EXPERT OPINION

Topical drug delivery using chitosan nano- and microparticles

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Introduction: Topical drug delivery offers important benefits for improving the therapeutic effect and reducing systemic side effects of the administered compounds. In addition, utilization of biopolymeric material-based systems can play a key role in developing new topical dosage forms and their applications. This review describes the advances that have been made, new strategies and as well as possible challenges of particular systems of chitosan used in topical drug delivery, including challenging innovations in topical usage of these systems that can make significant impact on clinical practice.

Areas covered: The main area covered is hypothesis that particulate carriers based on chitosan and its derivatives can penetrate the topical barriers from the body. For this reason, the novel studies described emphasize the fact that chitosan-based particular systems are popular that can be tailor-made according to *in vitro* and *in vivo* characterization. Such parameters, which are known to influence their *in vivo* performance, can be modulated by adjusting the formulation conditions of the chitosan-based particular systems for topical application.

Expert opinion: The topical application of drugs with particulate systems comprising a natural polymer, chitosan, is one of the most popular drug delivery routes. The aim of topical use of chitosan particles is to improve the drug bioavailability by prolonging the residence time of drugs applied topically or by enhancing the passing of drugs through the epithelial cells by opening the tight junctions between epithelial cells and also to reduce the side effects of the drugs.

Keywords: biopolymer, chitosan, microparticle, nanoparticle, topical drug delivery

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1. Introduction

Over the last few decades, the treatment of disease has been accomplished by administrating drugs to human body via various routes. Topical application is the application of formulations to topical area such as skin, eye, nasal, buccal, lung to direct local treatment or the manifestations of a systemic disease with the intent of containing the pharmacological or other effect of the drug to the surface of the topical area or within the topical zone [1]. Recently, many research groups studied new technologies and topical drug delivery systems for simple delivery and highly efficient treatment [2]. Nanoparticles, as the name implies, are particles in the range of 10 to 1000 nm. On the other hand, microparticles are small spherical particles, with diameters of 1 μm to 1000 μm [3]. Chitosan is a cationic biopolymer and one of the most abundant polysaccharides present in nature, one of the most widely used polymer in topical drug delivery due to its many advantageous characteristics such as biocompatibility, non-toxicity, biodegradability, and low immunogenicity [4].

This paper reviews the new developments in the production of various chitosan particular systems for application to topical areas and challenges that are faced by

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chitosan nano-/microparticles for their use in topical drug delivery in clinical practice.

2. Chitosan

Chitosan, deacetylated chitin, is a linear polysaccharide composed of randomly distributed β-(1–4)-linked D-glucosamine and N-acetyl-D-glucosamine, which has already been proposed as a biomaterial, due to its apparent satisfactory biocompatibility. Indeed, chitosan appears to have no adverse effects after implantation in tissue and for this reason it has been used for a wide range of biomedical application such as artificial skin substitutes [5]. Chitosan has been studied in numerous drug delivery systems as well as in non-medical applications. The positive charge of the chitosan molecule enables its interaction with polyanions, a process that has been used to obtain drug carrier systems through complexation forming nano-/microparticles [6]. It has also been investigated as a superior mucoadhesive cationic polymer due to its ability to develop molecular attraction forces through electrostatic interactions with negatively charged mucin glycoproteins, which is determined by the formation of either hydrogen bonds, or ionic interactions between the positively charged amino groups of chitosan and negatively charged sialic acid residues of mucin depending on environmental pH [7]. Chitosan can effectively bind and agglutinate a wide variety of mammalian cell types. This is directly related to its cationic polyelectrolyte structure and the inherent negative charge present on the cell surface [8]. These properties make chitosan a good candidate for the development of particulate drug delivery system.

3. Chitosan nano-/microparticles

Particulate systems composed of naturally occurring biodegradable polymers have emerged as potential carriers of various therapeutic agents for controlled drug delivery through the topical route [9]. Chitosan, a cationic polysaccharide, is one of such biodegradable polymers, which has been extensively exploited for the preparation of nano-/microparticles to control the delivery of many therapeutic agents [10,11]. Chitosan particles, due to their biodegradability, biocompatibility, low toxicity, ease of preparation, can be a valuable tool to be employed in novel drug delivery systems [12]. Moreover, the studies reviewed emphasize the fact that chitosan-based particles are versatile systems that can be tailor-made according to required compositions, surface characteristics and particle size depending on required applications. Such parameters, which are known to influence their *in vivo* performance, can be modulated by adjusting the formulation conditions of the methods/technologies responsible for their formation, by incorporating additional materials in the preparation steps, and/or by using synthetically modified chitosans for topical uses [13,14]. Up-to-date, different techniques including ionotropic gelation, emulsification solvent diffusion, solvent evaporation, polyelectrolyte complexation, emulsification cross-linking, and complex coacervation methods have been used to prepare nano-/microparticles comprising chitosan or its derivatives [6,15].

4. Topical application of chitosan nano-/microparticles

4.1 Ocular drug delivery

4.1.1 Barriers to ocular drug delivery

The major barriers and determining factors in ocular drug delivery are firstly the physiochemical properties of the drug, its elimination from lacrimal fluid and corneal barriers [16]. The conjunctiva is the first contact site in ocular application of drugs. Structurally, conjunctiva is made up of two distinct types of epithelium cells, constituting the conjunctival and the corneal epithelium. The role of conjunctiva has been considered to be mainly protective and functioning as a passive physical barrier; therefore, it forms the second barrier. Corneal barrier is a major barrier in ocular drug delivery. Structurally, cornea has a wider surface area than the conjunctiva. It is a heterogeneous tissue with no blood vessels and is composed of multiple cell layers. While hydrophilic drugs enter the cornea via the paracellular route, lipophilic drugs are transported via the transcellular route. Several parameters such as solubility, hydrophilicity, molecular weight, charge, and ionization degree of active ingredient affect drug penetration through the cornea [17-20]. The lacrimal tear film is the most dynamic structure of the functional unit, and has several functions from protecting the eye against pathogens to eye cleaning and preventing penetration of foreign matter. It is thus another important barrier for ocular delivery. These barriers present an obstacle for drugs with small and large molecular mass [16]. In addition, delivery of drugs with high molecular mass and complex structure such as genes, proteins and peptides presents significant problems in ocular drug administration and these problems are difficult to overcome [21]. Nucleic acids, when delivered to the eye, break down with the lacrimal tear film and endonucleases. In addition, they are unable to pass the corneal barrier due to their negative charges and physicochemical characteristics and remain confined to the superficial epithelial layer. Furthermore, tight junctions between epithelial conjunctival cells limit paracellular passage for drugs of high molecular mass to a significant extent [22]. These issues in ocular delivery of high molecular mass drugs prompted ocular administration of drugs in nano-/microparticulate carrier systems fabricated with biopolymers such as chitosan. The eye is also a promising organ as a local administration site for new pharmaceutical dosage forms due to its dense vascular structure. Development of drug delivery systems administered locally to the eye has gained speed since 1970s with the understanding of the physicochemical structure of the cornea and tear [23,24]. Ocular drug delivery with cationic vectors has become a preferred route of drug administration. However, the physical barriers

and the low drug delivery capacity (< 5%) of topical route has brought along some problems. In addition, corneal electrostatic charge is another factor that should be taken into consideration. Epithelial cells have intrinsic electronegative charge. Corneal cells also contain other components including ionizable amino acid residues, stromal collagen, and proteoglycans. Those are thus negatively charged cells with isoelectric point of 3 that show higher permeability to cationic structures when compared with anionic ones [25]. Administrations of cationic particulate systems to cornea have therefore given successful results [10,26].

4.1.2 *Ocular application of chitosan nano-/microparticles*

Cationic particulate gene delivery systems have recently created great interest for corneal application [6,27-29]. Recent studies have reported the achievement of effective transgene expression after delivery of oligonucleotide, small interfering RNA and plasmid DNA via intrastromal injection, iontophoresis, electroporation, gene gun, and nanocarriers [6]. Remarkably, studies on non-viral applications of nanoparticles fabricated with chitosan oligomers and co-polymers have reported that plasmid DNA transfer through the cornea has been easily achieved. Nanoparticle suspensions containing model GFP-plasmid DNA prepared with combination of hyaluronan and chitosan oligomer (1:2) were administered to eyes of rabbits at a dose of 25 μg/eye. After administration of nanoparticles, the onset of expression was within 7 days. Increasing the dose of nanoparticles to 50 μg/eye resulted in increased expression [6]. In a similar study, plasmid DNA-loaded nanoparticles of hyaluronic acid and chitosan were able to transfect up to 15% of corneal epithelial cells following particle endocytosis mediated by the hyaluronan receptor CD44 [28].

