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The promise of chitosan microspheres in drug delivery systems

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Chitosan is a partially deacetylated polymer obtained from the alkaline deacetylation of chitin, which is a glucose-based, unbranched polysaccharide that occurs widely in nature as the principal component of exoskeletons of crustaceans and insects, as well as of the 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 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 the pharmaceutical and medical fields. This review takes a closer look at the biomedical applications of chitosan microspheres. Based on recent research and existing products, some new and potential future approaches in this fascinating area are discussed.

Keywords: bioadhesive, biocompatible, chitosan microspheres

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1. Introduction

Chitosan is a functional, linear polymer that can be derived by the partial deacetylation of chitin. It is the most abundant natural polysaccharide on the earth after cellulose and can be obtained from the exoskeleton of marine crustaceans, such as crabs, lobsters, shrimps and krill. Chitosan is a copolymer consisting of 2-amino-2-deoxy-d-glucose and 2-acetamido-2-deoxy-d-glucose units linked with β-(1-4) bonds [1]. Because of its excellent properties (e.g., non-toxicity, biocompatibility, mucus adhesion and biodegradation), chitosan has been developed for a variety of biomedical applications, including in wound dressings and drug delivery systems [2,3]. Some of the biomedical applications of chitosan microspheres discussed in this article include: cancer therapy, tissue engineering, imaging and targeted drug delivery by magnetic carrier technology, antiviral and antibiotic drugs, implantation, vaccination, nutraceutical encapsulation, gene delivery, enzyme immobilisation, embolisation, peptide and protein delivery, and nasal, parentral, gastric, pulmonary and colon-selective drug delivery.

2. Cancer therapy

Gadolinium neutron-capture therapy is considered to be a powerful tool for the treatment of certain incurable types of cancers located in the liver, brain and other organs, and is dependent on the delivery of a high concentration of gadolinium and its retention in the tumour during neutron irradiation. There is potential for the application of chitosan microspheres (CMs) in this field, and studies have shown that microspheres containing gadolinium have been shown to significantly suppress melanoma solid tumours in mice compared with its solution during radiation [4].

CMs are also used for encapsulation and controlled release of different anticancer drugs, such as capecitabine, which has been successfully loaded into a semi-interpenetrating network hydrogel of CMs-poly(ethylene oxide-*g*-acrylamide) by emulsion crosslinking using glutaraldehyde [5]. In addition, IL-2, which is used for cancer immunotherapy, has been incorporated into porous microspheres formed by the gelation of sodium alginate and chitosan, followed by lyophilisation from an external aqueous solution of IL-2. The sustained released IL-2 was shown to trigger induction of cytotoxic T lymphocytes more efficiently than free IL-2 [6].

Chitosan and egg-phosphatidylcholine have been used as a unique combination for the localised and sustained release of paclitaxel over a 4-month period in biologically relevant media. The biological activity of paclitaxel loaded in the chitosan-egg phosphatidylcholine was confirmed in SKOV-3 human ovarian cancer cells [7]. Drug-containing chitin and chitin-Pluronic F-108 microparticles were have been formulated as biodegradable systems for localised administration to solid tumours, also for the delivery of paclitaxel. *In vivo* studies in Lewis lung carcinoma-bearing mice showed that the tumour volumes after 6 days using paclitaxel-loaded chitin, chitin-Pluronic F-108 microparticles and untreated control were 458, 307 and 997 mm^3, respectively [8].

Doxorubicin is commonly used in cancer therapy, but produces undesirable side effects such as cardiotoxicity. With the aim of minimising these effects, the drug has been coupled with dextran and encapsulated in chitosan nanoparticles. The nanoparticles were shown to increase survival time compared with either the drug conjugate or the free drug alone – not only reducing the side effects of the drug, but also its therapeutic efficacy in the treatment of solid tumours [9].

The application of chitosan microspheres loaded with methotrexate has also been investigated, with better sustained plasma drug levels and antitumour activity in Ehrlich ascites tumour-bearing mice than methotrexate solution on oral administration [10].

