Potential applications of chitosan in veterinary medicine

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Received 20 June 2003; accepted 18 February 2004
Available online 2 April 2004

Abstract

Chitosan is a partially deacetylated polymer obtained from the alkaline deacetylation of chitin which is a glucose-based unbranched polysaccharide widely distributed in nature as the principal component of exoskeletons of crustaceans and insects as well as of cell walls of some bacteria and fungi.

Chitosan exhibits a variety of physicochemical and biological properties resulting in numerous applications in fields such as waste and water treatment, agriculture, fabric and textiles, cosmetics, nutritional enhancement, and food processing. In addition to its lack of toxicity and allergenicity, and its biocompatibility, biodegradability and bioactivity make it a very attractive substance for diverse applications as a biomaterial in pharmaceutical and medical fields, where it has been used for systemic and local delivery of drugs and vaccines. It also has bioactive properties in its own right.

This paper reviews current veterinary applications for chitosan including wound healing, bone regeneration, analgesic and antimicrobial effects. It also discusses the potential application of chitosan to drug and vaccine delivery in veterinary species. Given the restrictions imposed by financial and animal restraint considerations, especially in farming applications, the veterinary drug delivery areas most likely to benefit from chitosan are the delivery of chemotherapeutics such as antibiotics, antiparasitics, anaesthetics, painkillers and growth promotants to mucosal epithelium for absorption for local or systemic activity, and the delivery of immunomodulatory agents to the mucosal associated lymphoid tissue for induction or modulation of local immune responses. The properties of chitosan expected to enhance these functions are discussed, and the future research directions in this field are indicated.

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Keywords: Chitosan; Veterinary; Wound healing; Tissue regeneration; Drug delivery; Mucosal immunization

Contents

1. Introduction ..................................................... 1468
2. Properties of chitosan ................................................. 1468
   2.1. Physicochemical ................................................ 1468
   2.2. Biological ................................................... 1469
3. Veterinary applications ................................................ 1470
1. Introduction

Chitin, which is the most abundant biopolymer in nature after cellulose, is a polysaccharide widely distributed in nature as the principal component of exoskeletons of crustaceans and insects as well as of cell walls of some bacteria and fungi. Like cellulose, it is a glucose-based unbranched polysaccharide. It differs from cellulose at the C-2 carbon by having an acetamido residue in place of a hydroxyl group. Chitosan is a partially deacetylated polymer of acetyl glucosamine obtained after alkaline deacetylation of the chitin. It comprises copolymers of glucosamine and N-acetyl glucosamine and the term chitosan embraces a series of polymers which vary in molecular weight (from about 10,000 to 2 million Dalton) [1]. Chitosan is semi-crystalline and the degree of crystallinity is a function of the degree of deacetylation. Crystallinity is maximal for both chitin (i.e. 0% deacetylated) and fully deacetylated (i.e. 100%) chitosan. Minimal crystallinity is achieved at intermediate degrees of deacetylation. Currently, chitin and chitosan are manufactured commercially in large scale from the outer shell of shrimps, lobsters and crabs.

2. Properties of chitosan

2.1. Physicochemical

Chitosan is insoluble at neutral and alkaline pH, but forms water-soluble salts with inorganic and organic acids including glutamic, hydrochloric, lactic and acetic acids. Upon dissolution in acidic media, the amino groups of the polymer become protonated rendering the molecule positively charged. Recently, importance has been attached to chitosan derivatives with a defined degree of deacetylation and depolymerization because of their significantly different physicochemical properties [2]. The degree of acetylation represents the proportion of N-acetyl-D-glucosamine units with respect to the total number of units. The properties of chitosan (e.g. pKₐ and solubility) can be modified by changing the degree of deacetylation and formulation properties such as the pH and ionic strength. At neutral pH, most chitosan molecules will lose their charge and precipitate from solution. Chitosan exhibits a variety of physicochemical and biological properties, therefore it has found numerous applications in various fields such as waste and water treatment, agriculture, fabric and textiles, cosmetics, nutritional enhancement, and food processing. In addition to its lack of toxicity and allergenicity, its biocompatibility, biodegradability and bioactivity make it a very attractive substance for diverse applications as a biomaterial in pharmaceutical and medical fields [3–5].