Klausner *et al.* prepared positively charged oligomeric chitosan nanoparticles smaller than 100 nm with gWiz-luc and gWiz-GFP plasmids. Transfection efficiency of optimum formulation has been investigated in rat cornea. Injection of oligomeric chitosan–DNA nanoparticles into the stroma showed that luciferase gene expression was 5.4 times greater than that of polyethylenimine–DNA nanoparticles. The authors argued that their study lays the foundation for evaluating oligomeric chitosan–DNA nanoparticles as carriers in ocular gene therapy [29].

Another study investigating gene transfer from the cornea was that in which hyaluronic acid–chitosan nanoparticles containing pEGFP or pβ-gal plasmids were fabricated by ionotropic gelation technique. Transfection of corneal epithelial cells and conjunctival cells with nanoparticles containing plasmid DNAs was found to be more efficient than naked plasmid DNAs. As shown in **Figure 1**, two different cell lines have been studied: human corneal epithelial cells and normal human conjunctival cells. The extent of transfection in both cell lines varied depending on the mass ratio of hyaluronic acid and chitosan in nanoparticle formulation [27].

The nanoparticulate system exhibited low cytotoxicity, an ability to enter the corneal epithelial cells by CD44 receptor-mediated endocytic uptake, and the crucial capacity to deliver pDNA into both the corneal and the conjunctival cells, thus eliciting effective levels of protein expression. It was concluded that chitosan–hyaluronic acid nanoparticles could be considered a promising means of ocular gene delivery [27].

In another study, chitosan nanoparticles have been investigated for ocular delivery of low molecular weight drugs used for the treatment of glaucoma and several ocular diseases due to their *in vivo* tolerability, mucoadhesive properties, and minimal toxicity [18,30,31]. A quaternized derivative of chitosan, N-trimethyl chitosan, are known to increase the paracellular transport of hydrophilic drugs and macromolecules across cell monolayers, such as the intestinal epithelium, by opening the tight junctions, but the study from Zambito *et al.* demonstrated that the tight junctions of stratified epithelium at the cornea are not effectively opened by N-trimethyl chitosan microspheres, at least to such an extent as to allow penetration of hydrophilic tobramycin sulfate [19]. On the other hand, it has been demonstrated that N-trimethyl chitosan has good mucoadhesive properties and controls the release rate of active ingredients from nanoparticles [19].

Gatifloxacin, a broad-spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated in the chitosan–alginate nanoparticles and *in vitro* release studies showed that the drug was released from nanoparticles over 24 h in a sustained release manner, primarily following non-Fickian diffusion release model [19,32]. Similar results have been obtained with chitosan microspheres containing acyclovir. In this study, New Zealand albino rats have been treated with acyclovir-loaded microspheres made from chitosan and acyclovir suspension, and then acyclovir levels in aqueous humor have been monitored after a single instillation of each formulation into the conjunctival sac. *In vivo* studies showed that acyclovir-loaded chitosan microspheres have increased drug bioavailability to the eye compared with raw drug, by prolonging drug residence time in the eye due to mucoadhesive properties of chitosan [33].

Chitosan nanoparticles may represent an interesting vehicle to enhance the therapeutic index of clinically challenging drugs with potential application at extraocular level. In a study, chitosan nanoparticles containing cyclosporine A, an immunosuppressive agent, have been fabricated by ionic gelation technique and applied by topical instillation to the rabbit eye. *In vivo* studies showed that therapeutic concentrations of cyclosporine A in external ocular tissues such as cornea and conjunctiva were achieved at least for 48 h [34,35].

The nanoparticles prepared from amphiphilic chitosan have recently attracted increasing interest in pharmaceutical area since nanoparticle preparation through the self-assembly of amphiphilic chitosan is easy and hydrophobic components enable the nanoparticles to act as a reservoir of hydrophobic drugs. Hydrophobic components such as deoxycolic acid,

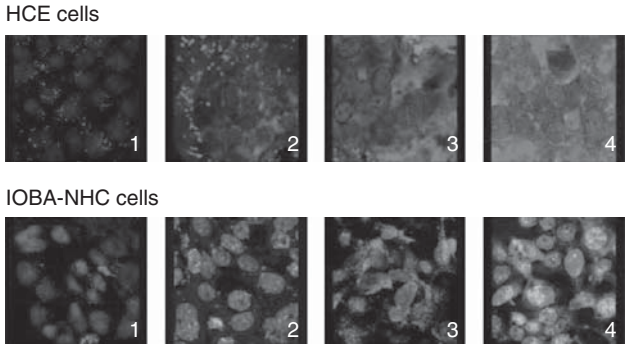

Figure 1. Confocal images showing the cellular uptake of (1) hyaluronic acid solution, (2) hyaluronic acid : chitosan oligomers mass ratio 1:2, (3) hyaluronic acid : chitosan mass ratio 1:2, or (4) hyaluronic acid : chitosan mass ratio 2:1 nanoparticles after 1 h of incubation with human corneal epithelial or normal human conjunctival cells. The images are the projection of x–y sections. Hyaluronic acid was labeled with fluoresceinamine (green), and the cell nuclei stained with propidium iodide (red). Magnification, ×63.

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cholesterol, and palmitic acid have been successfully used in the modification of chitosan [36]. In the study from Yuan *et al.* [37], self-aggregation of hydrophobically-modified chitosan with cholesterol nanoparticles was radiolabeled by ^{99m}Tc and their ocular distribution was investigated using single-photon emission computed tomography and scintillation counter. The nanoparticles showed a good spreading over the entire precorneal area shortly following the topical application. After 112 min, more than 70% of radiolabeled nanoparticles have remained at the ocular surface, indicating well retention ability of these nanoparticles at the precorneal area.

Lin *et al.* [38-40] investigated the efficacy of pilocarpine-loaded chitosan–carbopol nanoparticles using *in vivo* miotic tests and compared it with pilocarpine either in solution, gel, or liposomes. Pilocarpine-loaded chitosan–carbopol nanoparticles, 294 nm in size, showed the most significant long-lasting decrease in the pupil diameter of rabbits. It was reported that the pilocarpine-containing chitosan–carbopol nanoparticles might provide an excellent potential alternative as an ophthalmic sustained-release formulation of pilocarpine for clinical use due to the mucoadhesive properties of chitosan [38].

On the other hand, Tian *et al.* [41] studied on modified nanostructured lipid carriers (NLC) with partially deacetylated water-soluble chitosan (PDSC) as an efficient ocular delivery system to improve its transcorneal penetration and precorneal retention. The flurbiprofen-loaded nanocarrier systems were prepared by melt emulsification method. The spherical particles showed core-shell structure and a reversed zeta potential. Precorneal retention assessed by gamma scintigraphy in rabbits indicated that the area under the remaining activity–time curve of the PDSC-coated NLC was 1.3-fold of non-coated NLC and 2.4-fold of flurbiprofen solution. Moreover, *in vivo* study of ocular tolerance indicated that the PDSC-coated particles showed high potential for ocular drug delivery.

Corneal transplantation is one of the most commonly performed allografts, but this can cause immune graft rejection causing graft failure. The failure in suppression of graft rejection by topical administration usually results from the difficulty to achieve clinically effective drug concentrations in the cornea and anterior chamber. In the study of Yuan *et al.*, an immunosuppressive agent, rapamycin, loaded nanoparticles using polylactic acid and cholesterol-modified chitosan was prepared and their immunosuppressive activity in corneal transplantation was investigated. As shown in **Figure 2**, the nanoparticles have shown an excellent immunosuppressive effect when compared with the rapamycin eye drop [37].

Retinoblastoma is the third most common form of cancer in infants and is an ocular disease that requires attention as metastatic retinoblastoma is lethal. This type of cancer can be controlled with effective treatment in early stages of disease. The potential of carboplatin-loaded chitosan–alginate nanoparticles for the treatment of retinoblastoma was investigated. Nanoparticles improved the cellular uptake of carboplatin as compared with native carboplatin. It was also demonstrated that clathrin-mediated endocytosis played a key role in the internalization of nanoparticles in the Y79 cell line and biodegradable chitosan nanoparticles could be used as an effective ocular drug delivery system for sustained intracellular delivery of carboplatin for the treatment of retinoblastoma [42].