3. Tissue engineering

Damaged articular cartilage has very little capacity for spontaneous healing. Although many repair techniques have been proposed over the past three decades, all have failed to produce long-lasting repair tissue [11]. Recently, tissue engineering concepts have been applied to the development of a cell-based repair material for articular cartilage [12]. The use of a biomaterial similar to the target tissue's extracellular matrix may be useful. A three-dimensional (3-D) collagen–chitosan–glycosaminoglycan scaffold has been prepared in combination with TGF-1-loaded CMs. When microspheres were incorporated into the scaffold, they were shown to have the potential to enhance cartilage formation [13,14]. Another 3-D chitosan–poly(lactic-glycolic acid) (PLGA) composite porous scaffold was developed by sintering together these composite microspheres for bone tissue engineering applications. The presence of chitosan on the micosphere surfaces increased the alkaline phosphatase activity of the cells cultured on the composite scaffolds and upregulated gene expression of alkaline phosphatase, osteopontin and bone sialoprotein [15]. Chitosan scaffolds prepared by freeze drying encapsulated PLGA microspheres have also proven to be a promising cell scaffold for controlling the release of growth factors in tissue engineering [16].

Another type of composite microgranule is tricalcium phosphate–chitosan, which has been used as a bone substitute and tissue engineering scaffold, with the aim of obtaining high bone-forming efficacy. The microgranules were shown to have the ability to fill various types of defect sites with closer packing and were potential bone substitutes that had drug-releasing capacity and could act as an osteoblastic cell-culture scaffold [17].

4. Antiviral and antibiotic drugs

Multiple-unit, solid dosage forms using microspheres or beads have gained in popularity as oral drug delivery systems because they achieve a more uniform distribution of the drug in the gastrointestinal tract, a more uniform drug absorption, reduced local irritation and the elimination of unwanted intestinal retention of polymeric material, when compared with non-disintegrating single-unit dosage forms [18,19]. Acyclovir, an antiviral drug with limited water solubility, has been successfully encapsulated into semi-interpenetrating polymer network microspheres of acrylamide grafted to dextran and chitosan by an emulsion-crosslinking method. The grafting efficiency was 94% and the slow release of drug was extended up to 12 h [20]. In addition, *in vivo* ocular administration of acyclovir-loaded CMs to the rabbit eye resulted in prolonged high concentrations of acyclovir and increased AUC values [21].

Pentasodium tripolyphosphate crosslinked CMs [22] and a new derivative of chitosan, methylpyrrolidinone chitosan [23], have higher acid resistance and more stability in simulated gastric fluid, and have been shown to achieve the sustained release of ampicillin.

Chlorhexidine diacetate CMs prepared by a spray-drying technique have been developed for use in buccal tablets against bacterial infections. The chlorhexidine in the CMs dissolved more quickly *in vitro* than chlorhexidine powder, and was able to maintain or improve the antimicrobial activity of the drug, particularly against *Candida albicans*, due to the antimicrobial activity of the polymer itself [24].

Ciprofloxacin has also been loaded into CMs [25] and carboxymethyl CMs (an excellent biodegradable and bioacceptable polymer) [26] by a spray-drying method, and were shown to be more effective for the treatment of osteomyelitis than equivalent intramuscular antibiotics [25].

Microspheres of chitosan aspartate, glutamate and hydrochloride have been prepared by freeze drying and coated with stearic, palmitic, myristic and lauric acids using spray

drying. In these systems, vancomycin hydrochloride was used as a peptidic model drug, and its sustained release from the microspheres should minimise its inactivation in the upper part of the gastrointestinal tract [27].

Mucoadhesive CMs of clarithromycin provide a prolonged contact time to treat stomach ulcers. *In vitro* permeation studies across stomach tissue showed a higher accumulation of drug in the stomach tissue, with an increase in the bioavailability of clarithromycin from microspheres compared with simple drug suspension [28]. Tetracycline has also been loaded into CMs for maximum bioadhesivity and controlled drug release for the treatment of periodontitis. Antimicrobial studies showed that the concentrations of drug released were above the minimum concentration required for the inhibition of *Staphylococcus aureus* growth [29].

