Manufacturing of Large-Scale Functional Objects Using Biodegradable Chitosan Bioplastic[a]

Javier G. Fernandez, Donald E. Ingber*

Despite the urgent need for sustainable materials for mass-produced commercial products, and the incredible diversity of naturally biodegradable materials with desired structural properties, the use of regenerated biomaterials in modern engineering remains extremely limited. Chitin is a prime example: although it is responsible for some of the most remarkable mechanical properties exhibited by natural materials, including nacre, insect cuticle, and crustacean shells, and it is the most abundant organic compound on earth after cellulose, it has not been utilized in manufacturing strategies for commercial applications. Here we describe how analysis of differences in the molecular arrangement and mechanical properties of chitosan polymer that result from different processing methods led to development of a scalable manufacturing strategy for production of large three-dimensional (3D) objects of chitosan. This chitosan fabrication method offers a new pathway for large-scale production of fully compostable engineered components with complex forms, and establishes chitosan as a viable bioplastic that could potentially be used in place of existing non-degradable plastics for commercial manufacturing.

J. G. Fernandez, D. E. Ingber
Wyss Institute for Biologically Inspired Engineering, Harvard University, CLSB 5th floor, 3 Blackfan Circle, Boston, MA 02115, USA
E-mail: don.ingber@wyss.harvard.edu
D. E. Ingber
Vascular Biology Program, Departments of Surgery and Pathology, Children's Hospital and Harvard Medical School, Boston, MA 02115, USA
J. G. Fernandez, D. E. Ingber
Harvard School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02139, USA

Despite the urgent need for sustainable materials for mass-produced commercial products, and the incredible diversity of naturally biodegradable materials with desired structural properties, the use of regenerated biomaterials in modern engineering remains extremely limited. Chitin is a prime example: although it is responsible for some of the most remarkable mechanical properties exhibited by natural materials,[1] including nacre, insect cuticle, and crustacean shells, and it is the most abundant organic compound on earth after cellulose, it has not been utilized in manufacturing strategies for commercial applications. Here, we describe how analysis of differences in the molecular arrangement and mechanical properties of chitosan polymer that result from different processing methods led to development of a scalable manufacturing strategy for

---

[a] **Supporting Information** is available online from the Wiley Online Library or from the authors.

Macromol. Mater. Eng. 2014, 299, 932–938

DOI: 10.1002/mame.201300426

production of large three-dimensional (3D) objects of chitosan. This chitosan fabrication method offers a new pathway for large-scale production of fully compostable engineered components with complex forms, and establishes chitosan as a viable bioplastic that could potentially be used in place of existing non-degradable plastics for commercial manufacturing.

Referred as "the last biomass," about 10^{11} tons of chitin are produced by living organisms every year,[2] with most of this polysaccharide appearing as a highly acetylated polymer. The less acetylated form of the polymer, known as chitosan, can be produced at large scale using Mucoralean fungi (*Zygomycetes*) or other microbial cultures as a source. However, most of the worldwide production of chitosan comes from transformation of waste products from seafood processing, such as shrimp shells, which are highly enriched for chitin. Approximately 150 000 tons of industrially usable chitosan is obtained by this process every year.[3] Most of the chitosan is discarded or used in cosmetics, organic fertilizers, and dietary supplements,[4] while its natural structural properties have not be leveraged for engineering applications. This is because past chitosan fabrication methods that focused on freeze drying,[5] chemically modifying the polymer,[6] or blending it with thermoplastic polymers (e.g., polyesters),[7] failed to produce 3D materials with the organization, ecological integration, or structural properties of natural chitinous systems. As a result, there are no examples of large everyday objects made of pure chitosan for commercial production, and instead its use has been limited to thin films or scaffolds in microfluidic devices and surgical wound dressings.[8]

In contrast to cellulose production, which requires use of potentially toxic chemicals (e.g., carbon disulfide), chitosan's rich amine groups enable it to be dissolved in low concentrations of carboxylic acids (e.g., acetic, formic, or valeric acids) because protonation of these groups introduce repulsive interchain forces that are strong enough to disperse the polymer in solution.[9] Because this interaction is purely electrostatic, chitosan can be regenerated from its chitosonium form by neutralization of the acid solvent in a basic solution, or by spontaneous evaporation of the solvent at room temperature in the case of the small acetic acid molecules.[10,11] Both processes produce insoluble films of chitosan polymer; however, the films produced by coagulation through pH neutralization are brittle and opaque (ultimate strength = 21.6 ± 1.2 MPa; modulus of