Chemical derivatization of chitosan provides a powerful means to promote new biological activities and to modify its mechanical properties. The primary amino groups on the molecule are reactive and provide a mechanism for side group attachment using a variety of mild reaction conditions. The general effect of addition of a side chain is to disrupt the crystalline structure of the material and hence increase the amorphous fraction. This modification generates a material with lower stiffness and often altered solubility but the precise nature of changes in chemical and biological properties depends on the nature of the side group. In addition, the characteristic features of chitosan such as being cationic, hemostatic and insoluble at high pH, can be completely reversed by a sulfation process which can render the molecule anionic and water-soluble, and also introduce anticoagulant properties [6]. The variety of groups which
can be attached to chitosan is almost unlimited, and side groups can be chosen to provide specific functionality, alter biological properties or modify physical properties.

Due to its high molecular weight and a linear unbranched structure, chitosan is an excellent viscosity-enhancing agent in acidic environments. It behaves as a pseudoplastic material exhibiting a decrease in viscosity with increasing rates of shear. The viscosity of chitosan solution increases with an increase in chitosan concentration, decrease in temperature and with increasing degree of deacetylation, which is a structural parameter also influencing physiochemical properties such as the molecular weight, the elongation at break and the tensile strength [7]. Viscosity also influences biological properties such as wound-healing properties and osteogenesis enhancement as well as biodegradation by lysozyme [8].

Chitosan, which is polycationic in acidic environments, possesses an ability to form gels at acidic pH values because it is hydrophilic and can retain water in its structure. The acetylation of chitosan in hydro-alcoholic media allows the selective modification of the free amino groups and is responsible for a process of gelation. It has been shown that the charge density of the chain segments is an essential parameter for the formation of gels and all factors that lower this parameter favor deswelling and reversibility [9]. The high hydration, the physicochemical and physical properties, as well as the polyelectrolyte behavior of this kind of gel allow applications such as bioactive dressing for wound healing. Gels can also be used as a slow-release drug-delivery system.

The solubility of chitosan can be sharply reduced by cross-linking the macromolecules with covalent bonds using for example glutaraldehyde [10]. Swelling of the films decreases with an increase in the amount of cross-linking agent added. The swelling of enzyme-containing films decreases more significantly, probably because of formation of additional cross-links due to the participation of the functional groups of the enzyme in the reaction.

When chitosan is intended for contact with serous liquids, sterility becomes necessary. Heat is often employed to facilitate polymer processing and to sterilize the pharmaceutical and medical products. However, exposure to high temperatures can change the physical properties of chitosan, affecting its aqueous solubility, rheology, and appearance. It has been shown that the chitosan acetate membranes are less aqueous soluble upon heating in an oven for 1 h at 120 °C. One postulation, derived from NMR and thermal analyses, was a thermal conversion of the water-soluble chitosan acetate to water-insoluble chitin. Chitosan films were found to be less hydrophilic when autoclaved at 121 °C for 15–30 min. This reduction in solubility was related to the formation of the anhydrous crystal polymorph observed in chitosan samples heated in the presence of water. Unlike gamma irradiation, which caused main chain scissions and a dose-dependent decrease in viscosity [11], a more complex relationship was reported between the viscosity of the chitosan and the amount of heat supplied. The reduction in viscosity was concomitant with decreased aqueous solubility and the development of color in the chitosan samples. The cross-linking of chitosan chains by glutaraldehyde was characterized by similar coloration and decreased aqueous solubility. The rate and extent of the changes was accelerated with increasing temperatures and in the presence of saturated steam [12].

2.2. Biological

Chitosan has been used as a safe excipient in drug formulations over the last two decades [13]. This polymer also attracted the attention of pharmaceutical scientists as a mucoadhesive polymer. Chitosan in the swollen state has been shown to be an excellent mucoadhesive and as a natural bioadhesive polymer that can adhere to hard and soft tissues, it has been used in dentistry, orthopaedics, ophthalmology and in surgical procedures. It adheres to epithelial tissues and to the mucus coat present on the surface of the tissues. A variety of chitosan-based colloidal delivery systems have been described in the literature for the mucosal delivery of polar drugs, peptides, proteins, vaccines and DNA. Clinical tests carried out in order to promote chitosan-based biomaterials do not report any inflammatory or allergic reactions following implantation, injection, topical application or ingestion in the human body [14]. Biomedical applications and some bioactive properties of chitosan are summarized in Table 1. Chitosan is easily hydrolyzed by various
chitosanases, which are completely absent in mammals, and depending on degree of deacetylation, by lysozyme. Its biodegradation leads to release of aminosugars, which can be incorporated into glycosaminoglycan and glycoprotein metabolic pathways, or excreted.