Recently, chitosan has been used as coating materials in order to modify the surface characteristics of nano-/microcarriers [43,44]. Abdelbary [45] prepared mucoadhesive chitosan-coated liposomes to improve the ocular bioavailability of ciprofloxacin hydrochloride (CPX). *In vitro* drug release and *in vivo* results confirmed that chitosan-coated liposomal formulations have exhibited a higher retention of CPX due to the bioadhesive properties of chitosan and these results correlated with CS-coated liposomes could be a promising approach to increase the ocular bioavailability of CPX. Li *et al.* [44] obtained similar results with low molecular weight chitosan-coated liposomes (LCHL) designed for ocular drug delivery. Coating of liposomes prolonged drug retention on ocular surface and enhanced drug permeation.

In the last decade, particulate drug carrier systems fabricated with modified chitosans combined with some other biodegradable polymers such as PLGA were used for ocular drug delivery [45-47]. For this aim, Jain *et al.* [46] designed the PLGA–chitosan nanoplexes containing fluorescent rhodamine (Rd) with a diameter of 115.6 ± 17 nm, and evaluated their *ex vivo* and *in vivo* characteristics as well as their potential as ocular delivery system on rabbits. Rabbit corneas after treating with nanoplexes were evaluated for the *in vivo* uptake and ocular tolerance. Data from *ex vivo* and *in vivo* studies showed that the amounts of Rd in the cornea were significantly higher for

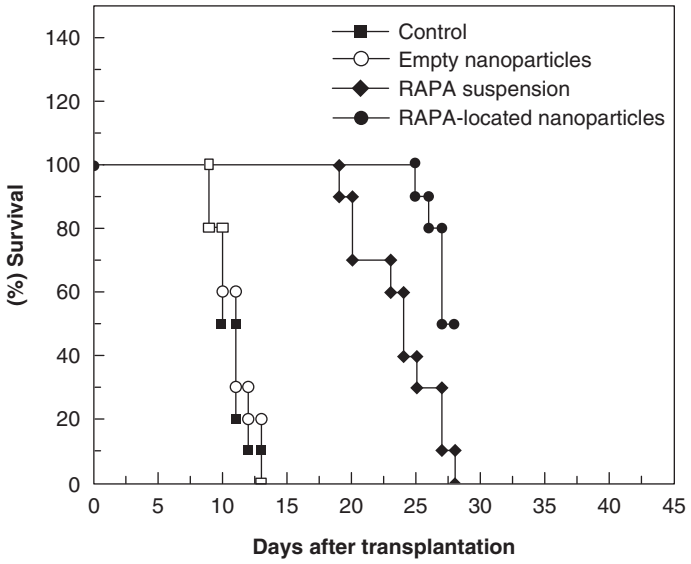

Figure 2. Survival curves of corneal allografts in rabbits treated with empty particles, RAPA suspension and RAPA particles.
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nanoplexes than that of Rd solution, these amounts being fairly constant for up to 24 h. Confocal microscopy of the corneas revealed paracellular and transcellular uptake of the nanoplexes. The uptake mechanism postulated was adsorptive-mediated endocytosis and opening of the tight junctions between epithelial cells. No alteration was microscopically observed after ocular surface exposure to nanoplexes. Taken together, these data demonstrate that nanoplexes are potentially useful as ocular drug carriers.

In conclusion, these illustrate successful clinical trials with chitosan nano-/microparticles.

4.2 Dermal and transdermal drug delivery

4.2.1 Barriers to dermal drug delivery

The foremost function of the skin is to protect the organism from the potential detrimental effects of the environment. Skin represents a formidable barrier for microorganisms, chemicals, radiation, and many allergens. The structure of the skin also prevents water loss from the organism. This barrier, however, allows partial permeability for several chemical and biological therapeutic agents. Basic knowledge relating to skin's physiology, function, and biochemistry is very necessary for designing dermal preparations. The skin is the heaviest single organ of the body, combines with the mucosal lining of the respiratory, digestive and urogenital tracts, which separates the internal body structures from the external environment. Acidity of the skin (pH 4 – 5.5) helps in limiting or preventing the growth of pathogens and other organisms [48,49].

The transepidermal pathway is principally responsible for diffusion across the skin. The resistance encountered along this pathway arises in the stratum corneum. Permeation by the transepidermal route first involves partitioning into the stratum corneum. Diffusion then takes place across this tissue. The current popular belief is that most substances diffuse across the stratum corneum via the intercellular lipoidal route. This is a tortuous pathway of limited fractional volume and even more limited productive fractional area in the plane of diffusion. However, there appears to be another microscopic path through the stratum corneum for extremely polar compounds and ions. When a permeating drug exits at the stratum corneum, it enters the wet cell mass of the epidermis and since the epidermis has no direct blood supply, the drug is forced to diffuse across it to reach the vasculature immediately beneath. Passage through the dermal region represents a final hurdle to systemic entry. This is so regardless of whether permeation is transepidermal or by a shunt route. Permeation through the dermis is through the interlocking channels of the ground substance. Diffusion through the dermis is facile and without molecular selectivity since gaps between the collagen fibers are far too wide to filter large molecules. Since the viable epidermis and dermis lack measure physiochemical distinction, they are generally considered as a single field of diffusion. Transfollicular absorption is the skin's appendages which offer only secondary avenues for permeation. Sebaceous and eccrine glands are the only appendages, which are seriously considered as shunts bypassing the stratum corneum since these are distributed over the entire body. However, the follicular route remains an important avenue for percutaneous absorption since the opening of the follicular pore, where the hair shaft exits the skin, is relatively large and sebum aids in diffusion of penetrants [50,51].

4.2.2 Dermal application of chitosan nano-/microparticles

The absorption-enhancing effects of chitosan and its derivatives have been intensively studied in recent years.

Chitosan is able to promote the dermal absorption of small polar molecules and peptide and protein drugs. The mechanism of action of chitosan in improving transport of drugs is thought to be a combination of bioadhesion and a transient opening of the tight junction in the cell membrane to allow polar drug to pass through. The effect of chitosan, most likely due to its positive charge, is able to interact with the opening mechanism of the tight junction of the dermal cells. On the other hand, its cationic polyelectrolyte nature provides a strong electrostatic interaction with negatively charged dermal cell surfaces and other macromolecules such as DNA [49,52]. Dermal applications also play a critical role in achieving genetic immunization since skin is a potent immunological site where it is fraught with antigen-presenting cells such as Langerhans cells and several methods including gene gun and needle-free devices and particulate systems have been utilized in delivery of pDNA. Previously, successful results have been obtained with chitosan particles for DNA delivery targeting Langerhans cells [53-57].

Lee *et al.* [55] investigated plasmid (EGFP) expression after administration of pDNA/chitosan and pDNA/chitosan/γ-glutamic acid nanoparticles to mice skin using low-pressure gene gun. The pDNA/chitosan/γ-glutamic acid particles were pH-sensitive and spherical shape with small particle size (146.0 – 174.5 nm), while pDNA/chitosan nanoparticles were of donut or rod like with heterogeneous size distribution (**Figure 3**). It was reported that EGFP expression was mainly localized to the suprabasal layers of epidermis after bombardment with pDNA/chitosan/γ-glutamic acid nanoparticles, while expression with pDNA/chitosan nanoparticles was limited to the superficial layer of epidermis. The pDNA/chitosan/γ-glutamic acid particles improved their penetration depth into the mouse skin and enhanced gene expression as compared with pDNA/CS nanoparticles in size of 229.3 nm.

In another study, gene expression efficiency of nanoscale DNA/chitosan complexes was tested in female C3H/HeN mice. The depth and expression levels of genes encoding either green fluorescent protein (GFP) reporter or JEV (Japanese encephalitis virus) E protein were measured in determining the delivery efficiency of the DNA/chitosan complex by the low pressure gene gun. The expression of GFP reporter gene was observable and traceable in epidermis and spleen for 3 days. The expressions of GFP and the activation of dendritic cells (DCs) were evident and co-localized in hair follicles and epidermis. The topical application of the DNA/chitosan complex on mice further demonstrated that the chitosan-based JEV DNA vaccine was capable of eliciting effective humoral immunity against the lethal dose challenge of JEV. It was concluded that transdermal immunization approach is a promising way in vaccination against microbial infections [56].

