the specific gravity of the alginate, 1.6.

Although, we believe that the chitin-calcium alginate fibers are strong enough to be used directly as a structural material for wound care, there appears to be room for improvement and optimization. We are currently investigating improving the strength of the fibers with a variety of techniques, including increasing the chitin content, optimization of fiber stretching (elongation) during spinning, possibly adding a structural polymer support (e.g., cellulose), or spinning multifilament fibers.

Water Uptake

There are no literature reports of water uptake for chitin fibers (since chitin itself is not used as a water absorbent), and we thus first measured the water absorption capacity of pure chitin fibers (spun from [C_2mim][OAc]) using a standard protocol.[68] At room temperature, 2.54 cm pieces of the fibers were weighed and immersed in 20 mL of DI water for 24 h. The average water absorption value for these fibers was 2.57 ± 0.31 g/g (average from three measurements) after 24 h.

The same procedure was used to test the chitin-calcium alginate fibers. Similarly to fibers made of neat chitin, water appeared to be absorbed into the fibers, causing the fibers to expand (swell) but still keeping their fibrous structure. The average water uptake value of 2.06 ± 0.30 (24 h) for chitin-calcium alginate fibers was slightly lower than that we obtained for pure chitin fibers. The literature reports that the absorption capacity of alginate-containing dressings depends mostly on the composition of the polymer (mannuronic and guluronic residues).[79]

Thermal Stability

Both the alginic acid and calcium alginate powders decompose at much lower temperatures than the chitin materials (Fig. 4, Table 2).

Table 2. Comparison of Thermal Properties

Fiber	T_{onset}, °C	$T_{5\%dec}$, °C	$T_{50\%dec}$, °C
Alginic Acid (commercial)	169.2	179.5	247.4
Calcium Alginate (commercial)			
Step 1	110.1	117.9	170.2
Step 2	220.1	232.2	271.7
Step 3	628.4	642.3	718.9
Regenerated Chitin Powder	257.2	283.5	347.5
Chitin Fibers	258.3	267.00	352.8
Chitin–Calcium Alginate Fibers	233.0	236.3	337.3

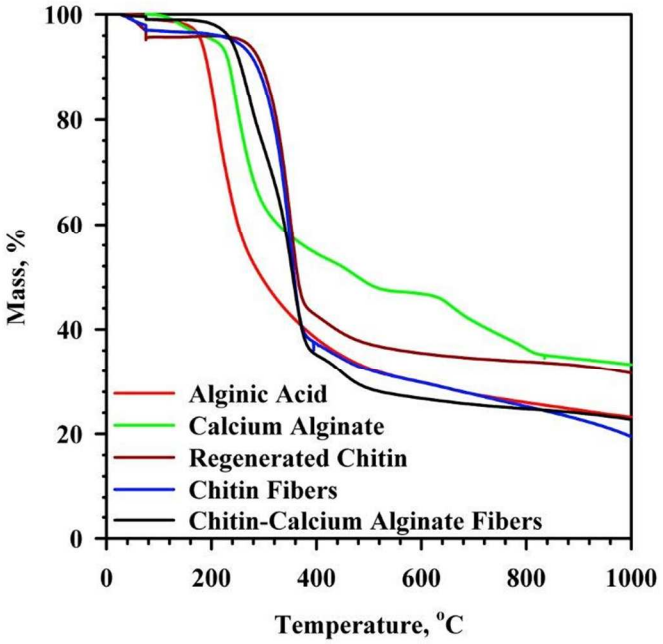

Fig.4 Comparison of TGA data for commercial alginic acid (red), commercial calcium alginate (green), regenerated chitin powder (dark red), chitin fibers (blue) and chitin-calcium alginate fibers (black).

The alginic acid decomposes in a single step (T_{onset} = 169.2 °C), while calcium alginate exhibits a three-step decomposition, with the first step at T_{onset} = 110.1 °C, due to dehydration,[80] the second step starting at 220.1 °C, and the third step at ca. 628 °C due to calcium carbonate decomposition. The decomposition temperatures of regenerated chitin and the pure chitin fibers are both similar with a smooth one-step decomposition, at almost the same T_{onset} values and slightly different $T_{5\%\ dec}$ values (chitin fibers are ca. 15 °C lower (Table 2)). The TGA decomposition profile for the chitin-calcium alginate fibers is noticeably different than observed for the pure chitin fibers (T_{onset} and $T_{5\%\ dec}$ are ca. 20-25 °C lower than pure chitin fibers) and appears to have features found in both the alginate data and the chitin data.