Recently, chitosan has shown promise as a carrier for delivery to the colon, results suggesting that degradation of chitosan by colonic bacterial enzymes might be an important factor in its successful targeting of the colon [15]. The degradation by the bacterial enzymes was shown to be dependent on both molecular weight and degree of deacetylation of the chitosan sample. Samples with a lower molecular weight and lower degree of deacetylation were found to be more susceptible substrates. On the other hand, chitosan was shown not to be affected by the environment of the small intestine [16].

It has been demonstrated that degree of deacetylation has no significant influence on the in vitro and in vivo cytocompatibility of chitosan films with keratinocytes and fibroblasts. The chitosan films with a low degree of deacetylation are very good biomaterials for superficial wound-healing [14]. Once placed on the wound, they adhere to fibroblasts and favor the proliferation of keratinocytes and thereby epidermal regeneration. The higher the degree of deacetylation of chitosan films, the lower was the adhesion to cells and the cell proliferation. It has been reported that the degree of deacetylation influences the strength as well as the elongation of films [17]. The chitosan films with a high degree of deacetylation were found to be more brittle and difficult to handle than those with a lower degree of deacetylation. Tissue responses to chitin and its derivatives were studied by implanting the films in the subcutaneous tissue of rats for different periods of time [17]. A relatively severe inflammatory reaction was observed in the tissue surrounding the chitin and chitosan films implanted for 1 week. However, this tissue response subsided 2 weeks after implantation. When films were implanted for 7 weeks, an encapsulation around the implanted films by a collagenous tissue was observed. Implanted films were more strongly encapsulated 12 weeks after implantation, as are conventional, non-biodegradable biomaterials. The surface properties of implanted biomaterials influence the tissue response. The film surface becomes less hydrophobic upon deacetylation of chitin to chitosan but the extent of hydration does not increase in proportion to the degree of deacetylation. In contrast to the hydration, the biodegradation of the films of chitin and its deacetylated derivatives does not seem to be governed by the physical microstructure but by the chemical structure of the films. The degradation kinetics of chitosan appear to be inversely related to the degree of crystallinity, which is controlled mainly by the degree of deacetylation. Highly deacetylated forms (e.g. >85%) exhibit the lowest degradation rates and may last several months in vivo, whereas samples with a lower level of deacetylation degrade more rapidly.

### 3. Veterinary applications

Recently, much attention has been given to the use of chitosan in veterinary applications, as a wound healing agent, antimicrobial agent, bandage material, skin grafting template, hemostatic agent and drug delivery vehicle. In this section, various investigations carried out recently on veterinary applications of chitosan are discussed as well as their potential uses in veterinary medicine.

<table>
<thead>
<tr>
<th>Table 1 Biomedical applications and bioactive properties of chitosan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial skin</td>
</tr>
<tr>
<td>Surgical sutures</td>
</tr>
<tr>
<td>Artificial blood vessels</td>
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<tr>
<td>Controlled drug release</td>
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<tr>
<td>Contact lens</td>
</tr>
<tr>
<td>Eye humor fluid</td>
</tr>
<tr>
<td>Bandages, sponges</td>
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<tr>
<td>Burn dressings</td>
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<td>Blood cholesterol control</td>
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<tr>
<td>Anti-inflammatory</td>
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<td>Tumor inhibition</td>
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<td>Anti-viral</td>
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<tr>
<td>Dental plaque inhibition</td>
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<tr>
<td>Bone healing treatment</td>
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<tr>
<td>Wound healing accelerator</td>
</tr>
<tr>
<td>Hemostatic</td>
</tr>
<tr>
<td>Antibacterial</td>
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<tr>
<td>Antifungal</td>
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<tr>
<td>Weight loss effect</td>
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</table>
3.1. Therapy

3.1.1. Wound healing

The chemical structure of chitosan, which is similar to that of hyaluronic acid, suggested that it would act as a wound healing agent [18]. Mechanisms of acceleration of wound healing have been investigated in vitro and in vivo by several groups [19–21]. Since 1985, Minami and his group have been developing chitin and chitosan products for veterinary use and they have reported their clinical effects on wound healing and their biological activities [22]. They found these polymers and products to accelerate wound healing, decrease treatment frequency, and give comfortable and painless wound surface protection. In addition to accelerating wound healing, chitosan is also capable of activating host defenses to prevent infection, thereby offering an alternative to the use of antibiotics.