Skin delivery of antisense oligonucleotides has exciting potential in the treatment of skin diseases such as psoriasis and Behçet's disease [58]. However, the therapeutic application of oligonucleotides is limited due to the instability of these molecules against nucleases, the dependence of their skin permeability to multiple factors and insufficient cellular uptake. In a study, skin delivery ability of chitosan nanoparticles for antisense oligonucleotides in adult and juvenile rats was investigated and results showed that gene expression was successfully inhibited by antisense oligonucleotide-loaded nanoparticles in both group of rats (**Figure 4**) [58].

N-trimethyl chitosan (TMC) is a quaternized derivative of chitosan, bears a permanent positive charge and water-soluble over a wide pH range [59]. TMC nanoparticles have frequently been used for mucosal immunization [60,61]. TMC has been shown to possess mucoadhesive and absorption enhancing characteristics and when formulated with vaccine greatly enhanced the efficacy of the intradermal administered vaccine in mice [62]. Due to charge interactions, simple mixing of negatively charged virus vaccine [61] with TMC leads to positively charged particles. In order to further improve its adjuvant effect, the structural properties of TMC, like the degree of quaternization, the degree of O-methylation, and the degree of N-acetylation, can be varied during synthesis. On the other hand, TMC nanoparticles loaded with hemagluttinin has been shown to be very immunogenic in mice [63]. It has been suggested that only an antigen-presenting cells that has taken up the antigen and the adjuvant in significant amounts is able to activate T cells, whereas an antigen-presenting cells that has only taken up either of the two components does not stimulate T-cell proliferation [64]. Therefore, combination of the antigen and the adjuvant in one entity may be a good strategy for future vaccine development. Bal *et al.* [62] administered diphtheria toxoid-loaded TMC nanoparticles to female BALB/c mice skin using micro-needles for cutaneous immunization. TMC nanoparticles improved the immune response of diphtheria toxoid due to their adjuvanicity.

Another area of dermal administration of chitosan nano-/microparticles is in wound and burn treatment [65]. There are several studies investigating administration of different cytokines, which have important role in healing, to the wound site. Chitosan-gelatin microspheres (bFGF-MS) containing the basic fibroblast growth factor (bFGF), which is responsible for many cellular activities and angiogenesis, were prepared and then incorporated into the porous chitosan-gelatin scaffolds and their potential as a tissue engineering scaffold were investigated by cell culture studies with human fibroblast cell line. The scaffolds with bFGF-MS significantly augmented the cell proliferation and glycosaminoglycan synthesis and possessed a promising potential as a tissue engineering scaffold to improve skin regeneration efficacy and to promote vascularization [66].

Sezer *et al.* [67] fabricated fucoidan and chitosan-based microspheres (fucosphere) by polyion complexation method and investigated their treatment efficiency in rabbit burn model. The authors demonstrated that re-epithelialization was significantly faster in group treated with fucospheres compared to the negative control group. The nucleolar organizer regions (NOR) were numerically higher up to day

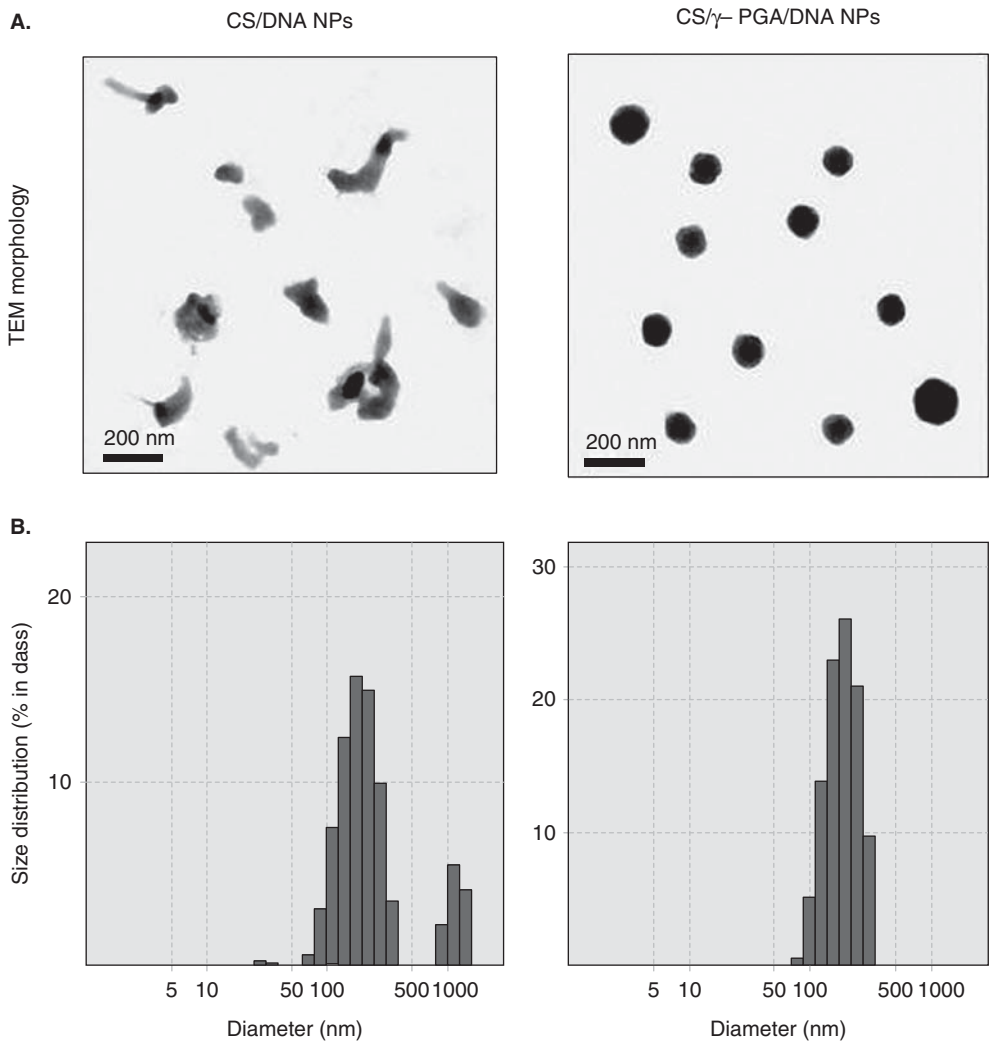

Figure 3. A. TEM micrographs of CS/DNA and CS/γ-PGA/DNA. **B.** Size distribution of CS/DNA and CS/γ-PGA/DNA obtained by dynamic light scattering.

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CS: Chitosan; γ-PGA: Poly-γ-glutamic acid.

14 for fucosphere-treated group, while increase of NORs was shown between days 14 and 21 in control groups. This indicated that proliferation of fibroblast cells in groups treated with fucospheres was faster up to day 14 with an earlier onset of healing process.

Administration of silver-containing formulations to the wound area to accelerate the healing process is a preferred approach. Silver nano-crystalline chitosan dressing (SNC), which is a nanoparticulate system, was developed for the treatment of wounds. The SNC dressing significantly increased the rate of wound healing and decreased the risk of silver absorption in comparison with silver sulfadiazine dressing. It was concluded that SNC would have extensive application in clinical settings [68].

Prolidase deficiency (PD) is a rare autosomal recessive disorder of the connective tissue, chronic in nature, progressive and debilitating due to the lack of prolidase. Previously, prolidase replacement therapy has been attempted for PD patients but this therapeutic approach has given only transitory effects caused by prolidase *in vivo* instability and its lacking cellular uptake [69]. In order to overcome these limitations, prolidase was encapsulated into the chitosan nanoparticles [70]. Then, fibroblasts from PD patients were incubated with prolidase-loaded chitosan particles and the enzymatic restored activity was determined by a capillary electrophoretic method. Encapsulation of the prolidase in chitosan nanoparticles protected the enzyme from degradation, provided the release of the enzyme in the active form into the cytoplasm and let to restore the prolidase activity into fibroblasts obtained from PD patients for a prolonged period of time [71].

Glucocorticoids are highly effective drugs widely used in dermatology for the treatment of inflammatory diseases.