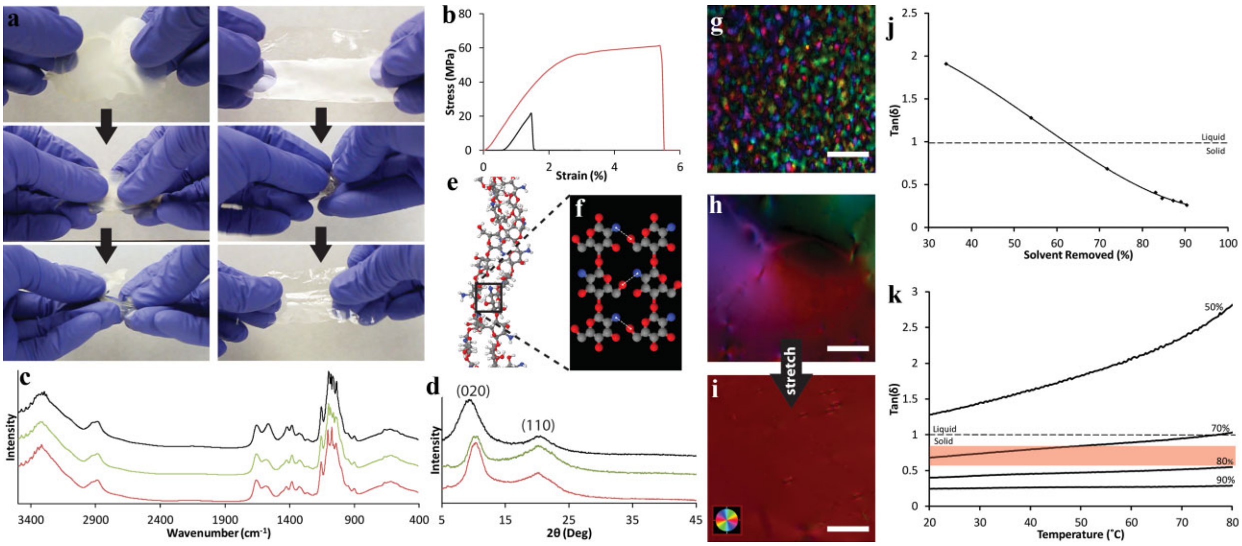

Figure 1. Characterization of cast chitosan films. a) Chitosan films produced by coagulation (left) are opaque and brittle, breaking easily when manipulated by hand (also see Supporting Information, Video S1), whereas films produced by evaporation (right) are optically clear and resilient when manipulated (see Supporting Information, Video S2). b) Mechanical stress–strain analysis of chitosan films produced by coagulation (black) versus evaporation (red), showing that the evaporated films exhibit much greater mechanical strength. c) FTIR analysis of evaporated films immediately after fabrication (red) or after being stretched by a 6% (green); similar analysis of a coagulated film is included as a control (black). d) X-ray diffraction pattern of the same samples. Notable is the absence of the shoulder at 21.5°, differencing tendon (from crab) and L-2 (from shrimp) chitosan polymorphs (color code of the lines is the same than previous panel). e) 3D representation of the twofold helical structure of chitin chains composed of C (gray), O (red), N (blue), and H (white) atoms. f) Detail of the interaction between adjacent chains shown in (e) with dashed lines indicating water-mediated bonds. Hydrogen atoms have been removed for clarity. g) Birefringence microscopy visualization of a coagulated film. Different colors represent different molecular alignment, characterized by the orientation of the slow axis of the material (bar is 0.3 mm). h) Same analysis of an evaporated film, showing much larger oriented crystal domains (bar is 0.5 mm). i) Rearrangement of the chains after unidirectionally stretching the film by 6% in length (bar is 0.5 mm). j) Variation of the G'/G'' (tan δ) measured for the chitosan solution with respect to polymer concentration. k) Variation of the G'/G'' ratio of a 3% chitosan solution of chitosan with the temperature. The percentage indicates solvent removed from the chitosan solution.

toughness $= 0.096 \pm 0.026\,\mathrm{J\,cm^{-3}}$) whereas films produced by evaporation are tough (ultimate strength $= 56.4 \pm 2$ MPa; modulus of toughness $= 2.29 \pm 0.4\,\mathrm{J\,cm^{-3}}$) and transparent (Figure 1a,b and Supporting Information, Video S1 and S2).