Chitosan enhances the functions of inflammatory cells such as polymorphonuclear leukocytes (PMN) (phagocytosis, production of osteopontin and leukotriene B4), macrophages (phagocytosis, production of interleukin (IL-1), transforming growth factor β1 and platelet-derived growth factor). As a result, chitosan promotes granulation and organization. Therefore, it is beneficial for healing of large open wounds in animals [23]. One of the most interesting effects of chitin and chitosan on wound healing is formation of granulation tissue with angiogenesis. Chitin and chitosan induce fibroblasts to release interleukin-8, which is involved in migration and proliferation of fibroblasts and vascular endothelial cells.

There have been many studies carried out by different groups showing that chitin and chitosan accelerated wound healing in clinical veterinary cases (Table 2) and many remedies using chitin and chitosan for treatment of wounds have already been marketed. In most of these studies, the chitin and chitosans have been used as filaments, powders, granules, sponges, or as a composite with cotton or polyester. The effects of chitin and chitosan on wound healing were demonstrated in small animals (dogs and cats), livestock (cows) and zoo animals using the chitin products (Chitipack S and Chitipack P), and a chitosan product (Chitopack C), which are commercialised in Japan [24]. In comparison with conventional therapy with irrigation and antibiotic administration to a wound, new treatments with chitin and chitosan products permitted a substantial decrease in treatment frequency with minimal scar formation.

The application of chitosan hydrogels has also been demonstrated to effectively interact with and protect the wound, ensuring a good, moist healing environment. An insoluble flexible hydrogel-like soft rubber was prepared following application of ultraviolet light irradiation to a photo-crosslinkable chitosan aqueous solution. The resulting gel was evaluated as a dressing for wound occlusion and an accelerator in the healing process, using a mouse model with full-thickness skin incisions. Application of the gel onto an open wound induced significant wound contraction and accelerated wound closure and healing. Furthermore, in cell culture studies, it was shown that the immobilized chitosan hydrogel in the presence of fetal bovine serum had a chemotactic influence on dermal fibroblasts and stimulated cell growth [25].

In addition to effects on superficial wound repair, the influence of chitin on the healing of sheep flexor tendons after repair with non-woven polyester fiber has been investigated. Chitin was found to have a beneficial effect on collagen differentiation around non-woven fabric tendon implants [26].

For more detailed information on the accelerating effects of chitin and chitosan on wound healing, readers are referred to the studies published by Minami and his group [27].