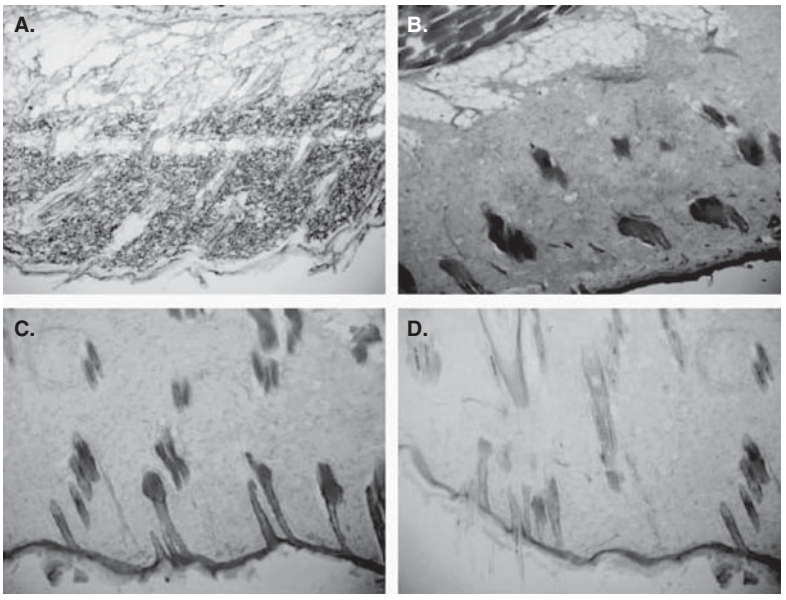

Figure 4. Histological photographs of the skin samples of adult rats stained with X-gal. A negative control. **B** Positive control [only applied pSV-b-Gal]. **C** Applied nanoparticles containing AsODNs [3 days]. **D** Applied nanoparticles with AsODNs [9 days].
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β-Gal: β-Galactosidase.

However, severe adverse effects often accompany their long-term use. Recently, studies have focused on strategies to optimize the potency of steroids while minimizing adverse effects due to drug absorption across the skin. Senyigit *et al.* showed that lecithin–chitosan nanoparticles increased the physical and chemical stability as well as the skin accumulation of clobetasol-17-propionate [72].

Chitosan–tripolyphosphate nanoparticles as a skin drug delivery system for an antiviral agent, acyclovir, were developed. The study demonstrated that incorporation of acyclovir into nanoparticles significantly improved its chemical stability and permeation of drug through porcine skin depended on particle size and chitosan content [73].

On the other hand, combinations of chitosan with different polymers such as lecithin, glutamic acid have frequently been studied for skin drug delivery [72,74,75]. Hafner *et al.* [75] prepared lecithin/chitosan nanoparticles as colloidal nanosystem for transdermal melatonin delivery. The particle size and zeta-potential values of nanoparticles were changed between 113.7 and 331.5 nm and between 4.6 and 31.2 mV, respectively, due to the type of lecithin (Lipoid S45 and S100) used to fabricate nanoparticles. The potential of lecithin/chitosan nanoparticles to enhance transdermal melatonin delivery was investigated by determining the drug flux across porcine skin and its skin deposition. Lecithin/chitosan nanoparticles provided 1.3 – 2.3-fold higher flux compared to melatonin solution. Possible cytotoxicity of nanoparticles was evaluated using human skin keratinocytes and fibroblasts. It was demonstrated that lecithin/chitosan nanoparticles can be applied to skin cells at concentrations up to 200 μg/mL without inducing plasma membrane damage or cell viability decrease.

The microparticulate systems can penetrate into skin via transfollicular route due to the size and surface characteristics of particles. Many researchers focus on this issue for increasing the penetration of microparticulate system through the skin and the drug bioavailability. Gelfuso *et al.* [76] fabricated porous chitosan microparticles, in a size of 3 μm, containing minoxidil sulfate (MXS) at different MXS/chitosan ratios and evaluated their efficacy for the treatment of alopecia. The results showed that chitosan microparticles had a potential to improve topical therapy of alopecia with minoxidil.

Cosmetic applications of chitosan particles create a new area. To this end, mPEG-phthaloylchitosan derivative and mPEG-phthaloylchitosan derivative grafted with a UVB absorptive chromophore, the 4-methoxycinnamoyl group, have been synthesized and UV-screening nanoparticles containing a model drug, 2-ethylhexyl-4-methoxycinnamate, have been fabricated with these polymers [77]. In another study, a microparticulate delivery system incorporating the hydrophilic sunscreen agent, phenylbenzimidazole sulfonic acid was prepared for better in-use performance. Incorporation of the sunscreen in chitosan microparticles improved its *in vitro* UV screening effect [78].

Several formulations incorporating chitosan microparticles were also tested in skin care and acne management. Retinoic acid, an analogue of vitamin A, is an agent used in acne treatment. Unfortunately, local irritation reactions strongly limit its topical use [79]. Lira *et al.* investigated the efficacy of chitosan-treated alginate microparticles in acne treatment

and showed that microparticles sustained the release of retinoic acid and improved the topical delivery of drug since microparticles delivered retinoic acid preferentially in deeper layers of the skin [80].

Chitosan-coated liposomes and microspheres containing glycolic acid were also used in cosmetics as exfoliant and moisturizer [81].

Catechins are major antioxidants in green tea and cannot penetrate into the skin. Wisuitiprot *et al.* [82] investigated the influence of chitosan microparticles to cutaneous absorption of catechins and showed that chitosan microparticles significantly improved the skin permeation of catechins.

4.3 Nasal drug delivery

4.3.1 *Barriers to nasal drug delivery*

The nasal mucosa has been a potential administration route for the low molecular weight drugs to achieve high level of drug absorption due to the large surface area, rapid blood flow, porous and thin endothelial membrane of nasal epithelium and the avoidance of first-pass metabolism [83].

Despite the many advantages, some limitations may prevent drug absorption through the nasal mucosa [84]. Among the major disadvantages of nasal route are the limited application volume (max. 250 µL), the difficulty of high molecular weight drugs (HMW) (> 1000 Da) to pass through the nasal mucosa, the presence of pathological conditions, mucociliary clearance, enzymatic barriers and irritation of nasal mucosa [84]. The nasal administration of HMW drugs including peptides, proteins, and vaccines for systemic medication has been widely investigated in last decade. Due to the hydrophilic structure of protein and peptide, their nasal bioavailability is generally less than 1%. Besides their week mucosal membrane permeability and enzymatic degradation in nasal mucosa, protein and peptide drugs are rapidly cleared from the nasal cavity due to the mucociliary clearance [13,83].

Recent studies focus on the anatomical and physiological aspects of the nasal membrane, including its vascular nature, as they relate to drug delivery. On the other hand, many attempts have been made in the recent past to increase the residence time of drug formulations in the nasal cavity, resulting in improved nasal drug absorption. Researchers became interested in the nasal route for the systemic delivery of medication due to high degree of vascularization and permeability of the nasal mucosa [13,84].

4.3.2 *Nasal application of chitosan nano-/microparticles*

Particulate systems delivering the drug via the nasal route have become a focus of investigations lately [85]. In particular, administration of nano-/microparticles prepared with chitosan, a polycationic biopolymer, to the nasal region resulted in successful outcomes with several types of drugs. Protein and peptide drugs, particularly insulin, were administered to the nasal cavity benefiting from the mucoadhesive property of chitosan.

In different studies, insulin was encapsulated in nanoparticles fabricated with modified chitosans such as PEG–chitosan, thiolated chitosan and N-trimethyl chitosan to enhance the bioavailability of the drug [86,87]. *In vivo* studies concluded that insulin, which was encapsulated in the PEG–chitosan nanoparticles, was predominantly absorbed from nasal cavity. PEG–chitosan nanoparticles improved the nasal absorption of insulin compared with insulin–PEG–chitosan suspension and insulin solution (**Figure 5**). The changes in the composition and molecular weight of polymer clearly influenced the nasal absorption of insulin (**Figure 5**) [88].

Nasal route has many advantages for either local or systemic drug delivery. Low molecular mass drugs are rapidly absorbed through the nasal mucosa. The main reasons of this are wide absorption area, high permeability, porous, and thin endothelial basement membrane of the nasal epithelium. For these reasons, intranasal delivery is an alternative route for low molecular mass drugs such as nonsteroidal anti-inflammatory drugs [89,90] macrolide antibiotics [91], and beta-blockers [92].

Sun *et al.* [93] investigated the effect of chitosan molecular mass on the drug delivery in nasal route. The researchers tested low (LMW, 40,000 Da), medium (MMW, 480,000 Da) and high- (HMW, 850,000 Da) molecular mass chitosans with the same degree of deacetylation (96%). The molecular mass of chitosan noticeably influenced the micromeritic properties, size and distribution, encapsulation efficiency, controlled release behavior, and mucoadhesive properties of methotrexate-loaded microspheres. Nasal ciliatoxicity studies indicated only minor cilia irritation after microsphere application and it was found that the particles were suitable for nasal drug delivery.

