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Paper or Plastic?

The Commercial Future of Chitosan

By Charlotte Seid

The grocery shopper’s simple choice of paper or plastic now represents a dilemma of global proportions, as today’s consumers weigh the impacts of petroleum prices, deforestation, and carbon emissions on their everyday decisions. Plastics, in particular, seem indispensable to the packaged food industry, yet the disposable wrappers are notoriously slow to biodegrade. Two of the most common packaging materials, polyethylene and polypropylene, are derived from petroleum and are thus nonrenewable resources (1). Recent engineering, however, has introduced an enticing alternative, the natural biopolymer chitosan.

Chitosan derives from the more familiar compound chitin, the tough polysaccharide in the exoskeletons of arthropods and fungi, although in pure form both are much more flexible than a beetle’s wing. Compared to chitin, chitosan has fewer acetyl groups (2). Appropriately, chitosan shares key characteristics with paper and plastic. Like both, it is a polymer, composed of repeating linked units of the simple sugar glucose. Its chemical structure is identical to the cellulose in paper, except that chitosan contains amine groups (NH$_3^+$) on each subunit.

As much as chitosan resembles its supermarket counterparts, however, it far surpasses them in renewability and diversity of commercial and medical applications.

A major advantage is chitosan’s antimicrobial properties, which have been acknowledged since the 1970’s but only recently probed in detail. Ricardo Muzzarelli, a pioneer of chitosan research, observed in 1990 that chitosan drastically inhibited the growth of 298 bacterial cultures. In Gram-positive bacteria it disrupted the cell walls, while in Gram-negative species, which have an outer lipid layer that prevents Gram-staining, it distended the space between membranes. From electron micrographs Muzzarelli’s group suspected that chitosan’s many positive charges interfered with the negatively charged acids on cell walls, causing them to leak (3). This explanation is accepted as the most likely mechanism, although later studies have indicated that chitosan also inhibits bacterial toxin production by binding to the metal ions necessary for toxin synthesis (2).

When chitin was discovered in 1811 by the French chemist Henri Braconnot, it was appropriately named for the Greek word for “envelop,” a role that may now extend from crabs and mushrooms to food products and medicine. As late as 2005, a Japanese review foreshadowed, “only limited attention has been paid to chitin, in contrast to the comprehensive studies hitherto done on cellulose. Chitin thus remains an almost unutilized biomass resource.” (4)

But if chitosan resembles its supermarket counterparts in structure, it far surpasses them in sustainability, environmental low-impact, and versatility.

Food preservation

The commercial applications may come slowly, but an early 2007 review proposed that chitosan-derived films might replace plastics as environmentally friendly and edible food packaging (5). Considering the impractical biodegradation time of plastics, the continued and gratuitous use of petroleum-based materials is not ecologically sustainable. Chitosan, in contrast, can be recovered
en masse from shrimp and crab shells, which contain up to 40% chitin in addition to proteins and calcium carbonate (4). This preexisting supply would give chitin products the additional benefit of recycling seafood processing waste.

As described in a January 2007 report, powdered chitosan can be dissolved in acetic acid, filtered through a membrane, and dried into a clear film less than a millimeter thick. Not only is transparency ideal for consumers inspecting products, but the film completely inhibited growth of *Aspergillus niger*, the common black mold that infects fruits and vegetables. Since chitosan is thought to interfere with nutrient uptake by the mold spores, it could help prevent large-scale food contamination, especially when fresh produce is stored in the close conditions that otherwise favor fungal growth (1).

In a related experiment that assessed consumer appeal, Develieghere and colleagues at the University of Ghent, Belgium, tested an edible chitosan coating directly applied to the food. Strawberries dipped in a solution of chitosan and lactic acid showed significantly lower microbial growth over 12 days versus untreated strawberries, and the amount by weight of mold-infected berries was nearly a quarter of that for untreated ones (6). The chitosan coating could thus be expected to reduce produce waste due to spoilage and allow for longer shipping or storage times.