Molecular characterization of these materials using infrared spectroscopy (Figure 1c and Supporting Information, Figure S1) and X-ray diffraction (Figure 1d) reveal that both the evaporated and coagulated forms of chitosan, are the hydrated L-2 allomorph that forms sheets of molecules stabilized by water (Figure 1e,f),[12] which is characteristic of chitosan from shrimp shells (*Pandalus borealis* in our case).[13] However, birefringence microscopic analysis of these different chitosan materials revealed that they differ greatly in their crystal organization. The evaporated chitosan samples contain oriented crystal domains that often reach several square millimeters in size (Figure 1h and Supporting Information, Figure S2), and which are separated by smooth transitions where the intervening polymer chains gradually rotate from the characteristic alignment of one domain to the next. On the other hand, the crystal domains are much smaller ($49.17 \pm 24.69\,\mu\mathrm{m}^2$, s.d.) in the coagulated samples, and they are limited by sharp boundaries, which explain the opacity of the films due to internal light scattering (Figure 1g and Supporting Information, Figure S2).

Importantly, under an external force, the polymer chains in the evaporated films align parallel to the force, giving rise to surfaces with anisotropic alignment (Figure 1i) whereas in the coagulated films, the apparently independent domains break without any appreciable conformational change. This reorientation of polymer chains under an external force is well known in synthetic polymers[14] and, as observed in other structural biopolymers[15] and composites,[16] the mechanical properties of the processed chitosan strongly depends on small changes in its molecular organization, which are governed by the fabrication method, rather than depending on its chemistry alone.[17]

To better understand how the material regeneration process affects the macroscale material properties of chitosan materials, we monitored the ratio of the storage modulus (G') to the loss modulus (G'') of the material. This analysis revealed that removal of solvent by evaporation produces a chitosan liquid crystal, which exhibits every state between the liquid and solid forms without any discontinuous phase transition (Figure 1j). Additionally, while chitosan undergoes thermal degradation prior to melting, the properties of the solution depend on temperature, even at high concentrations (Figure 1k). This is consistent with past observations showing that altering temperature can produce a fast (but limited) change in the viscosity of a polymer solution when the intermolecular interactions characteristic of the crystal form are already present.[18] This was an important observation because it allowed us to exploit this property to produce a malleable material that can be adapted for component manufacturing by varying polymer concentration and temperature.

We concentrated an initially dilute solution of chitosan in acetic acid (i.e., $\rho < 30\,\mathrm{kg\,m^{-3}}$) so that it formed pliable liquid crystal material that could be employed in casting or injection processes (Figure 2a). By casting this liquid crystal material in an appropriate mold, we found that components with virtually any 3D form can be manufactured with chitosan polymer that retains its natural microscale crystalline organization (Figure 2b,c and Supporting Information, Figure S4). The high definition of the process enables accurate reproduction of the smallest features of the mold employed for the casting. For example, we were able to reproduce letters on the model that are 0.7 mm wide and 50 μm tall (Figure 2d). This high fidelity of feature reproduction is consistent with previous studies that employed chitosan for nanotechnology applications in which patterns have been produced with $<$100 nm features.[19] Most importantly, with an ultimate tensile strength of about 60 MPa and ultimate strain of 6.2%, the properties of these functional chitosan objects are similar to those of common manufacturing polymers (e.g., polyethylene terephthalate or polystyrene).