Intracutaneous Testing for Biocompatibility

Based on the anticipated duration of exposure and the route of administration of the final product, an intracutaneous irritation test was chosen to evaluate the biocompatibility of the chitin-calcium alginate fibers, using chitin fibers for comparison. The purpose of this evaluation was to determine if any chemicals that may leach or be extracted from the test articles are capable of causing local irritation in the dermal tissues of rabbits. Both a polar [normal saline (NS)] and non-polar [cottonseed oil (CSO)] were used as extraction vehicles for each fiber preparation, using extraction conditions described in the experimental section.

At the start of the extraction, the solutions appeared clear and free of particulates and fibers appeared intact with no macroscopically observable degradation. The fibers were observed after all extractions to be intact with no macroscopically observable degradation. The CSO extracts were clear, while the NS extracts were clear and pale brown in color. Each extract was then injected intradermally into New Zealand white rabbits, with additional injections of vehicle only (NS or CSO). The injection sites were scored for erythema and edema at 24, 48, and 72 h following injection, as described in the Experimental section. A summary of the results is provided in Table 3, with individual scores given in ESI Tables S8-S15.

All animals appeared normal during the 72 h test period. After injection with the NS chitin fiber extracts two of the three rabbits demonstrated some very slight erythema/edema at 24 and 48 h. Only very slight erythema/edema was observed in one animal at 72 h after injection with the NS chitin-calcium alginate fiber extractions.

While all animals demonstrated some very slight erythema and/or edema in response to the CSO extracts, the observations were found equally among the tested fibers extracts and the CSO vehicle control, since this is a common observation in response to CSO alone. Based on the ISO 10993-10 guidelines,[69] which defines a material as an irritant if receiving a score greater than 1.0, neither the chitin nor chitin-calcium alginate fibers is considered an irritant. Overall, the erythema/edema scores were lower for the chitin-calcium alginate fibers than for the chitin fibers.

Table 3. ISO Intracutaneous Reactivity Test - Dermal Observation [a]

0.9% Normal Saline (NS)			
Treatment	Average Score	Treatment	Average Score
Chitin	0.4	Chitin-calcium alginate	0.1
Vehicle Control	0	Vehicle Control	0
Difference[b]	*0.4*	*Difference*	*0.1*
Cottonseed Oil (CSO)			
Treatment	Average Score	Treatment	Average Score
Chitin	1.0	Chitin-calcium alginate	1.1
Vehicle Control	1.0	Vehicle Control	1.2
Difference [b]	*0*	*Difference*	*0* [c]

[a]Detailed dermal observations/results for reactivity tests of fiber leachates are provided in ESI† Tables S8-S9 (NS chitin), S10-S11 (NS chitin-calcium alginate), S10-S11 (CSO chitin), and S14-S15 (CSO chitin-calcium alginate); [b]Difference of tested material extract minus vehicle control, where > 1.0 is considered an irritant per ISO 10993-10; [c]Negative values were rounded up to 0.

Rat Model of Wound Healing

The potential for the chitin-calcium alginate fibers to promote wound healing was investigated using a rat full-dermal thickness wound model and compared to chitin fibers without alginate. The chitin or chitin-calcium alginate fibers were cut to a uniform 1 cm length and placed on the adhesive side of Tegaderm film to create the wound dressings (Fig. 5).

Fig 5. Representative patch of fibers used for wound healing assessment.

These dressings were then applied to freshly-created wounds, 8 mm in diameter, created by sterile dermal punches on the backs of Sprague Dawley rats. Photographs and wound measurements were obtained post-wound creation, and on Days 0, 3, 7, 10, and 14. (The detailed procedure is provided in the ESI†). The dressings were maintained on the wounds for the duration of the study, unless they became damaged. Because the Tegaderm dressing is transparent, measurements and photographs could be obtained through the dressing, though the fibers partially obstructed optimal viewing of the wound edges during measurements.