CMs with sizes < 3 µm have been developed in order to improve the oral bioavailability of Polymyxin B, and were shown to be taken up by Modified-cells of Peyer's patches for drug delivery to the gut-associated lymphoid tissue [30].

Encapsulated ofloxacin in hollow *N*-methylated CMs (2 – 5 µm) has also been shown to be released more rapidly, at pH 7.4 than at pH 1.2, and reaches 90 wt % within 8 h. [31].

Alginate CMs encapsulating three antituberculous drugs: rifampicin, isoniazid and pyrazinamide, have been developed. A subtherapeutic dose of these microspheres showed sustained release and an increase in the bioavailability of the drugs. In *Mycobacterium tuberculosis* H37Rv-infected guinea-pigs, administration of a therapeutic dose of microspheres spaced 10 days apart, produced a clearance of Bacilli equivalent to conventional treatment for 6 weeks. By using microspheres, the total weekly dose of the drug was reduce to half [32].

In another study, cefradine-containing ethylcellulose microparticles coated with chitosan maintained the plasma concentration of the drug for 24 h, and were useful for the prolonged intestinal absorption of cefradine [33].

5. Implantation

Biodegradable CMs containing vancomycin hydrochloride, implanted in the proximal tibia of rats with methicillin-resistant *Staphylococcus aureus* osteomyelitis, have been shown to be more effective than intramuscular administration for the treatment of experimental osteomyelitis [34]. In another study, after 6 months of implantation of cytarabine-loaded CMs, embedded in a PLGA film, most of the microsphers seemed to be intact, the comatrix appeared to be surrounded by conjunctive tissue and small blood vessels and nerve packets were detected in the periphery of the implant [35]. Film- and stick-type implantable dosage forms of chitosan and hydroxypropyl-chitosan containing uracil anticancer drugs also showed a sustained release of uracil *in vitro* and *in vivo*, and potential as biocompatible and biodegradable vehicles for implantable, sustained-release dosage forms of anticancer drugs [36].

Endothelial cell growth factor stimulates vascularisation. However, its relatively short half-life requires this angiogenic factor to be frequently administrated by non-specific and uncontrolled methods. Chitosan–albumin microspheres have been shown to be a potential delivery system for its controlled and localised release. *In vivo* studies demonstrated a high degree of neovascularisation for implants, starting from 7 days post-implantation, compared with control animals that received the injected form [37].

6. Peptide and protein delivery

CMs have important applications in the controlled release of protein and peptide drugs because they show excellent mucoadhesiveness [38] and a permeation-enhancing effect across the biological surfaces.

Superoxide dismutase, the most potent antioxidant enzyme, has been encapsulated into CMs to obtain suitable sustained protein delivery based on the complex coacervation process. The addition of polyethylene glycol to the protein solution or change of pH enhanced the encapsulation efficiency for controlled release [39].

Luteinizing hormone-releasing hormone (LH-RH), a decapeptide, is a naturally-occurring hormone that controls human sex hormones. Numerous LH-RH analogues (TX46) have been synthesised to manipulate the menstrual cycle and treat various steroid-dependent disorders, sex-hormone-dependent cancers and gynaecological conditions [40]. A novel kind of CMs have been prepared to prevent TX46 degradation by the proteases or other enzymes [41].

Insulin-loaded CMs have been prepared by combining a membrane emulsification technique and a step-wise crosslinking method. The CMs showed high encapsulation efficiency (80%), high chemical stability of insulin (> 95%), low burst release and steady release behaviour [42]. Chitosan gel beads prepared with chelated copper (II) ions are vehicles for the delivery of peptide and protein drugs, and these too have been studied for the release of insulin. In this study, insulin was scarcely released from the chitosan gel beads *in vitro*, which was proposed to be due to the interactions occurring between insulin, chitosan and the copper (II) ions. The efficacy of insulin released from the chitosan gel beads was confirmed by implantation into diabetic mice [43].

Human growth hormone encapsulated in CMs has also shown to be effective in early bone consolidation in distraction osteogenesis [44].