Chitosan can absorb and retain large amounts of water and, although the structures made of chitosan are not conceived for applications in direct contact with water. However, insects naturally protect most of their cuticle from hydration by employing a waxy epicuticle. Thus, we explored whether artificial oily coatings, such as beeswax, or parylene-c, can stabilize the fabricated chitosan materials against water. These studies revealed that while bees wax was only minimally effective, samples coated with parylene-c do not show a significant strength difference whether dry or wet ($p = 0.16$, two-tailed t-test; Figure 2f). Using this method, the molded chitosan structures were made highly water-resistant (Supporting Information, Video S3), and thus this manufacturing process can be used to produce composites that even retain their structural properties in an aqueous environment.

By dissolving chitosan at high concentrations (where the polymer shows a plastic behavior) and raising the temperature to about 80 °C, we also can produce chitosan bioplastic components using injection molding techniques (Supporting Information, Figure S3). In the case of large structures produced by injection molding, polymer shrinking can result in failure to maintain the original desired shape of the component. But by combining the chitosan liquid crystal solution with "filler" materials, shrinkage is easily prevented. For example, the use of wood flour (a wood processing waste product), resulted in production of a tough composite of wood particles interconnected by a chitosan-based organic matrix (Figure 2g,h and Supporting Information, Video S4). This material can be processed (e.g., drilled,