3.1.2. Antimicrobial activity

In addition to the requirements for wound healing, it is important to control any infection of a wound under dressing. Infectious organisms preferentially target wounds beneath the dressing materials and elicit serious infections that frequently require removal of the wound dressing. For these reasons, the treatment of wounds requires the suppression of bacterial growth. Chitosan has been shown to provide inhibition of bacterial proliferation in the treatment of infected wounds [28]. The antimicrobial activity of chitosan has been recognized against several bacteria and fungi, and is influenced by a number of factors that include the type of chitosan, the degree of polymerization and some of its other
<table>
<thead>
<tr>
<th>Chitin/chitosan type</th>
<th>Form</th>
<th>Animal model</th>
<th>Application</th>
<th>Effect</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>Cotton type (Chitopack C)</td>
<td>Small animals</td>
<td>Topical</td>
<td>Wound/infection healing</td>
<td>Disappearance of purulence, formation of granulation tissue, re-epithelization, decrease in treatment frequency</td>
<td>[24]</td>
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<td></td>
<td></td>
<td>Large animals</td>
<td>Injection into pleural cavity</td>
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<td></td>
<td></td>
<td>Wild and zoo animals</td>
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<tr>
<td>Chitin</td>
<td>Sponge-like chitin (Chitipack S)</td>
<td>Small animals</td>
<td>Topical</td>
<td>Wound healing</td>
<td>Decrease in treatment frequency with minimal scar formation</td>
<td>[36]</td>
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<td></td>
<td>Composite chitin sheet (Chitipack P)</td>
<td>Small animals</td>
<td>Topical</td>
<td>Wound healing</td>
<td>Good wound contraction, re-epithelization, granulation tissue formation</td>
<td>[36]</td>
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<tr>
<td></td>
<td></td>
<td>Large animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chitin</td>
<td>Non-woven fiber and chitin complex (Chitipack P)</td>
<td>Sheep</td>
<td>Implantation</td>
<td>Tendon healing</td>
<td>Increased immature and intermediate fibroplasia</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>Freeze-dried soft sponge</td>
<td>Rabbit</td>
<td>Topical</td>
<td>Bone healing</td>
<td>Induced bone repair</td>
<td>[35]</td>
</tr>
<tr>
<td>Methylpyrrolidone chitosan</td>
<td>P-chitosan containing calcium phosphate cements</td>
<td>Rabbit</td>
<td>Implantation</td>
<td>Bone healing</td>
<td>Osteoinduction with relatively low doses of inducing signals</td>
<td>[36]</td>
</tr>
<tr>
<td>Phosphorylated chitosan (P-chitosan)</td>
<td>N,N-dicarboxymethyl chitosan</td>
<td>Soft spongy and hydrophilic material</td>
<td>Sheep</td>
<td>Topical</td>
<td>Bone healing</td>
<td>Accelerated regeneration of bone tissue</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Powder</td>
<td>Dog</td>
<td>Topical</td>
<td>Bone healing</td>
<td>Enhancement of bone healing</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td>Solution</td>
<td>Rat</td>
<td>Injection into knee</td>
<td>Tissue regeneration (chondrogenic)</td>
<td>Intra-articular fibrous tissue formation, articular chondrocyte proliferation, reduced decrease in the epiphyseal cartilage thickness</td>
<td>[39]</td>
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chemical and physical properties [29,30]. Chitosan more effectively inhibits growth of bacteria than chitosan oligomers but the inhibitory effects differ with regard to the molecular weight of chitosan and the type of bacterium. Chitosan generally exerts stronger bactericidal effects against gram-positive than gram-negative bacteria. The antibacterial activity of chitosan is affected by pH (pH 4.5–5.9 range tested), with greater activity being found at the lower pH values. In dilute acid solutions, the positive charges of chitosan interfere with the negatively charged residues of macromolecules at the bacterial cell surface, presumably by competing with Ca⁺⁺ for electronegative sites on the membrane without conferring dimensional stability, rendering the membrane leaky [31].

3.1.3. Tissue regeneration

Cellular interactions of chitosan with mammalian tissues have been positive from the tissue repair and regeneration standpoint. One of chitosan’s most promising features is its excellent ability to be processed into porous structures for use in cell transplantation and tissue regeneration [32,33]. Porous chitosan structures can be formed by freezing and lyophilizing chitosan solutions in suitable molds. The mechanical properties of chitosan scaffolds depend largely on the pore sizes and pore orientations [34]. Such scaffolds can enhance bone repair by supporting the proliferation of osteoblastic cells as well as their differentiation. Chitosan and its derivatives have been reported to have osteogenic activity and promote bone mineralization on artificially made bone defects in animal

<table>
<thead>
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<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitin</td>
<td>Suspension</td>
<td>Mouse</td>
<td>Intraocular injection</td>
<td>Analgesic</td>
<td>A dose-dependent decrease of the abnormal behaviors due to pain</td>
<td>[40]</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Granule</td>
<td>Dog</td>
<td>Topical</td>
<td>Wound healing</td>
<td>Re-epithelialization in chitin and chitosan groups (no statistical difference between chitin and chitosan groups)</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>Cotton type</td>
<td>Dog</td>
<td>Topical</td>
<td>Wound healing</td>
<td>Accelerated infiltration of PMN cells at the early stage, followed by the production of collagen by fibroblasts</td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td>Cotton type</td>
<td>Dog</td>
<td>Implantation</td>
<td>Immunopotentiator</td>
<td>Enhanced activity of polymorphonuclear cells, increase in number of white blood cells</td>
<td>[44]</td>
</tr>
<tr>
<td></td>
<td>Solution</td>
<td>Mouse</td>
<td>Intravenous infusion</td>
<td>Anti-inflammatory</td>
<td>Potentially useful in chitosan-induced acute respiratory distress syndrome</td>
<td>[45]</td>
</tr>
</tbody>
</table>

DD, degree of deacetylation; MW, molecular weight (Da); PMN, polymorphonuclear cells.
models [35–37]. In a study investigating the bone healing property of chitosan in dogs, the time required for bone repair was shorter by 1 week when compared to controls without chitosan, resulting in reduced animal stress and medical cost [38].