7. Vaccination

CMs have been developed and studied recently for various vaccines, such as Influenza, Pertussis and Diphtheria antigens [45]. Immune-stimulating factor of *Bordetella bronchiseptica* dermonecrotoxin, a major virulence factor of a causative agent of atropic rhinitis, has been loaded in CMs. *In vivo* activity of immune induction was investigated by intranasal administration

of the loaded CMs into mice. *Bordetella bronchiseptica* dermonecrotoxin-specific IgA titers in the nasal cavity were time- and dose-dependently increased by the administration. Similar phenomena were observed with the analysis of systemic IgA and IgG *in sera* that suggests direct vaccination via the nasal cavity is effective for targeting nasal-associated lymphoid tissues, and that CMs are an efficient adjuvant in nasal mucosal immunity for atropic rhinitis vaccine [46]. CMs prepared by an ionic gelation process with tripolyphosphate were also used for loading *Bordetella Bronchiseptica* dermonecrotoxin. TNF-α and nitric oxide from RAW264.7 cells that were exposed to the loaded CMs were gradually secreted with time, suggesting that the antigen released from the CMs had the immune-stimulating activity of atropic rhinitis vaccine [47].

A single injection of PLGA or CM containing tetanus toxoid could maintain the antibody response from days to over months at a level comparable to the booster injections of conventional aluminium hydroxide adsorbed vaccines. Hence, CMs have potential application in replacing the expensive polymer PLGA in vaccine delivery [48].

Porous CMs suitable for the delivery of antigen have been prepared using a wet phase-inversion method, and were chemically modified with 3-chloro-2-hydroypropyltrimethyl-amonium chloride. The antigen of the Newcastle disease vaccine was immobilised into the pores of CMs. Sustained-release of Newcastle disease vaccine's antigen could was achieved through an adsorption–desorption release test [49].

Recombinant *Streptococcus mutans* glucan-binding protein D has also been incorporated into PLGA microspheres for intranasal administration, and were then surface-coated with chitosan. The microspheres were shown to be potentially useful for antigen delivery in dental caries vaccination in rats [50].

Adenoviral vectors were encapsulated into a micro-particulate system for mucosal delivery. Microencapsulation of the vectors was performed by ionotropic coacervation of chitosan, with bile salts as counter-anions. Not only was the adenovirus protected from the low pH of the external medium, but also their release was delayed and dependent on cell contact, which is an advantage for mucosal vaccination purposes. The adenoviral infectivity was maintained and the onset of delivery was host controlled [51].

For the future development of microparticles for oral vaccine delivery, a Modified-cell *in vitro* model has been developed. Using this model, commercially available FluoSpheres® (Molecular Probes, Inc.) and CMs (1.7 µm) have been shown to be transported at a significantly higher amount by the human Modified-cell model compared with transport by using a Caco-2 cell monoculture [52,53]. This *in vitro* model improves the study of the targeting of Modified-cells of human origin.

8. Nutraceutical encapsulation

Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health by increasing humoral immunity and improving the balance of intestinal microflora [54-57]. Lactic acid bacteria used as probiotics are commonly incorporated into foods to provide a wide variety of health benefits [58] and are required to reach their site of action alive and in certain numbers [58]. However, a major barrier to the survival of ingested microorganisms is the acidic environment (pH 2) of the stomach [59]. Micro-encapsulation of lactic acid bacteria with alginate and chitosan offers an effective way of delivering viable bacterial cells to the colon and maintaining their survival during refrigerated storage [60]. In addition, polyphenolic compounds exhibit highly potent antioxidant activity, and the polyphenolic compounds from olive-leaf extract have been encapsulated into CMs by spray drying [61].

9. Nasal delivery

The nasal route is used both for the local and systemic administration of drugs, as well as for the delivery of peptides and vaccines. Mucoadhesive microspheres composed of hydroxypropyl methylcellulose, chitosan, carbopol 934P (and combinations of polymers) [62], as well as chitosan-poly(methyl vinyl ether-co-maleic anhydride)-microparticles [63] were prepared by spray drying, for the nasal delivery of propranolol HCl. These microspheres affected the integrity of tight junctions, without causing cell damage, relative to their swelling and charge of polymer. Cell viability was not affected, except with CMs, but this was recoverable [62].