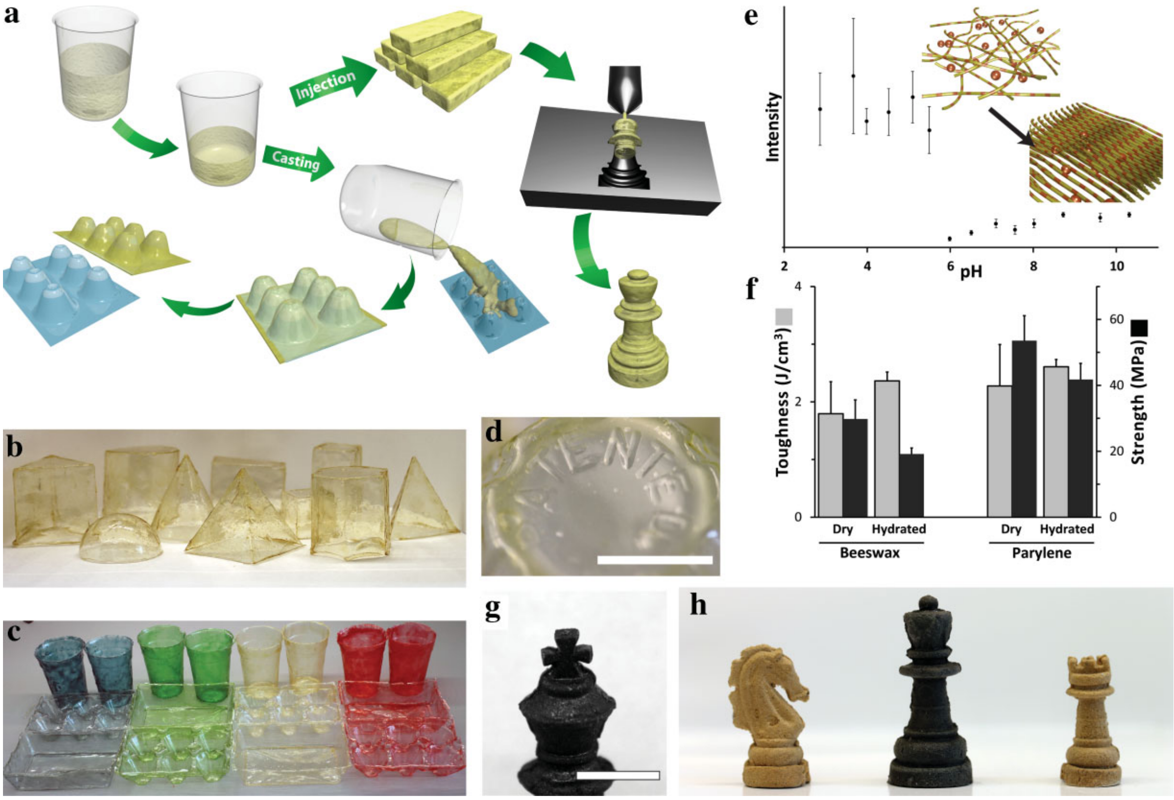

Figure 2. Fabrication of 3D objects with chitosan. a) Diagram of the fabrication process. An initial solution of 3% chitosan is concentrated until reach the viscosity necessary to be molded. To cast the solution, it is warmed up to decrease the viscosity and poured over the mold. The viscosity increase at room temperature helps to keep the polymer on the walls of the mold; the final crystallized form of chitosan is separated from the mold after the remaining solvent evaporates. In the case of injection molding, the polymer (by its own or mixed with a filler) is concentrated to a plastic state and warmed up at 80 °C before being injected in the mold. Just after the injection, the mold is open and the fabricated objects removed. b) Examples of geometric 3D shapes fabricated from chitosan by the casting method. c) Examples of cast chitosan objects that have been colored with water soluble dyes (i.e., tartrazine, allura red, brilliant blue FCF and fast green FCF). d) Detailed photograph of a casted object. Letters are 50 μm tall (bar is 10 mm). e) Color intensity, measured by spectrophotometry, of the solvent where a piece of red colored chitosan is submerged. Release of the colorant is high in low pH, and negligible when at high pH (>6.0). Insets show graphic representations of the retaining or removal of the color dye molecules (red spheres) depending on the pH of the environment. f) Mechanical properties (strength and toughness) of the material measured before (dry) and after 12 h under water (hydrated) when treated with different organic waterproof coatings of beeswax or parylene-C (parylene) (also see Supporting Information, Video S3). g) Detailed picture of a wood/chitosan king chess piece made by injection molding. Maltese cross is 5.8 mm wide and 2 mm thick (bar is 10 mm). h) Example of chess pieces (dyed black and colorless) made by injection molding using wood flour as filler. Queen is 41.08 mm tall (see also Supporting Information, Figure S4).

carved, sawed, and sanded) much like natural wood. While the chitosan-wood filler composite is less tough than the pure chitosan material, the shrinkage due to solvent evaporation of this less expensive material is greatly reduced ($S = 1.21 \pm 0.68\%$ s.d. with a 1:5 chitosan:wood ratio by weight). This property makes it much more usable for injection molding procedures with a fidelity of reproduction suitable for many applications depending of the filler grain size (Figure 2g, king's cross is 5.8 mm wide and 2 mm thick). Other fillers, such as sand or calcium carbonate, also can be used (Supporting Information, Figure S4); however, objects produced with these fillers are less tough due to coagulation of the chitosan resulting from the increase of pH produced when mixed with a salt.

By introducing dyes during the fabrication process, chitosan objects can be produced in a variety of colors (Figure 2c,e,h). Current use of dyes in plastics is complicated by the fact that discarded objects of the same color have to be collected and grouped before they can be recycled. For consumer products with many small parts consisting different types and colors of plastics, the resources it requires to separate the plastics far exceed their value, and hence these items are often discarded. By contrast, chitosan can capture and retain small molecules, a property that has been leveraged in the past to develop novel controlled-release molecular delivery systems.[20] Thus, we employed this property of chitosan to retain and program the controlled release of colorants to avoid the requirement of labor-intensive color-based sorting of these bioplastic objects before recycling.

The dye molecules are retained in chitosan in moderate acid and basic solutions, while these colors are released in more acidic environments (Figure 2e). Thus, it is possible to recycle non-dyed (Figure 3a, cycle A) and color (Figure 3a, cycle B) dyed pieces simply by varying pH. The fact that the dye is introduced and discarded in each recycling step (Figure 3a, cycle C) enables the reuse of the polymer independently of the colorants, which differs from conventional plastics where the colorant is covalently bonded to the structure. Importantly, because there are no chemical modifications during the process, objects made of chitosan do not show any detectable ($p > 0.05$, two-tailed t-test) degradation of their mechanical properties even after 20 rounds of recycling (Figure 3b).