As judged by a trained panel of six tasters, the chitosan coating did not reduce the culinary quality of the strawberries. Immediately after coating, the berries were reported to taste bitter, but this effect disappeared after three days of storage. Visually, the chitosan coating was undetectable, and after 12 days, the coated berries even exceeded noncoated ones for texture ratings (6).

Chitosan is not a universal preservative, however. To determine its actual antimicrobial effectiveness when combined with food, the same study added starch, whey protein, table salt, or oil (representing common food components) before inoculating chitosan solutions with different microbe strains. At low concentrations of food additives and for all concentrations of oil, chitosan continued to inhibit microbial growth, but at certain thresholds for starch, protein, and salt (30%, 10%, and 1%, respectively), chitosan had no effect. The negatively charged groups in starch and protein most likely competed with the bacterial cell walls for interaction with chitosan, while salt, which dissociates into positive and negative ions, could have formed ionic bonds with both chitosan and bacterial membranes. Oil, as a nonpolar compound, had no effect. Chitosan's antimicrobial activity was also only significant at acidic pH, in which the amino groups are protonated (6). Thus, while the antimicrobial loss may make meat and bread products suboptimal products for such packaging, chitosan is an ideal preservative for fruits and vegetables, which have low pH and negligible salt or protein, and remains overall an attractive, renewable alternative to plastics.

**Gene Therapy**

In addition to wrapping food, chitosan can be a component of gene therapy, which targets genetic disorders with DNA-based treatments instead of conventional drugs. Chitosan nanospheres, tiny hollow globes under a micron in diameter, readily encapsulate plasmid DNA that is engineered to encode specific proteins (7). Since chitosan is non-toxic, it shows special promise as an oral vehicle for DNA vaccines, and its pH-sensitivity allows for controlled release of the contents. In the acidic environment of the stomach, chitosan
peared to untreated mice and those that had been given only DNA without chitosan. Over a 60-minute period, the chitosan-immunized mice took 10 minutes longer to develop symptoms related to peanut intake, including faster breathing, raised fur, and/or eye irritation. Furthermore, symptoms never exceeded level 2. By the end of the hour, the symptoms of no-chitosan and untreated mice stabilized around 3.5 (cyanosis or blue skin discoloration from lack of oxygen) and 4 (convulsions or unresponsiveness), respectively. Although all mice recovered within two hours, the experiment indicated vaccination using chitosan could reduce this allergy’s acute symptoms to a mere irritation (9).

While potential applications of chitosan as delivery vehicles in humans will require further study, this experiment might foreshadow a preventative treatment for severe allergies (9). An oral vaccine would be painless and easy for patients to self-dose, and it could reduce the number of allergy-related deaths. With sufficiently early treatment, perhaps DNA vaccines could eliminate allergic reactions altogether, increasing the lifestyle choices of those with food and pet allergies.

Disease vaccines, too, might benefit from chitosan oral delivery. In 2000, McNeela et al. tested chitosan nanoparticles in booster vaccines for diphtheria in response to a resurgence of the disease during the 80’s and 90’s in Europe. Mice that had already been immunized with the traditional injected vaccine were administered a booster of either a second, non-chitosan injection or an intranasal dose of chitosan particles. The two treatments were equally effective in raising antibody levels, so with additional research and mass-production, oral chitosan vaccines might replace traditional booster shots (10). Intranasal spray vaccines are particularly ideal for young children receiving their pre-kindergarten immunizations, and adults might be more likely to take booster vaccines that they can self-administer.

More recent studies have confirmed the effectiveness of chitosan in oral vaccines and addressed the molecular path to immunity. Chitosan is known to adhere to mucus membranes, which may help its passage through the barriers between epithelial cells such as in those in the small intestine (11, 10). Chew et al., who vaccinated mice against dust mite allergens, speculated that the particles are absorbed by lymphoid cells scattered in the small intestine called Peyer’s patches. After passing into the lymphatic system, the encoded antigens could be presented to helper T cells for antibody production (11). A 2005 study by Bowman et al. supported this model; the researchers found plasmid DNA was expressed in the ileum, Peyer’s patches, liver, and spleen, indicating that the nanoparticles had entered the lymphatic system via intestinal epithelials (12).