Nagda *et al.* [89,90] developed mucoadhesive chitosan microspheres containing ketorolac for nasal administration and observed any severe damage on the integrity of nasal mucosa in *ex vivo* studies.

On the other hand, intranasal delivery is an alternative method for targeting therapeutics to the central nervous system. In a present study, the influence of chitosan glutamate-based mucoadhesive microspheres on rokitamycin absorption into the bloodstream and cerebrospinal fluid was evaluated after their nasal administration in rats. The *in vivo* results obtained after nasal administration of chitosan microspheres were compared with those of intravenous administration of free drug. *In vivo* studies showed that, after intravenous administration, rokitamycin could not cross the blood–brain barrier and reach to cerebrospinal fluid from the bloodstream. On the contrary, drug was transported into cerebrospinal fluid and the bloodstream only after nasal administration of chitosan glutamate microparticles [91].

Nanoparticles prepared with modified chitosans such as thiolated chitosan and N-trimethyl chitosan (TMC) were used for nasal application of drugs and vaccines [94-97]. It was reported that TMC nanoparticles were frequently used for nasal immunization and increased the immunogenicity of

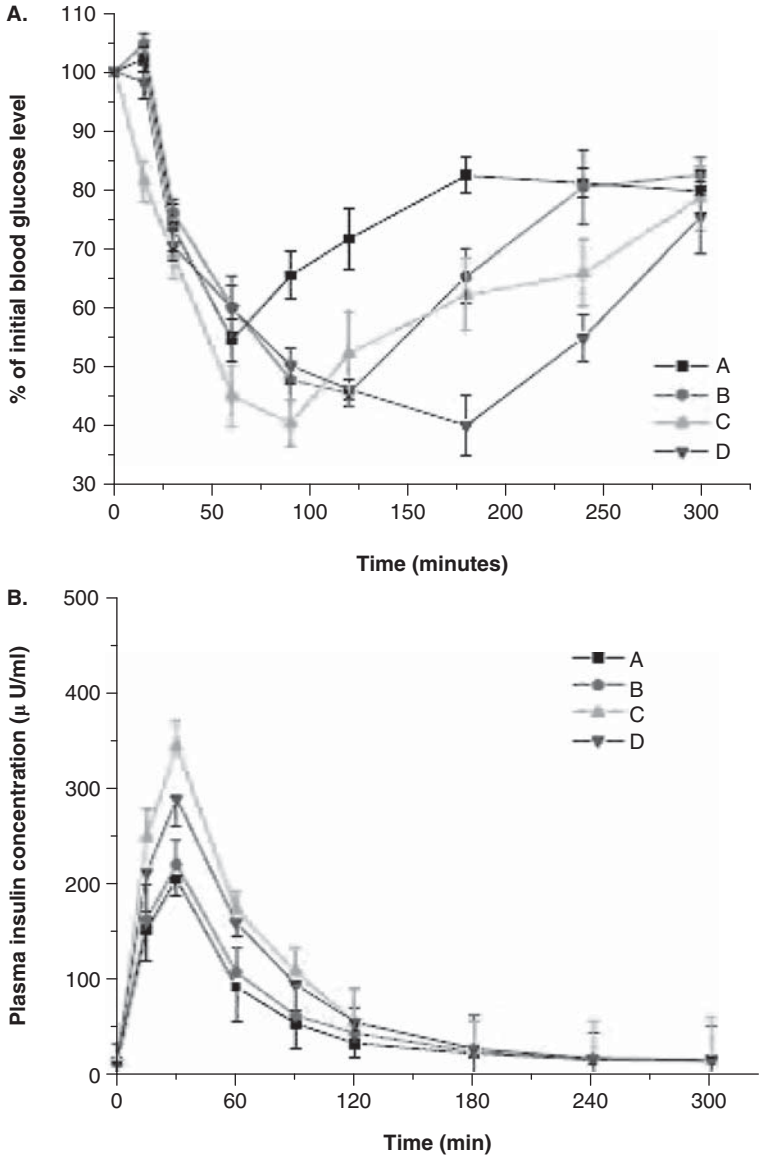

Figure 5. *In vivo* study. A Plasma glucose levels in rabbits following nasal administration (at pH 7.4) of insulin-loaded PEG-g-chitosan nanoparticles suspended in PBS: A, PE3gC24 prepared with chitosan 20.000 Da and PEG 350 with the molar ratio of 0.4; B, PE3gC28 prepared with chitosan 20.000 Da and PEG 350 with the molar ratio of 0.8; C, PE3gC64 prepared with chitosan 6.000 Da and PEG 350 with the molar ratio of 0.4; D, PE7gC64 prepared with chitosan 6.000 Da and PEG 750 with the molar ratio of 0.4. **B** Plasma insulin concentration after administration of insulin-loaded PEG-g-chitosan nanoparticles: A, PE3gC24; B, PE3gC28; C, PE3gC64; D, PE7gC64. Data are the mean ± SD (n = 6).
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subunit antigens [96]. In a study, the capability of a thiolated chitosan derivative as nasal delivery system for insulin in comparison to a delivery system based on unmodified chitosan was investigated and thiolated chitosan nanoparticles resulted in a significantly higher bioavailability than those comprising unmodified chitosan [97]. On the other hand, thiolated chitosan (chitosan-thioglycolic acid) and unmodified chitosan nanoparticles (NPs) containing leuprolide were developed and their nasal bioavailability was evaluated. Leuprolide was released over 6 h from thiolated NPs, which might be attributed to inter- and/or intramolecular disulfide formation within the NPs network. Unmodified NPs and thiolated NPs provoked increased leuprolide transport through porcine nasal mucosa by 2.0- and 5.2-folds, respectively, when compared with leuprolide solution. While the relative nasal bioavailability of leuprolide from thiolated NPs *vs* subcutaneous injection calculated on the basis of AUC_{0-6} was 19.6%, for leuprolide solution was 2.8% [95].

Hyaluronic acid (HA) is the most popular biopolymer, used in combination with chitosan derivatives for mucosal applications. Ovalbumin-loaded stabilized TMC-HA particles demonstrated superior immunogenicity [94]. Nano-/microparticles of chitosan and its derivatives were also investigated to enhance the nasal absorption of antiemetic drugs including metoclopramide hydrochloride [98,99], ondansetron hydrochloride [100], antiasthmatic drugs such as salbutamol sulfate [101], antiepileptic drugs such as carbamazepine [102], antihypertensive drugs such as carvedilol [103], antibiotics including rokitamycin [91] and gentamicin sulfate [104].

Chitosan is one of the most extensively studied vaccine carriers. Its absorption-promoting effect is believed to improve mucosal immune response. Chitosan acts as an adjuvant for systemic vaccine delivery [84]. A chitosan-based DNA flu vaccine was developed by Illum *et al.* that showed high antibody level in mice after intranasal administration [105]. Chitosan was shown to form colloidal particles and entrap macromolecules through a number of mechanisms, including ionic cross-linking, desolvation, or ionic complexation, though some of these systems have been realized only in conjunction with DNA molecules. An alternative involving the chemical modification of chitosan has also been useful for the association of macromolecules with self-assemblies and vesicles. Up-to-date, the *in vivo* efficacy of these chitosan-based colloidal carriers has been reported for two different applications: while DNA–chitosan hybrid nanospheres were found to be acceptable transfection carriers, ionically cross-linked chitosan nanoparticles appeared to be efficient vehicles for the transport of peptides across the nasal mucosa [98,106]. A study introduced a chitosan-based siRNA nanoparticle delivery system, with the formation of interpolyelectrolyte complexes between siRNA duplexes (21-mers) and chitosan, for RNA interference *in vitro* and *in vivo*. The particle size changed between 40 and 600 nm. Rapid uptake (1 h) of Cy5-labeled nanoparticles into NIH 3T3 cells, followed by accumulation over a 24 h period, was visualized using fluorescence microscopy. Nanoparticle-mediated knockdown of endogenous enhanced green fluorescent protein (EGFP) was demonstrated in both H1299 human lung carcinoma cells and murine peritoneal macrophages (77.9% and 89.3% reduction in EGFP fluorescence, respectively). Effective *in vivo* RNA interference was achieved in bronchiole epithelial cells of transgenic EGFP mice after nasal administration of chitosan/siRNA formulations (37% and 43% reduction compared to mismatch and untreated control, respectively). These findings highlighted the potential application of this novel chitosan-based system in RNA-mediated therapy of systemic and mucosal disease [105].