Less than 5% of the conventional plastics produced is currently recycled worldwide,[21] and the rest is either incinerated or it accumulates in landfill and marine habitats.[22] This challenge to sustainability is driving a rebirth of interest in the use of biological materials for commercial products that are fully biocompatible and recyclable, such as ones composed of polylactic acid or polyhydroxyalkanoates. In contrast to these synthetic polymers, biological polymers are produced and assimilated in one or several natural cycles. Chitosan, for example, is widely available in unexploited natural sources,[4] its production does not compete with the resources for basic human needs, and it is rapidly broken down in compost (<2 weeks),[23] releasing nutrients that enhance plant growth.[24] In fact, we confirmed that even after undergoing the manufacturing processing we describe here, ground up pieces of our chitosan molded objects can serve as nutrient source for California black-eyed peas (i.e., *Vigna unguiculata*); this nitrogen-rich material supported plant growth to a similar degree as regular planting soil alone (Figure 3c,d and Supporting Information, Table S1).

To fully exploit the properties of biological materials (included their ecological integration) in technological and commercial applications, it is critical to use these natural constituents taking into account their native ultrastructure, and to do this by using commercially viable manufacturing processes. The results presented here demonstrate the ability to manufacture everyday products using highly abundant chitin-based materials, which are responsible for the unique material properties of some of the most remarkable structural systems in nature, spanning from fungal cell walls to the shells of arthropods and mollusks. Chitosan also provides many features required for production of consumer products, including optical

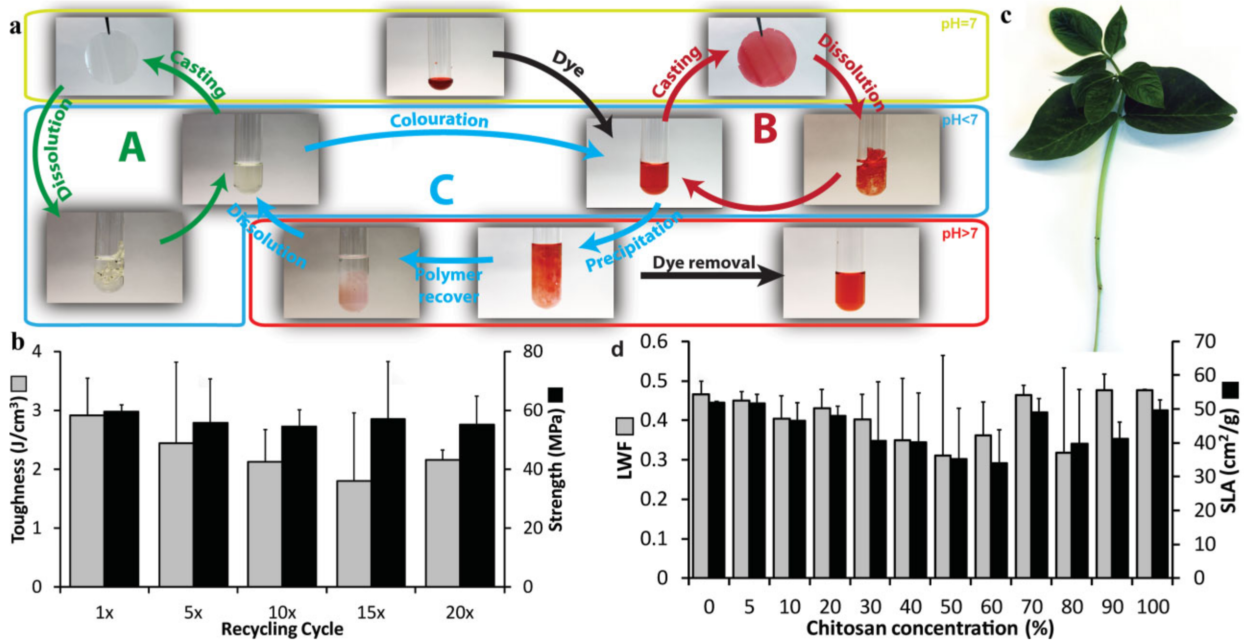

Figure 3. Recycling of manufactured chitosan objects. a) Diagram overview of the chitosan polymer recycling process. "A" indicates the main recycling process (casting–dissolution–casting) of an uncolored chitosan film using an acidic solution. "B" is similar to process "A" but a dye is introduced in the chitosan solution to produce a colored film. Both cycles (A and B) are connected by the process "C," where the colored chitosan is precipitated in a basic solution to separate dye and polymer, being the later susceptible to be employed again in process A or dyed in a different color in B. b) Strength of the polymer films after each recycling process (cycle A in Figure 3a). Each cycle comprises the casting of a film, removal of the remaining acetic acid, re-dissolution of the film, and its casting again. c) Example of a plant growing in a soil composed of chitosan. d) Leaf weight fraction (LWF) and specific leaf area (SLA) of California black-eyed peas after two weeks culture in different soils based on ground chitosan objects (also see Supporting Information, Table S1).