The dressings were maintained on the wounds for the duration of the study, with only one exception, in which one animal in the chitin-calcium alginate group had compromised the material, which was then replaced. In all other cases, the material stayed on the wound for the fourteen-day duration without need for re-application. Tables S16-S17 (ESI†) provide observations on the 7 and 14 Day chitin and chitin-calcium alginate treated wound sites. Photographs of the wounds over time (Days 3, 7, 10, and 14 post wounding) are presented in Fig. 6.

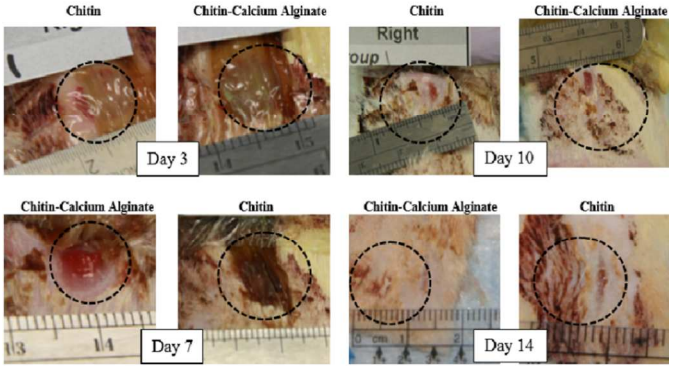

Fig. 6 Representative images of the wounds sites taken on days 3, 7, 10, and 14.

Wound closure, based on wound area measurement over time expressed as percentage of the full wound area on Day 0, are summarized Fig. 7, with detailed measurements provided in Table 4. The wound sites treated with the chitin–calcium alginate fibers had slightly faster time-to-closure when compared to the chitin, but all wound sites achieved 95-99% closure by Day 10, with full closure (100%) by Day 14 (Table 4).

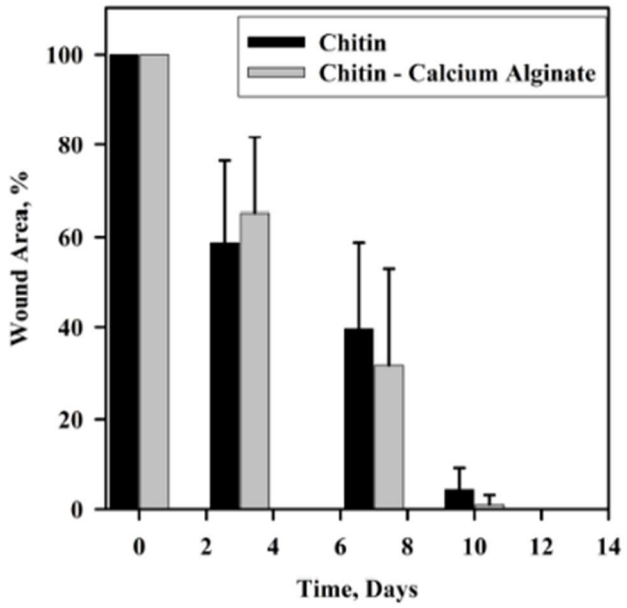

Fig. 7 Wound closure results expressed as percentage of initial mean wound area (averaged per group). The wound sites were treated with chitin fibers (black) or chitin–calcium alginate fibers (grey).

Both chitin and chitin-calcium alginate dressings demonstrated comparable healing based on wound area measurements over time, with wound closure of ca. 35 and

42%, respectively by Day 3 (Table 4). By Day 7, chitin fibers demonstrated approximately 60% wound closure, while chitin-calcium alginate fibers were slightly better performing (70% wound closure). Chitin and chitin-calcium alginate patches resulted in almost complete healing (99 and 95%, respectively). There were no statistically significant differences in wound areas between chitin and chitin-calcium alginate fibers at any time point.