Mangal *et al.* [107] prepared TMC nanoparticles and investigated the adjuvant properties of the polymer. The authors synthesized TMCs using mild (TMC-M) and conventional (TMC) methods and evaluated their efficacy as nasal vaccine delivery vehicles. TMC-M nanoparticles showed the lowest nasal clearance rate when compared with chitosan (CS) and TMC nanoparticles. The immunogenicity of nanoparticle-based delivery system(s) was assessed by measuring anti-HBsAg antibody titer in mice serum and nasal secretions after intranasal administration. Results indicated that alum-based HBsAg induced strong humoral but negligible mucosal immunity. However, TMC-M nanoparticles induced stronger immune response at both of the fronts as compared to generated by CS or TMC nanoparticles. This study demonstrated that TMC-M could be a better carrier adjuvant for nasal subunit vaccines.

Atherosclerosis is considered to be one of the most common causes of morbidity and mortality in cardiovascular patients all over the world. Cholesteryl ester transfer protein (CETP) plays an important role in lipid metabolism [108]. When CETP is overexpressed or its enzymatic activity is excessively high, it may lead to atherosclerosis by decreasing high-density lipoprotein cholesterol (HDL-C) and increasing low-density lipoprotein cholesterol (LDL-C). The inhibition of CETP activity by vaccine-induced antibodies to modulate the lipoprotein profile is considered to be a promising way against atherosclerosis [109]. In a study, the plasmid pCR-X8-HBc-CETP (pCETP)-loaded nanoparticles were prepared to elicit serum anti-CETP IgG antibodies, to modulate the plasma lipoprotein profile, and to attenuate the development of atherosclerosis after intranasal administration in the cholesterol-fed rabbits. Intranasal administration of nanoparticles significantly increased the antiatherogenic HDL-C/TC and reduced the proatherogenic LDL-C/TC, which is beneficial to attenuate the progression of atherosclerosis (**Table 1**). The authors indicated that nasal vaccination with nanoparticles could be a convenient and non-invasive route for the delivery of DNA vaccines against atherosclerosis [109].

Encapsulation of different active agents including ((RS)-1-benzyl-4-[5,6-dimethoxy-1-indanone)-2-yl]-methylpiperidine [110], 17β-estradiol [111], N^6-cyclopentyladenosine [112], which are not absorbed into the brain from the bloodstream, into the chitosan nanoparticles improved the amount of drug transported into central nervous system.

Intranasal administration is a potential route to enhance systemic and brain delivery. Chitosan has been utilized to improve the brain targeting efficiency by the direct nose to brain pathway especially for drugs for treatment of central nervous system disorders. Furthermore, it was used to combine the active drug for targeting to the olfactory region with controlled release bioadhesive characteristics for maintaining the drug on the absorption site [113-115].

Didanosine, an HIV reverse transcriptase inhibitor, exhibits several delivery-related disadvantages which contribute to a significant clinical problems including low and highly variable bioavailability after oral administration and limited central nervous system (CSF) penetration [116]. Application of didanosine-loaded chitosan nanoparticles significantly enhanced the systemic absorption of the drug from the nasal cavity and its concentration in brain tissue, olfactory bulb and CSF was relatively higher than that of intravenous administration of didanosine solution [117].

Table 1. Effects of intranasal immunization with chitosan/pCETP nanoparticles or intramuscular immunization with pCETP solution on plasma lipids and aortic lesions in cholesterol-fed rabbits (n = 8).

	Weeks	Normal	Saline control	CS/PCETP/i.n.	PCETP solution/i.m.
TC (mM)	1	1.38 ± 0.56	1.55 ± 0.39	1.42 ± 0.67	1.34 ± 0.21
	28	1.42 ± 0.22	47.77 ± 6.96[a]	27.21 ± 12.88[b,c]	19.10 ± 3.29[b]
HDL-C (mM)	1	0.32 ± 0.19	0.38 ± 0.33	0.41 ± 0.16	0.35 ± 0.24
	28	0.35 ± 0.18	7.83 ± 2.54[a]	7.82 ± 2.27[d,c]	6.18 ± 1.22[d]
LDL-C (mM)	1	1.16 ± 0.27	1.23 ± 0.65	1.08 ± 0.53	1.04 ± 0.43
	28	1.15 ± 0.28	33.32 ± 5.56[a]	15.74 ± 7.46[e,c]	11.84 ± 2.82[b]
HDL-C/TC (%)	28	24.73 ± 10.95	16.56 ± 5.75[a]	32.84 ± 13.07[e,c]	32.46 ± 4.16[b]
LDL-C/TC(%)	28	74.94 ± 5.62	69.93 ± 8.08[a]	59.64 ±13.54[d,c]	61.70 ± 6.16[d]
Atherogenic index	28	3.69 ± 1.89	5.95 ± 3.10[f]	2.39 ± 0.11[e,c]	2.12 ± 0.42[e]
Atheromatous area (%)	28	-	71.0 ± 14.4	29.0 ± 10.9[b,c]	21.2 ± 14.4[b]

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Values are mean ± S.D. (n = 8). Differences (t-test) are indicated as: [a]$p < 0.05$ *vs* normal group; [b]$p < 0.01$ *vs* saline control group; [c]$p > 0.05$ *vs* i.m. group; [d]$p > 0.05$ *vs* saline control group; [e]$p < 0.05$ *vs* saline control group; [f]$p > 0.05$ *vs* normal group.

Atherogenic index: Ratio of non-HDL-C to HDL-C; CS/pCETP NP: Chitosan/pCETP nanoparticles; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol.

4.4 Buccal drug delivery

4.4.1 Barriers to buccal drug delivery

Most biological surfaces of interest for drug administration are mucosal surfaces and these are generally covered with a layer of mucus [118]. For drug administration, the first and the biggest barrier in buccal area is the mucus layer. On the other hand, controlled or sustained release of the drug is not achieved as the drug resides for short time periods. Moreover, low permeability of the buccal membrane, smaller surface area and the continuous secretion of saliva leading to subsequent dilution of the drugs are the another disadvantages of the buccal application [119,120]. The buccal mucosa acts as a barrier for macromolecular drugs such as peptide and protein because of the tight junction of the epithelial cells of the surface [121].

4.4.2 Buccal application of chitosan nano-/microparticles

Mucoadhesive properties of polymers are highly important point for mucosal delivery of active agents. Chitosan is a positively charged natural mucoadhesive biopolymer with permeability enhancement properties, has been widely used for mucosal drug delivery. These properties of chitosan justify its use for overcoming the reduced efficacy of conventional treatments of oral diseases. Various tests simulating the buccal environment have described controlled drug release profile and significant activity against buccal pathogens by chitosan nano-/microparticles entrapped antimicrobial agents [122,123]. On the other hand, early diagnosis and treatment are vital in management of serious conditions such as several types of cancer and in preventing metastasis. In this context, buccal application of bioadhesive particulate systems provides high absorption potential, avoids hepatic first-pass effect, protects from degradation of several gastrointestinal enzymes and offers ease of application [121,124].

In a study, the penetration enhancement property of chitosan hydrochloride (HCS) both as a polymeric solution and as a nanoparticulate system was compared with that of TMC hydrochloride solution on buccal mucosa. Fluorescein isothiocyanate dextran with a molecular weight of 4.4 kDa was used as a macromolecular hydrophilic drug. Absorption properties of the formulations were investigated on pig buccal mucosa and porcine buccal tissue. Morphological analysis suggested a similar mechanism of penetration enhancement for both HCS and TMC solutions and for HCS nanoparticles. The authors indicated that such a mechanism probably involved a repackaging of the epithelial cells up to the basal membrane and a partial disarrangement of desmosomes. TMC solution and HCS nanoparticles were able to increase dextran permeation across buccal epithelium to a greater extent than the HCS solution [125].