clarity and efficient ecological integration, in addition to mechanical properties similar to synthetic plastics. Moreover, we show here that functional objects with complex 3D shapes suitable for mass-scale consumer products can be fabricated from chitosan bioplastic (Supporting Information, Video S3 and S4) using conventional manufacturing processes (e.g., casting, injection, and molding), and that these objects can be rapidly and efficiently recycled. Chitosan components created with this manufacturing approach might also be well suited for commercial medical products as foams composed of this biopolymer are already approved by the FDA for wound healing applications.[25] Importantly, in contrast to most other bioplastics (e.g., thermoplastic scratch and polylactic acid) that come from croplands or forest, chitosan is abundantly available as waste from a broad range of marine sources. Therefore, this sustainable material can be used without having a negative impact on the food supply or being limited by production capability (e.g., with algae), which have been restricted exploitation of these technologies in the past.[26,27]

One of the potential limitations of this approach is that chitosan is three to four times more expensive than non-specialized commodity plastics. However, use of composites of chitosan and wood filler drop the cost of the material to the range of commodity plastics ($\approx 2\,\$\,\mathrm{kg}^{-1}$). Moreover, it is likely that the efficiency of this manufacturing approach will increase when it is applied at the industrial scale, which will further lower its cost, as has been observed during the refinement of many other putative manufacturing technologies (e.g., the cost of plastic injection molding has dropped drastically over time). This would allow this fabrication strategy to be used for manufacturing of less expensive engineered components that can require strength and toughness, but for the first time, also can be fully compostable.

Experimental Section

Industrial grade chitosan (Sigma–Aldrich, USA), with a range of molecular weights between 190 and 310 kDa and a deacetylation degree 80.46 ± 0.6%, was dissolved in a 1% v/v acetic acid water solution. For the casting of positive and/or hollow structures, 60% of the solvent was removed by evaporation in a 37 °C chamber (the process last about 6 h) and stored at this concentration in a close container to prevent further evaporation. Before its use, the solution is warmed up to about 40 °C and poured on the mold at room temperature. The evaporation of the remaining solvent gives rise to a hard positive replica of the mold. The samples are then treated with a fast immersion in 4% w/v water solution of NaOH, which neutralize any remaining acetic acid and the associated "vinegar" smell.

The polymer can be used directly in an injection molding process. From the initial 3% w/v of chitosan solution 80% of the solvent is removed, giving rise to a plastic solid, which is molded in bars for its storage upon use. The injection molding is performed by decreasing the viscosity of the material at 80 °C, and then cast with a manual benchtop injection molder in an epoxy mold. The technique, which is based in a moderate pressure, ensures also the absence of bubbles due to the elimination of the Harvey nuclei in the process.[28] The resulting negative replicas of the mold (Figure 2 and Supporting Information, Figure S4) are removed and stored at 37 °C for about 3–10 h (depending on the size and geometry of the fabricated object), where the remaining solvent is evaporated.

The removal of the water has associated a shrinking of the whole structure about 1/3 of its original volume. In structures requiring high precision in the final result, this effect can make unviable the technique. To compensate this effect, we add calcium carbonate, wood flour, or sand to the initial 3% solution of chitosan, while the rest of the injection molding process still the same. Error bars indicate standard deviation (s.d.) throughout. More detailed information on the experimental procedures can be found in the Supporting Information.

Acknowledgements: The authors would like to thank Dr. Kenny Roberts for his helpful comments, Cindy Tian for assistance with the colorimetric studies, and Kristopher Lofgren for his assistance with the birefringence measurements. This work was supported by the Wyss Institute for Biologically Inspired Engineering at Harvard University.
Author Contributions: J.G.F. and D.E.I. conceived the project; J.G.F. designed and performed the fabrication and characterization experiments; J.G.F. and D.E.I. analyzed the results and wrote the paper.