Table 4. Wound Closure Results Summary[a]

Materials	Days			
	Day 3	Day 7	Day 10	Day 14
	Wound Area Measurement (mean mm^2 ± SD[b])			
Chitin	37.3 ± 10.5	25.2 ± 12.3	2.7 ± 2.8	0 ± 0
Chitin-Calcium Alginate	42.1 ± 9.7	19.7 ± 11.7	0.6 ± 1.2	0 ± 0
	Wound Closure, %			
Chitin	41.8	60.6	95.8	100
Chitin-Calcium Alginate	35.5	69.6	99.1	100

[a]Details for measurement of dermal wound width and epidermal gap of chitin and chitin-calcium alginate treated wound sites are provided in ESI† Tables S23-S26; [b]SD – standard deviation.

Histopathological Evaluation

This study compared the wound healing response after topical application of the chitin-calcium alginate fibers or the chitin fibers to full thickness dermal wounds for 7 and 14 days. The tissue response found in the 7 and 14 day wounds from both groups consisted of normal wound healing. The dermal region of the 7 day wounds in both groups was filled with maturing granulation tissue composed of prominent neovascularization and fibroblasts admixed with chronic granulomatous inflammation and scattered mature collagenous fibers arranged in a random pattern (Fig. 8). New hyperplastic epidermal tissue (re-epithelialization) was migrating from the wound edges of both groups, to cover the dermal granulation tissue, but only the chitin-calcium alginate covered wounds were consistently completely covered by the new epidermis at 7 days. Focal central regions of the 7 day chitin covered wound sites still had areas of epidermal ulceration.

At 14 days postoperatively, all of the wound sites (covered with chitin and chitin-calcium alginate fibers) were completely covered by a minimally hyperplastic epidermis and the dermis consisted of maturing fibrous connective tissue. The 14 day dermal fibrosis had a decrease in the number of fibroblasts, neovascularization, and inflammation compared to the 7 day dermal granulation tissue and the fibroblasts within the 14 day wound sites were more elongated, like the fibroblasts found in normal dermal tissue. Admixed with the dermal fibrosis of the 14 day wound sites were variable thick bundles of mature collagen fibers arranged in a normal parallel fashion to the epidermis (Fig. 8 and ESI Fig. S4). There was no regeneration of adnexal structures in any of the 7 day or 14 day healing wound sites covered with either chitin or chitin-calcium alginate, which would be an expected finding for full thickness dermal wounds.

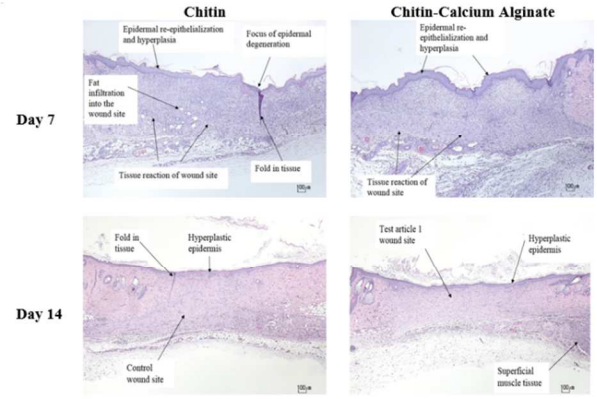

Fig. 8 Representative photos of chitin-covered and chitin–calcium alginate covered wound sites on Day 7 and 14. Tissue sections were stained with hematoxylin and eosin (H&E) and are shown with 40x magnification.

The chitin and chitin-calcium alginate covered wounds at 7 days and 14 days consisted of normal healing wounds containing components of maturing fibrous connective tissue with neovascularization and chronic inflammation in the dermis covered by hyperplastic epidermis. The differences in the chitin-calcium alginate and chitin covered wound responses at 7 and 14 days were in the amount of epidermis at 7 days and wound contraction at 14 days. There was consistently complete coverage of the dermal fibrosis with hyperplastic epidermis in the 7 day chitin-calcium alginate covered wound sites, and inconsistent coverage of the dermal fibrosis with hyperplastic epidermis in the 7 day chitin covered wound sites. Areas of the 7 day chitin covered wound sites had a focal area of epidermal ulceration. The 14 day wound sites covered with either chitin or chitin calcium alginate contained the same tissue components – maturing fibrosis and collagen fibers covered completely with epidermis; however, the difference in the 14 day wounds was in the width of the dermal fibrosis (wound contraction). The chitin covered wound sites had greater wound contraction than the 14 day chitin-calcium alginate covered wound sites. Detailed histopathology data of chitin and chitin-calcium alginate treated wound sites are provided in ESI†, Tables S18-S21.