Giunchedi *et al.* [126] developed the buccal tablets based on chitosan microspheres, which were prepared by spray-drying method, containing chlorhexidine diacetate. The antimicrobial activity of drug-loaded, drug-empty microparticles, and chlorhexidine (as powder) was evaluated. The results of microbiological tests, expressed as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), are reported in **Table 2**. Against the microbial strains, chlorhexidine as powder exhibited MIC values that range between 0.9 and 21.9 mg ml^{-1} and MBC values that range between 3.9 and 125 µg ml^{-1}. After buccal administration of the tablets, chlorhexidine concentration in saliva was determined. The results showed that the tablet formulations prolonged the release of the drug in the buccal cavity and had more potential for intraoral drug delivery than those of free chlorhexidine and microspheres alone [126].

Similar results were obtained by Yedurkar *et al.* [123]. A multiple-unit system comprising mucoadhesive bilayer buccal tablets of carvedilol-loaded chitosan microspheres (CMs) was prepared to improve therapeutic efficacy of the drug. CMs were compressed into bilayer tablets. Optimized mucoadhesive bilayer buccal tablet formulation showed maximum

Table 2. MIC and MBC values expressed as μg ml^{-1} of chlorhexidine (powder) or of spray-dried microspheres (n = 3; SD within approximately 3%).

Microbial strains	Chlorhexidine (powder)	Drug-empty microspheres (Spray-dried chitosan)	Microspheres with a chlorhexidine-to-chitosan ratio of 1:2 (batch A)	Microspheres with a chlorhexidine-to-chitosan ratio of 1:4 (batch B)
Staphylococcus aureus	0.9 (3.9)	208 (1000)	2.4 (13.0)	3.9 (7.8)
Escherichia coli	1.9 (15.6)	1000 (> 1000)	6.2 (11.7)	7.8 (31.2)
Pseudomonas aeruginosa	21.9 (125)	1000 (> 1000)	62.5 (1000)	93.8 (> 1000)
Candida albicans	7.8 (7.8)	1000 (1000)	15.6 (15.6)	31.3 (31.3)

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mucoadhesive force (50 ± 1.84 dyne/cm^2), and demonstrated zero-order kinetics with non-Fickian release mechanism. Pharmacokinetic studies in rabbits showed significantly higher C_{max} (71.26 ± 6.45 ng/mL), $AUC_{0\text{-}10}$ (390.75 ± 5.23 ng/mL/h), and $AUC_{0\text{-}\infty}$ (664.72 ng/mL/h) for mucoadhesive bilayer buccal tablets than those of conventional carvedilol oral tablet. Multiple-unit system exhibited enhanced bioavailability and sustained release of carvedilol, indicating its improved therapeutic potential for the treatment of hypertension.

Mazzarino *et al.* [120] designed new mucoadhesive nanoparticles prepared with blend of polycaprolactone and chitosan for buccal delivery of curcumin. Particles were prepared by nanoprecipitation method and then coated with different molar masses and concentrations of chitosan. Mean diameter of nanoparticles was changed between 114 and 125 nm with positive surface charge confirming the decoration of the nanoparticles with the mucoadhesive chitosan. The particles have obtained high encapsulation efficiency and showed a great ability to interact with mucin indicating also their suitability for buccal applications.

4.5 Periodontal drug delivery

4.5.1 Barriers to periodontal drug delivery

Periodontal region covers the soft (periodontal ligament) and hard tissues (bones) surrounding the teeth. The teeth are not directly attached to the bone in the alveolar protrusion they reside [127]. A tissue of fibers called the periodontal ligament lies between the teeth and bone. This structure is covered with the gingiva. There is approximately 1 – 1.5 mm space between the teeth and the gingiva, which is called the "periodontal pocket" and many medicines to treat dental conditions are administered usually from this site. Absorption of active agents in a dosage form is rapid from periodontal pocket bypassing the hepatic first pass clearance. It is also convenient route for local treatment of gingival diseases such as gingivitis and periodontitis [127-129].

4.5.2 Periodontal application of chitosan nano-/microparticles

Efficacy of chitosan microspheres containing tetracycline in dental infections and periodontal diseases was investigated in many previous studies [127,128]. Chitosan sponges cross-linked with tripolyphosphate were formulated with tetracycline and *in vitro* characterization studies were carried out. The antibacterial efficacy of sponge formulation against *Staphylococcus aureus* and *Escherichia coli* was found to be higher than that of free drug and it was concluded that chitosan sponges was suitable as a slow-release device for tetracycline for periodontal applications [129].

Periodontal ligament cells play a crucial role in the regeneration of periodontal tissues and an undifferentiated mesenchymal cell subset is thought to exist within this population. Inanc *et al.* [130] assessed the osteogenic differentiation potential of human periodontal ligament fibroblasts (hPDLFs) in a three-dimensional osteogenic culture environment following encapsulation in chitosan–hydroxyapatite (C/HA) microspheres in a size range of 350–450 μm. The results suggested that hPDLFs content of C/HA microspheres could be induced to differentiate into osteogenic cells with high efficiency in cell culture conditions. It was concluded that the cell-containing microspheres might serve as a suitable model for developing strategies for periodontal tissue engineering applications.

Recently, much attention has been paid to tissue engineering and local gene delivery systems in periodontal tissue regeneration. Gene-activated matrix (GAM) blends these two strategies, serving as a local bioreactor with therapeutic gene expression and providing a structural template to fill the lesion defects for cell adhesion, proliferation and synthesis of extracellular matrix. A novel GAM with embedded chitosan/plasmid nanoparticles encoding platelet-derived growth factor (PDGF) was designed based on porous chitosan/collagen composite scaffold. The chitosan/collagen scaffold acted as a three-dimensional carrier and condensation of chitosan with plasmid DNA formed nanoparticles. The plasmid DNA entrapped in the scaffolds showed a sustained and steady release over 6 weeks and was effectively protected from degradation by chitosan nanoparticles. Cytotoxicity studies demonstrated that periodontal ligament cells (PDLCs) cultured into the novel GAM achieved high proliferation. The histological results confirmed that PDLCs maintained a fibroblast shape and the periodontal connective tissue-like structure formed in the scaffolds after 2 weeks. It was

concluded that the novel GAM had potential in the application of periodontal tissue engineering [131].

5. Conclusion

Chitosan is a versatile biofunctional polymer with which controlled release of administered drug can be achieved. Diverse chitosan nano-/microspheres prepared by different methods prolong the residence time of drug at the site and so enhance its therapeutic effect, show excellent physical, chemical and biological properties and many different therapeutic applications for topical application. The potential role of size and surface modification cannot be ignored during designing formulations for topical use of chitosan nano-/microspheres. All these capabilities of chitosan and its nano- and microsphere systems suggest that this biopolymer has a very bright future in the field of topical drug delivery.

6. Expert opinion

In this section, important points are summarized.

1) Purpose of topical use of chitosan particles. Various efforts in topical drug delivery have been made to improve the bioavailability and to prolong the residence time of drugs applied topically. Besides its low toxicity and good topical tolerance, chitosan exhibits favorable biological behavior, such as bioadhesion and permeability-enhancing properties, and also interesting physico-chemical characteristics, which make it a unique material for the design of topical drug delivery vehicles. On the other hand, these particles can be rapidly fabricated under extremely mild conditions with their ability to incorporate bioactive compounds used topically.
2) Therapeutic significance in chitosan particles' topical delivery. Chitosan particulate drug carrier system may be applied in liquid form like eye drop solutions, where upon their interaction with the glycoprotein of the cornea and conjunctiva can form a precorneal depot resulting in prolonged release of the bound drug. Nano- and microparticle-based topical drug delivery is also very efficient in crossing membrane barriers, such as the blood–retinal barrier in the eye, tight junctions in dermal surfaces, etc. Topical drug delivery based on chitosan particles can function as excellent systems for chronic local diseases requiring frequent drug administration, for example in ophthalmic diseases like chronic cytomegalovirus retinitis, psoriasis.
3) Future of the chitosan particles in topical uses. Micro- and nanoparticles for topical application are presently being researched based grossly on nanotechnology in which drugs can be administered on all topical areas. Also poorly water-soluble or insoluble drugs can be successfully prepared as effective systems to provide easy administration to buccal, ocular, dermal and nasal tissues. Particulate systems provide great convenience to the patient for adjusting the drug dose and frequency. Chitosan can be combined with drugs in such a way that the drug is released in spesific area in a very precise and controlled manner. At present, clinical results proved the effectiveness of chitosan particles in the treatment as compared with other topical drug carrier systems. These promising results require further investigation regarding topical usage of chitosan particles and these approaches await validation of drug release for topical applications and most probably a combination of technologies holds the key to success in the near future.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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