Note: Section heading numbers were removed after initial online publication. None of the content of the article has been changed.

Received: October 31, 2013; Revised: December 2, 2013; Published online: February 26, 2014; DOI: 10.1002/mame.201300426

Keywords: bioplastic; chitin; manufacture; recycle; sustainable

[1] J. G. Fernandez, D. E. Ingber, *Adv. Funct. Mater.* **2013**, *23*, 4454.
[2] K. Kurita, *Prog. Polym. Sci.* **2001**, *26*, 1921.
[3] H. Teftal, *Master of Science, Department of Agricultural Economics*, McGill University, Montreal, **2000**.
[4] M. N. V. Ravi Kumar, *React. Funct. Polym.* **2000**, *46*, 1.
[5] S. V. Madihally, H. W. T. Matthew, *Biomaterials* **1999**, *20*, 1133.
[6] V. K. Mourya, N. N. Inamdar, *React. Funct. Polym.* **2008**, *68*, 1013.
[7] V. M. Correlo, L. F. Boesel, M. Bhattacharya, J. F. Mano, N. M. Neves, R. L. Reis, *Mater. Sci. Eng. A* **2005**, *403*, 57.
[8] D. W. Lee, H. Lim, H. N. Chong, W. S. Shim, *Open Biomater. J.* **2009**, *1*, 10.
[9] M. Rinaudo, G. Pavlov, J. Desbrières, *Polymer* **1999**, *40*, 7029.
[10] A. Yamamoto, J. Kawada, T. Yui, K. Ogawa, *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1230.
[11] K. Ogawa, T. Yui, M. Miya, *Biosci. Biotechnol. Biochem.* **1992**, *56*, 858.
[12] K. Okuyama, K. Noguchi, M. Kanenari, T. Egawa, K. Osawa, K. Ogawa, *Carbohydr. Polym.* **2000**, *41*, 237.

[13] K. Sakurai, T. Shibano, K. Kimura, T. Takahoshi, *Sen'i Gakkaishi* **1985**, *41*, 361.
[14] H. Kang, Q. Wen, P. A. Janmey, J. X. Tang, E. Conti, F. C. MacKintosh, *J. Phys. Chem. B* **2009**, *113*, 3799.
[15] Z. Shao, F. Vollrath, *Nature* **2002**, *418*, 741.
[16] J. G. Fernandez, D. E. Ingber, *Adv. Mater.* **2012**, *24*, 480.
[17] W.-J. Chung, J.-W. Oh, K. Kwak, B. Y. Lee, J. Meyer, E. Wang, A. Hexemer, S.-W. Lee, *Nature* **2011**, *478*, 364.
[18] J. S. Chang, K. L. B. Chang, M. L. Tsai, *J. Appl. Polym. Sci.* **2007**, *105*, 2670.
[19] J. G. Fernandez, C. A. Mills, J. Samitier, *Small* **2009**, *5*, 614.
[20] J. G. Fernandez, J. Samitier, C. A. Mills, *J. Biomed. Mater. Res. A* **2011**, *98A*, 229.
[21] C. J. Moore, *Environ. Res.* **2008**, *108*, 131.
[22] Y. Mato, T. Isobe, H. Takada, H. Kanehiro, C. Ohtake, T. Kaminuma, *Environ. Sci. Technol.* **2000**, *35*, 318.
[23] K. Ohta, A. Taniguchi, N. Konishi, T. Hosoki, *HortScience* **1999**, *34*, 233.
[24] F. Burrows, C. Louime, M. Abazinge, O. Onokpise, *Am. -Eurasian J. Agric. Environ. Sci.* **2007**, *2*, 103.
[25] T. Kean, M. Thanou, *Adv. Drug Deliv. Rev.* **2010**, *62*, 3.
[26] E. Waltz, *Nat. Biotechnol.* **2013**, *31*, 12.
[27] V. Dornburg, I. Lewandowski, M. Patel, *J. Ind. Ecol.* **2003**, *7*, 93.
[28] J. G. Fernandez, C. A. Mills, M. Pla-Roca, J. Samitier, *Adv. Mater.* **2007**, *19*, 3696.