An emulsifying method used for the production of ethyl-cellulose–CMs suitable for the nasal delivery of loratadin was shown to result in improved drug entrapment and moderate swelling, compared with conventional CMs [64]. Chitosan-4-thiobutylamidine (TBA) microspheres have also displayed the controlled release of fluorescein isothiocyanate-labelled insulin over 6 h and have the potential to be a useful formulation for the nasal administration of peptides. Chitosan–TBA–insulin, chitosan–insulin and mannitol–insulin microparticles led to an absolute bioavailability of 7.24 ± 0.76%, 2.04 ± 1.33% and 1.04 ± 0.27%, respectively, in rats [65].

The loading of carbamazepine in chitosan–glutamate microspheres, produced by spray drying, has been shown to increase the amount of the drug absorbed through the nose, when compared with the nasal administration of the pure drug as a powder (C_{max} = 800 and 25 ng/ml for CMs and pure drug as a powder, respectively) [66].

Microspheres composed of sodium alginate, chitosan hydrochloride, or both, have been obtained using a spray-drying method for the nasal administration of metoclopramide hydrochloride. *Ex-vivo* studies showed that drug permeation through the mucosa from CMs was higher than from those consisting of alginate alone. This can be related to the penetration-enhancing properties of chitosan via the opening of tight junctions. The complexation of chitosan with alginate led to controlled drug release [67].

CMs crosslinked by ascorbyl palmitate have been prepared by an emulsification process for the nasal delivery of insulin. They caused a 67% reduction in blood glucose compared with the intravenous route, and the absolute bioavaliability of insulin was 44% [68].

In addition, the *in vivo* performance of mucoadhesive microspheres of chitosan citrate containing salbutamol has shown a prolonged and controlled release of salbutamol via the nasal route, compared with the oral administration of conventional dosage forms [69].

In order to to improve the nasal delivery of gentamicin, the application of microparticles composed of hyaluronan and chitosan hydroglutamate, as well as microparticles consisting of both polymers, has been demonstrated. In one study, the rabbit bioavailabilities of gentamicin incorporated in hyaluronan, chitosan and mixed microparticles were increased 23-, 31- and 42-fold, respectively, compared with the control intranasal solution. This indicated that these microparticles were retained for longer periods on the nasal mucosa, thereby improving drug absorption, which may also be partly due to the penetration-enhancing effects of CMs [70].

A nasal morphine formulation based on CMs has also provided a highly increased absorption, with five- to sixfold increase in bioavailability over a simple morphine solution in a sheep model [71].

10. Parentral drug delivery

The disadvantage of an oral route of administration is the requirement daily ingestion and the subsequent daily variations in blood concentration. Various intramuscular or subcutaneous controlled drug delivery systems in the form of implants or microparticles have been developed based on biodegradable polymers such as PLGA. Recently, CMs have been successfully used for the treatment of brain edema. The efficiency of dexamethasone-loaded CMs in the treatment of cold-injury-induced brain edema was significantly higher compared with the systemic and topical application of dexamethasone by the intra-peritoneal route in Sprague–Dawley rats [72].

The intramuscular injection of smooth, highly spherical, glutaraldehyde crosslinked CMs (45 – 300 µm) loaded with progesterone in a rabbit model has also acheived a plasma concentration of 1 – 2 ng/ml, maintained up to 5 months, without a high burst effect. It has been suggested that these microspheres would be a suitable system for the long-term delivery of steroids [73].

11. Enzyme immobilisation

Chitosan is known as an ideal support material for enzyme immobilisation because of its many characteristics, such as improved mechanical strength, resistance to chemical degradation, protection of enzymes from the action of metal ions and antibacterial properties. In one study, laccase immobilised on magnetic CMs improved the performance of a fibre optic biosensor of oxygen consumption in relation to analyte oxidation, and has potential applications for medical examinations and diagnostics [74]. Catalase is another another enzyme that has been immobilised, by a phase-inversion method, [75] with sulphoxine as a chelating resin fixed to CMs acting as a support matrix [76].