Tissue repair and engineering
While gene therapy addresses the human body at the unseen molecular level, chitosan’s potential in external applications is equally remarkable. Due to their antimicrobial activity, chitosan bandages and sutures are popular in medical settings, notably by U.S. troops in Iraq and Afghanistan (4). In Japan, a related compound sold as Beschitin® serves as a burn coating (13). Furthermore, chitosan could help rebuild damaged tissues by serving as a scaffold for cell culture.

Nerve grafting, for example, is a critical surgical challenge. Under current procedures, a piece of nerve is...
transplanted from a healthy site to the damaged area, but the nerve diameters must be near-identical and the donor area risks cell death. Furthermore, for the nerve to function properly, its axon must be surrounded by Schwann cells, which produce the myelin that serves as electrical insulation. Attracted by chitosan’s flexibility and biodegradability, Cao and colleagues tested the polymer as a potential nerve regeneration scaffold in 2005. Previous studies had indicated that pure chitosan collapsed too quickly, so various cross-linking agents were added; these compounds reinforced the strength and enzyme resistance of chitosan films by forming additional bonds (14).

Hexamethylene diisocyanate (HDI) proved the most effective additive and the only cross-linker that did not inhibit Schwann cell proliferation. HDI-chitosan films showed significantly higher growth of rat Schwann cells compared with growth on chitosan alone; moreover, there was an increase in tensile strength, ensuring HDI-chitosan scaffolds are less likely to rupture under muscle movement. These properties make HDI-chitosan a promising material for nerve grafts, since the growth of Schwann cells incorporates the new segment into the original tissue (14).

Additionally, HDI cross-linking slowed the film’s degradation by lysozyme, a ubiquitous body enzyme. Normally, lysozyme destroys bacteria by hydrolyzing bacterial cell walls, but the chemical similarity between chitosan and bacterial peptidoglycan also makes chitosan a target. While immediate degradation would prematurely destroy the growing nerve, slow degradation allows the old scaffold to disintegrate as new tissue forms, thereby circumventing the need for surgical removal of the scaffold (14).

Chitosan may make similar improvements in the treatment of disc degeneration, in which the cushioning cartilage between vertebrae degrades. The existing available options are imperfect; removing the disc or a spinal fusion restricts the patient’s mobility and can strain the remaining discs. In a 2007 study, Shao and Hunter successfully cultured annulus fibrosus cells from the intervertebral disc on a scaffold of chitosan and alginate gel. Alginate, a gumlike substance extracted from seaweeds, has been used previously in cartilage repair, but it was not considered stable enough for the mechanical demands of the spine; chitosan was selected to complement the alginate, as they form ionic bonds and stabilize each other. Canine disc cells were grown in culture plates on this hybrid gel, and after five days, they produced an extracellular matrix, a network of proteins that anchors connective tissue cells. The matrix is essential for permanent cell growth and indicated that the alginate-chitosan scaffold was a viable growth substrate (15). With subsequent in vivo studies to test these results under natural conditions, chitosan may earn a permanent role in disc replacement and improve the ease and success of spinal surgeries.

**Conclusion**

Will the next decades mark an emerging era of chitosan in hospital, supermarket, and home applications? In 2003, Tharanathan and Kittur published a review with an unequivocal title: “Chitin: The Undisputed Biomolecule of Great Potential.” They described chitin and its derivatives as “the most under-exploited biomass resource available on Earth” and predicted, “of the truly abundant polysaccharides in Nature, only chitin has yet to find utilization in large quantity” (16).

This biopolymer may present a medical, commercial, and environmental panacea once it receives public recognition; it could signal the end of traditional plastic packaging, the end of fatal allergic reactions, and the end of risky transplant surgeries. If the next generation of customers comes to purchase gene-therapy nanopills and pathogen-free produce wrapped in chitosan film, this remarkable bio-polymer of choice could make the classic grocer’s question—and much more—obsolete.

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**References**