Recently, it has been shown that the chitosan solution injected into the knee articular cavity of rats causes a significant increase in articular cartilage chondrocyte density, suggesting that chitosan could act on the growth of epiphyseal cartilage and wound healing of articular cartilage [39]. These biochemical and morphological results coupled with favorable biological properties of chitosan indicate that chitosan is a promising scaffold for cartilage tissue engineering. It is also very important that the chitosan scaffold possesses the necessary mechanical properties in order to provide seeded chondrocytes with a proper biomechanical environment. Development of a chitosan-based material that can support chondrogenesis may be significant not only in terms of the quality of neocartilage produced, but also in terms of the ability of that tissue to integrate with the host matrix. Combination of chitosan-based cartilage scaffolds with gene therapy and the use of growth factors may improve the long-term outcomes of cartilage repair in clinical settings [6].

### 3.1.4. Analgesia

The analgesic effects of chitin and chitosan on inflammatory pain were evaluated using the acetic acid-induced writhing test in mice [40]. Following intraperitoneal administration of chitin and chitosan solutions prepared in acetic acid solution, a dose-dependent decrease in the number of the abnormal behaviors (writhing) due to pain, including extension of the hind legs, abdominal rigidity, and abdominal torsion was observed. This effect was greater in the animals administered the chitosan than those administered the chitin. The level of bradykinin in the peritoneal lavage fluid was lower in the animals administered chitin than those receiving chitosan. The chitin particles absorbed bradykinin more extensively than chitosan. The main analgesic effect of chitosan was suggested to be due to the absorption of proton radicals released in the inflammatory site, while that of chitin was due to the absorption of bradykinin.

Chitosan and N-O-carboxymethyl chitosan incorporated with morphine have been used to develop a product to provide 8–12 h of effective analgesia and which is inexpensive, non-irritating at the site of injection and devoid of side effects other than those normally associated with morphine. The use of opioids for the provision of pain relief in animals is common in both veterinary practice and laboratory animal medicine. Most opioid drugs have a relatively short half-life and duration of action in most companion animal species, necessitating multiple injections at 4–6-h intervals if adequate analgesia is to be maintained. Although not being directly used for its analgesic effect, chitosan as a gel matrix was demonstrated to provide more prolonged absorption, lower maximal serum concentrations and more sustained serum morphine concentrations than conventional morphine sulfate solutions [41].

In Table 2, the recent studies carried out on applications of chitosan for therapy in veterinary medicine are summarized [24,26,35–40,42–45]. However, there are some reported complications of chitosan applications. It is reported to cause lethal hemorrhagic pneumonia in dogs, following a subcutaneous high dose of chitosan [46]. In spite of application of chitosan to various species, this finding was observed only in dogs. Secondly, intratumor injection of chitosan on mice bearing tumors was found to increase the rate of metastasis and tumor growth. The development of excessive granulation tissue and accumulation of exudates was also encountered when large amounts of chitin and chitosan were administered in subcutaneous tissue [47]. Suitable levels of chitin and chitosan for organization of the implants in the rat were found to be 1–10 and 0.1–1 mg/ml, respectively. Therefore, it is important to consider these effects of chitosan prior to drug delivery.

### 3.2. Drug delivery

Chitosan is not currently used as a drug delivery system for veterinary species but its characteristics briefly described above suggest that it has considerable potential for such an application, although there will be restrictions imposed by animal restraint considerations, especially in farming applications. The veterinary areas most likely to benefit from chitosan are discussed in this section.
3.2.1. Delivery of chemotherapeutics

There is a need for delivery of chemotherapeutics such as antibiotics, antiparasitics, anaesthetics, painkillers and growth promotants to mucosal epithelium for absorption for local or systemic activity. Potential sites for application are buccal, nasal, conjunctival, mammary, vaginal and large intestinal mucosae. Oral administration intended for intestinal absorption may be possible, but would require formulation to cope with dilution and degradation in the stomach(s); this is particularly complex for ruminants where the physiological conditions of each of the stomachs are different, and the system is designed for microbial degradation of ingested substances [48]. Should chitosan be shown to enhance permeability of skin stratum corneum and epithelium, there would also be potential application to the transdermal delivery of drugs by backline application. A potential role has been identified in companion animals for delivery of chemotherapeutics to oral mucosal epithelium for local effect [49] and in ruminants for systemic regulation of reproduction [50] and to mammary mucosal epithelium for local effect [51]. In the future, when interfering RNA (RNAi) becomes a useful tool, chitosan may permit its mucosal delivery for local activity.