12. Embolisation

Embolisation, the process by which a blood vessel or organ is obstructed by the lodgement of a material mass, has been used as a form of cancer treatment [77]. The introduction of embolic materials into the blood vessels that supply a tumour starves the tumour of blood. As an effective therapy for hepatocellular carcinoma, hepatic arterial chemo-embolisation therapy utilising cisplatin–CMs has been trialled; angiograms revealed a remarkable decrease in the number of arterioles in the liver, and histopathological specimens displayed nodular necrosis and hepatic cell degeneration in the embolised region [78].

CMs (100 – 400 µm) coated with alginate have been loaded with mitomycin–chitosan for chemoembolisation [79]. The renal angiograms obtained before/after embolisation and the histopathological observations showed the feasibility of the chitosan-coated alginate microspheres as chemo-embolisation agents [80].

13. Imaging and targeted drug delivery by magnetic carrier technology

Magnetically controlled drug targeting is one of the various possibilities of drug targeting. This technology is based on binding drugs with magnetic nanoparticles, which concentrate drugs in the area of interest by means of magnetic fields. The most popular applications of magnetic carrier technology are bioaffinity chromatography, waste water treatment, the immobilisation of enzymes or other biomolecules and the preparation of immunological assays [81-84]. High mechanical resistance, thermal stability, resistance to solvents and microbial attack, ease of manufacture and an excellent shelf life make inorganic materials ideal for the carriers, but they have limited functional groups for selective binding. Instead, polymers such as calcium alginate, polystyrene, polyacrylamide, polyvinyl alcohol, nitrocellulose and polyvinyl butyral with a variety of surface functional groups have been used in the preparation of magnetic carriers [85-89]. Well-shaped, spherical CMs of 100 – 250 µm have been prepared by suspension crosslinking for application as magnetic carriers [90].

Stable, chitosan–polyacrylic, polymer, magnetic microspheres with high Fe_3O_4 loading content were developed by electrostatic adsorption and subsequent polymerisation of acrylic acid onto the chitosan-coated Fe_3O_4 cores. A continuous release of the entrapped ammonium glycyrrhizinate in the

microspheres occurred, which confirmed their potential applications for the targeted delivery of drugs [91].

Superparamagnetic iron oxide nanoparticles have also been developed for clinical applications in MRI contrast enhancement [92-94]. In other studies, such nanoparticles were embedded in CMs (100 – 150 µm) by a sonochemical method. The CMs were then injected into the kidney of a New Zealand white rabbit via an angiographic catheter, and detected in MRIs of the kidney [95,96].

14. Gene delivery

The potential of chitosan as a polycationic gene carrier for oral administration has been explored since the 1990s. Chitosan has been shown to effectively bind DNA in saline or acetic acid solution and protect DNA from nuclease degradation. In one study, plasmid DNA (pDNA) was encapsulated in CMs using a complex coacervation process, and animal studies revealed exogeneous gene expression after oral administration of pDNA–CMs [97].

The encapsulation of an IL-2-encoding gene in CMs might be a useful strategy to increase the expression and to control the delivery of this cytokine gene into cells. CMs loaded with an IL-2 expression plasmid have been evaluated for gene-based immunotherapy. It was shown that the structure of plasmid remained unchanged during the encapsulation process. A high level of IL-2 expression was obtained with plasmid-loaded CMs, and showed similar IL-2 production as lipofectin. The molecular weight of the chitosan used and the amount of plasmid influenced the *in vitro* IL-2 production in the cells [98].

Evidently, polyplexes of chitosan and DNA significantly improve transfection efficiency. However, these polyplexes are not capable of achieving the sustained-release DNA and, thus, prolong gene transfer. In order to achieve the prolonged delivery of DNA, chitosan polyplexes have been formulated by physically combining PEG-grafted chitosan with PLGA using a modified conventional emulsion solvent evaporation method [99].