In conclusion, full thickness dermal wounds in rats treated with chitin-calcium alginate or chitin showed that the wounds in both groups were undergoing normal wound healing at 7 days and 14 days – maturing dermal fibrosis with re-epithelialization (epidermis). The new epidermis overlaying the dermal fibrosis was consistently complete (fully covering the dermis) in the chitin-calcium alginate wound sites and partially complete in the chitin covered wound sites; but the chitin wounds had greater wound contraction (as indicated with the width of the dermal fibrosis) at 14 days postoperatively, than the chitin-calcium alginate wound sites.

Conclusions

By taking advantage of the ability of some ionic liquids to extract high molecular weight chitin directly from shrimp shells and the mutual solubility of many natural biopolymers in these same ILs, we were able to prepare the first example of chitin–calcium alginate biocomposite fibers and tested these for their utility in wound care. While fibers could be prepared with consistent reproducibility and blend-homogeneity, conversion of alginic acid into calcium alginate during spinning was incomplete suggesting that there was not enough time for $CaCO_3$ to diffuse inside the fibers while in the coagulation bath. Longer immersion times in the $CaCO_3$ bath might be necessary to achieve full conversion, if indeed full conversion is necessary.

The chitin-calcium alginate composite fibers prepared met the technical specifications (strength, water sorption) needed for wound care fibers. However, if improved strength is required for a specific application, the mechanical properties could be improved in a number of ways, including increasing the polymer load of the spinning dope, modification of the spinning process to increase elongation during coagulation, making braided or multifilament fibers, or even by addition of another structural biopolymer).

Biological testing conducted to evaluate the fibers utility in wound healing, provided evidence that both chitin and chitin-calcium alginate fibers accelerated wound closure. Interestingly, only one dressing/per animal/per wound site was required during the entire 14 day duration of the study.

The difference in the chitin-calcium alginate and chitin covered wounds was in the amount of epidermis after 7 days of treatment, and wound contraction after 14 days of treatment. Even though both chitin-calcium alginate- and chitin–covered wounds underwent normal wound healing and complete re-epithelialization by the end of the study, coverage of the dermal fibrosis with hyperplastic epidermis after 7 days of treatment was consistently complete for chitin-calcium alginate covered wound sites, and only partially complete in the chitin covered wound sites. Contrarily, the chitin covered wound sites had greater wound contraction than chitin-calcium alginate covered wound sites by the end of the study. Both the wound healing response and the reduced number of dressing which had to be applied (indicating cost effectiveness) suggested that the fibers might be effective and versatile wound dressings.

The use of composite materials based on chitin for wound care applications has a great promise due to the biocompatibility and efficacy of the chitin biopolymer. Nonetheless, we realize that optimization is needed, for example in full conversion of alginic acid to alginate, in improvement in fiber strength, and in obtaining the optimal ratio of calcium alginate and chitin needed to obtain the best healing properties. We would also suggest that the ability of ionic liquids to directly dissolve high molecular weight chitin and many other biopolymers, this technology could represent a platform of biocomposite fibers useful in medicine.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Other Notes

Dr. Robin D. Rogers has partial ownership of 525 Solutions and is a named inventor on related patent applications. Drs. Gabriela Gurau and Julia Shamshina are part time employees of 525 Solutions. The University of Alabama maintains approved Conflict Of Interest Management Plans.

Electronic Supplementary Information (ESI) available: [the biopolymer loadings, ratios of chitin to alginic acid, solutions viscosity, fiber technical characteristics, FT-IR spectra, optical and electron microscopy images, and biological tests results (irritation tests, wound closure results, and histopathology)]. See DOI: 10.1039/b000000x/

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Chitin-Calcium Alginate Composite Fibers for Wound Care Dressings Spun from Ionic Liquid Solution

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