3.2.2. Delivery of immunomodulatory agents

Chitosan may provide for delivery of immunomodulatory agents such as vaccines, adjuvants and anti-inflammatory agents in veterinary medicine. There is a growing need, particularly in livestock industries, for effective mucosal immunomodulation as a sustainable disease control alternative to chemotherapeutics which are becoming less effective because of pathogen resistance or less acceptable because of environmental concerns. The target sites would include follicular associated epithelium and M cells of Peyer’s patches, and possibly also the absorptive epithelium, not associated with organized lymphoid tissue, of the small and large intestines and epithelium of respiratory tract, mouth, eye and vagina. The aim would be to deliver the agents to the mucosal associated lymphoid tissue for induction or modulation of local immune responses. The targets of such vaccines would include bacterial, viral and parasitic pathogens, reproductive hormones and ingested plant toxins, for the induction of either immune responsiveness or tolerance. Bowersock and Martin [52] identified the following issues as needing to be addressed by future veterinary vaccine formulation and delivery:

- Stimulation of immunity at mucosal surfaces. This is highly desirable, as most pathogens enter by this route, but the practical difficulties involved mean that it is yet to be achieved. One difficulty is that systemic immune responses are not necessarily effective at mucosal sites, and systemic vaccination usually produces poor mucosal immune responses. Local delivery may therefore be required, however, oral delivery for intestinal uptake has inherent problems such as enzymic and bacterial degradation and dilution in the stomach(s) and intestine, and interference by pH and mucus. There is thus a need for practical and effective delivery of inactivated vaccines so as to induce protective mucosal immune responses.

- Oral alternatives to systemic vaccines, in order to avoid hide and carcass damage, and restraint and handling difficulties. Oral vaccines for systemic effect would also be useful for feral animal control, in terms of reproduction, zoonotic disease, and disease transmissible to domestic livestock and companion animals. They may also permit priming of the neonate immune system in the presence of maternal antibodies [53]. In order to generate systemic immunity, the target pathway of antigen uptake and processing may differ from that desired for local immunity.

- Controlled release of inactivated vaccines. Given the cost of individual vaccination, especially in livestock species, there would be potential application for single administration biocompatible implants or devices, which were long-acting or self-boosting.

The desired destination of the drug or vaccine will influence the mechanism of uptake required and therefore the formulation of the delivery system. Drugs for local or systemic effect are likely to be less specific in their transport requirements, and where drug uptake depends on permeability of the intercellular tight junctions site and type of epithelium may be unimportant. In laboratory species, chitosan particles have been shown to be an effective
delivery system for the transport of a range of macromolecules (including peptides and DNA) across nasal, tracheal, oral and ocular epithelium [54,55]. Chitosan solutions, powder and nanoparticles have enhanced absorption across nasal mucosa, enhanced efficacy and prolonged action time of drugs [56–59]. However, vaccines will need to be delivered to appropriate lymphoid tissues.

**Mucosal associated lymphoid tissues (MALT)**

The anatomy and physiology of the mucosal immune system of the veterinary species has been comprehensively reviewed [60]. These species have a common mucosal immune system in that cells primed at one mucosal site recirculate to other mucosal sites. In general, these species have both diffuse lymphoid cells, especially in the lamina propria of the small intestine, and organized lymphoid tissue associated with conjunctiva, bronchi, small and large intestines. This organized tissue may be solitary nodules, often reactive rather than constitutive, or complex constitutive tissues such as tonsils, Peyer’s patches and lymph nodes. Immune responses to foreign antigens are initiated in these organized tissues. Peyer’s patches have specialized lympho-epithelium which includes M (microfold or membranous) cells with a capacity for active particle uptake, the subsequent processing of which differs between ileal and jejunal patches [61]. Fish are an exception in that they lack Peyer’s patches, the skin functions as a mucosal barrier and skin, gill and hindgut are all capable of macromolecular uptake.

Uptake of antigen by M cells is dependant on particle size, surface hydrophobicity and targeting ligands [62,63] and on the physiological characteristics of the M cells [64]. Thus microparticles may enhance the uptake of vaccines by M cells and therefore target them more effectively to lymphoid sites of immune induction [56,57,65]. Although microparticles are not commonly used in veterinary vaccines, a number have been investigated for veterinary applications [52]. Soluble antigens are thought to be transported through and between the absorptive epithelia of the gastrointestinal tract to local dendritic antigen presenting cells thus reaching the draining lymph nodes, or to liver. The site and type of immune response generated by mucosally delivered antigens may therefore differ with the solubility of the antigen [60].