Encapsulation of adenovirus in chitosan–bile-salt microparticles has been shown to maintain adenovirus infectivity and permit a delayed release of the biologically active particles; the onset of delivery was host-controlled. The results displayed the beneficial properties for this system for mucosal adenovirus delivery [100]. Studies on DNA complexes with cationic polymers have been prompted by the search for non-viral DNA carriers for gene therapy. Poly(L-lysine) is able to deliver DNA as a bolus, but without long-term release. pDNA:poly(L-lysine) complexes encapsulated into CMs is an alternative. Their *in vitro* release and transfection characteristics, as well as the pDNA integrity and stability against serum and DNase I, has shown this system to potentially be a good alternative for polycation-based gene carriers [101].

Two different pDNAs (pGL2 and pMK3) have been encapsulated in CMs without affecting their structural and functional integrity. Sustained high protein production was obtained [102].

15. Gastric delivery

Floating hollow CMs would be an interesting gastroretentive controlled-release delivery system for drugs. The oral administration of tetracycline CMs prepared by chemical crosslinking in fasted gerbils show that they provide a longer residence time than either tetracycline solution or microspheres prepared by ionic precipitation [103].

The release of the drug from floating microcapsules containing melatonin prepared by the ionic interaction of chitosan and sodium dioctyl sulfosuccinate was greatly retarded in simulated gastric fluid, and the microspheres maintained their integrity for > 3 days compared with non-floating microspheres, where drug release was almost instant [104].

A stomach-specific drug delivery system using CMs has been developed to increase the efficacy of tetracycline against *Helicobacter pylori* by ionic crosslinking [105]. However, the high aqueous solubility of chitosan restricts the utility of CMs for gastric drug delivery. Reacetylated CMs were prepared with suitable properties for the controlled release of amoxicillin and metronidazole in the gastric cavity and, hence, for the eradication of *Helicobacter pylori* in gastric ulcers and possibly gastric carcinoma [106].

16. Pulmonary delivery

The pulmonary tract tends to be considered as a very promising and attractive route for the administration of active substances intended for local delivery and for the treatment of systemic diseases (e.g., diabetes).

Non-crosslinked and glutaraldehyde crosslinked CMs have been found to be potential candidates for carriers of proteins, peptides and pDNA to the lung via a pressurised metered-dose inhaler system [107].

Betamethasone-loaded CMs containing gelatin and Pluronic F68 have demonstrated good drug stability (1% less hydrolysis product), a high entrapment efficiency (95%) and a positive surface charge (37.5 mV). *In vitro* drug release from the CMs displayed a prolonged release pattern for 12 h that was suitable for pulmonary delivery [108-110].

Spray drying is a very valuable technique for producing dry powders adequate for thepulmonary delivery of drugs. Chitosan–tripolyphosphate nanoparticles that promote peptide absorption across lung mucosal surfaces are used to microencapsulate insulin-loaded chitosan nanoparticles using typical aerosol excipients, such as mannitol and lactose [111].

Surface-modified PLGA nanospheres containing chitosan are also used for the pulmonary delivery of elcatonin. After pulmonary administration, chotosan-modified PLGA nanospheres have been shown to be more slowly eliminated from the lungs than unmodified PLGA nanospheres, and

reduced blood calcium levels to 80% of the initial calcium concentration with prolonged pharmacological action to 24 h [112].

Pulmonary administration of a new pDNA, encoding eight HLA-A*0201-restricted T cell epitopes from *Mycobacterium tuberculosis*, has been incorporated in chitosan nanoparticles to produce a pulmonary vaccine. Results showed it induced increased levels of IFN-γ secretion compared with the pulmonary delivery of plasmid in solution or the more frequently used intramuscular immunisation route [113].