Thus, a mucosal delivery system for drugs or vaccines should provide protected delivery, delivery to a specific target site, optimal uptake by the target route, and for vaccines, appropriate immunomodulation. Chitosan has a number of properties which can address these requirements.

- **Mucoadhesion.** Adherence of macromolecules to mucosal surfaces has been shown for various mucosae and may permit enhanced or prolonged drug absorption [66,67]. This would address the problems of washout and dilution effects, and permit maximal availability to the mucosal epithelium. In the case of chitosan, this adherence is generally the result of physicochemical interactions, and is thus not limited to specific ligands [68–70], but specific glycosylation can result in binding to specific lectins on epithelial cells [71]. Rapid turnover of gastrointestinal mucus may still be a problem.

- **Enhanced mucosal epithelial permeability.** Chitosan has been shown to enhance permeability of small polar molecules and peptide/protein drugs through nasal mucosa in animal models and human volunteers [72]. Other studies have shown an enhancing effect on penetration of compounds across the intestinal mucosa, buccal mucosa and cultured Caco-2 cells [73–76]. Several mechanisms have been proposed such as increasing cell permeability by opening of intercellular tight junctions, and favoring paracellular and intracellular transport.

- **Capacity to customize particle size, protection, release rates, administration formats and biodegradability.** A number of microparticular delivery systems have been used to administer drugs and vaccines via mucosal routes in order to make use of the characteristics of the macromolecular uptake by epithelial cells of MALT [62]. The size of particles influences susceptibility to gut washout and trans-epithelial uptake, with nanospheres more readily taken up than microspheres [70]. There is currently a great interest in the use of biodegradable polymers for the delivery of vaccines, both parenterally and to mucosal surfaces. Entrapment of antigens in particles prepared from biodegrad-
able polymers such as copolymers of polylactide and polyglycolide esters, and alginates for mucosal immunization, have given successful outcomes in animals [52]. Chitosan can be formulated as micro- or nano-particles and chitosan microparticles have been shown to be taken up by epithelium of murine Peyer's patches [77]. Chitosan can also be protected from degradation by glutaraldehyde or formaldehyde cross-linking, although this reduces its mucoadhesive properties [78,79]. Release rates from chitosan formulations can be varied by altering the chitosan properties and the initial drug concentration [80].

- Adjuvant effects. Chitosan for mucosal vaccination has been reported to enhance local and systemic immune responses. Possible mechanisms suggested have been induction of cytokine release by epithelial cells, shifting of the Th1/Th2 balance, activation of macrophages, or simply by increased absorption of antigen [56,57,81]. Chitosan has been shown to enhance local and systemic immune responses to influenza, tetanus toxoid, diphtheria and pertussis vaccines when delivered intranasally [82–85], and to DNA when delivered orally [86] to laboratory species. Chitosan given in an emulsion also enhanced both T and B lymphocyte responses to the poorly immunogenic βhCG delivered by intraperitoneal injection [87]. Subcutaneous implantation of chitosan alone in dogs increased the number of white blood cells, particularly neutrophils and activated both polymorphonuclear cells and macrophages, suggesting that chitosan may be useful as an immunopotentiator for prevention of immunosuppression as well as enhancement of vaccination [44].

4. Conclusions and future directions

Chitosan, which is a biodegradable, non-toxic biopolymer, is currently being used for therapy in veterinary medicine due to its various biological properties. It is capable of activating host defenses to prevent infection and accelerate wound healing as well as repairing the tissues. Studies are continuing on new derivatives of chitosan, which would not only exert new biological activities but also provide a better form for application to the site of effect. Little work has been published with chitosan for veterinary drug or vaccine delivery systems. Given the potential usefulness of this system, studies in livestock and companion animals are required. The most rewarding applications would appear to be for mucosally effective vaccines, oral vaccines for control of disease and reproduction in feral animals and possibly for sustained delivery of low doses of drugs such as hormones. Future studies need to include the stability of drugs and vaccines in chitosan formulations, and stability of the complex in the presence of gut bacteria; the optimal epithelial site of uptake for a given purpose; the optimal particle size for uptake by the desired cell type; and the requirement for bolus versus sustained delivery of mucosal vaccines.

References


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