17. Colon-selective drug delivery

The colon is a site for the administration of protein and peptides that are degraded by digestive enzymes in the upper gastrointestinal tract. Along with many applications in local and systemic delivery of drugs, the colon-specific drug delivery systems can delay the absorption for the treatment of diseases that exhibit circadian rhythm, such as nocturnal asthma, angina and rheumatoid arthritis. 5-aminosalicylic acid, a cyclo-oxygenase inhibitor and an anti-inflammatory drug effective in Crohn's disease and ulcerative-colitis, is rapidly absorbed from the small intestine. Eudragit-coated CMs (200 µm) have been developed by an emulsion-solvent evaporation technique based on a multiple water/oil/water emulsion to deliver it specifically to the colon [114]. Also for colon-specific delivery, mucoadhesive alginate–CM containing prednisolone have been prepared. Depending on the preparation method, the particles displayed varying mucoadhesiveness [115].

Albendazole was also delivered specifically into the colon, in another study, by microspheres of chitosan hydrochloride, and drug release in 24 h was 48.9% and 76.5% in colonic fluid without and with rat caecal contents, respectively [116].

18. Conclusion

CMs represent a very promising drug delivery systems, which can be considered for a wide range of applications. Optimisation of the production procedure combined with the development of new, well-characterised and biodegradable derivatives of chitosan has enabled the development of CMs with increased acceptance and potential. The most important reasons for the increased bioavailability and effectiveness of drugs coupled with CMs is the bioadhesiveness and the absorption-enhancing effect of chitosan through the opening of tight junctions at the affected sites of administration.

19. Expert opinion

Chitin and chitosan have both attracted considerable attention as new non-toxic biological polymer materials, having favourable characteristics such as immune adjuvant activity, biological compatibility and biodegradability. CMs have been used for their mucoadhesive properties, for colon-specific drug delivery, nasal delivery, peptide and protein delivery and for the maintenance or improvement of the antibacterial activity of drugs, especially for *H. pylori* infections through gastroretentive controlled-release delivery systems, and in the future have promise as gene-delivery vehicles. The enhanced bioavailability of drugs loaded in CMs could be explained by the bioadhesive properties of such carriers to the mucosal tissues and the related increased diffusion of the drug into the vascular compartment. However, the drug release properties are not the only consideration of different delivery systems; for example, the bioavailability of protein drugs at the site of delivery delivery needs to be addressed. Over the last few years, the absorption of therapeutic macromolecules administered by the pulmonary route has received great attention. CMs have recently been proposed for pulmonary administration, once they can be designed to achieve appropriate morphological and aerodynamic characteristics for that purpose. After contact with the aqueous environment of the lung, CMs degraded by lysozymes are expected to be able to achieve the release therapeutic macromolecules. Thus, this system is proposed for systemic delivery of these macromolecules, considering the known properties of CMs to promote peptide absorption. In the future, they may also be used as a tool for the treatment of lung diseases, such as cystic fibrosis or cancer. Through certain small modifications in the chemical structure of CMs, they can be made to perform their duty of protein delivery in a more precisely controlled manner. As research is progressing in the area of oral protein delivery with natural, pH-sensitive polymers, we can expect many novel and innovative applications in the future that make use of the unique properties of CMs. The use of drug-absorption enhancers in drug delivery systems is likely to enhance therapeutic efficacy. Studies on drug absorption have focused on transporters that mediate drug influx and efflux and agents which can enhance drug absorption. It has been shown that chitosan can enhance drug absorption by the opening of tight junctions. Therefore, more research on the specificity of drug uptake by different derivatives of chitosan at the site of delivery is necessary. Such studies will be significant in advancing the targeted delivery of therapeutics in the future. Although the entrapment of hydrophilic macromolecular compounds (e.g., proteins, DNA, oligonucleotides) still faces some limitations, the many possible variations in the structure and type of chitosan derivatives allows the efficient entrapment of a great number of molecules. For CM technology, as it is now quite well mastered, the main objective is the improvement of their targeting properties following parentral administration. The capability of CMs to form polyplexes with pDNA to enhance their transport across cellular membranes through endocytosis makes them good candidates for gene therapy and biotechnological products in the near future. The strategy that is actually gaining a widespread interest is based on the design of CMs with tailored surface characteristics. More than ever, the achievement of pharmaceutical CM formulations for human use depends on the successful exploitation of multidisciplinary knowledge.

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