Asian Journal of Chemistry; Vol. 34, No. 4 (2022), 787-792

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2022.23548

MINI REVIEW

A Short Review on Recent Applications of Chitosan Biopolymer in Gene and Drug Delivery

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Received: 26 September 2021; *Accepted*: 30 November 2021; *Published online*: 10 March 2022; AJC-20718

The battle of the human race with genetic disorders was prevailing from the time immemorial. Revolutions and modernizations in science and technology have clearly improved our understanding regarding the molecular basis of genetic disorders thereby aiding in designing new therapeutic interventions in their treatment and prevention. With the advent and development of gene therapy in the last few decades, promising windows were opened for treatment and prevention of genetic disorders and cancer. Despite of its remarkable significance in medicine, the common practice of using viral vectors as gene delivery agents has created controversies and concerns among the scientific community. This made the research focus on biobased polymers as alternative non-viral vector systems for gene and drug delivery for treating genetic disorders and cancer. Chitosan is a cationic polymer that can be easily tailored to serve as gene and drug delivery due to their biocompatibility and biodegradability. Their structural integrity and stability have made them widely used for various applications in the biomedical field. Chitosan and its derivatives have gained more attention as vectors for gene delivery and cancer therapy in the past decade. The amenability of structural modification, non-toxicity and high biodegradability of chitosan derivatives can make them prospective carriers for controlled drug delivery in future.

Keywords: Chitosan, Gene therapy, Drug delivery, Biopolymers, Gene delivery, Vectors.

INTRODUCTION

Chitosan is a commercially important biopolymer with a wide variety of industrial applications especially in pharmaceutical industries. The structural integrity and stability of the compound has attracted many scientists for its application in medical fields such as wound healing, drugs and gene delivery [1]. One of the most important aspects of gene delivery is the use of a carrier compound that when incorporated in the body can be targeted properly and can endure enzymatic degradation [2]. Many substances are available today for the gene delivery application but its instability and undesirable efficiency causes other side effects in the body. Chitosan has been studied and reported in various reports as a potential drug delivery device due to properties of controlled drug release, mucoadhesion, antimicrobial features and more [3].

The use of different polymer materials like hydrogels have also been experimented with chitosan as a scaffold material for the purpose of drug delivery. Both invasive and non-invasive routes are used for the drug administration [4]. The advantages and disadvantages of different routes of administration of chitosan are described in Table-1. Scientists have attempted the use of chitosan nanoparticles for improving efficiency in the gene delivery methodology. Using the biodegradable property of chitosan, the drugs are encapsulated by chitosan nanoparticles which degraded later to slowly release the drug into the system can be seen in Fig. 1.

Ameeduzzafar *et al.* [5] in their study have used chitosan nanoparticles (CH-NPs) for encapsulating levofloxacin, which acts as an antibacterial agent for the treatment of ocular infection. The study concluded that chitosan was a potential carrier for the antibacterial drug delivery [5]. Marciello *et al.* [6] used chitosan-based nanoparticles for its application as vaginal drug delivery for the purpose of peptide based vaccines in order to treat different sexually transmitted diseases. The study was able to prove the successful delivery of mucoadhesive nanoparticles and peptides using chitosan nanoparticles. Another study used the natural polymer, chitosan for the delivery of curcumin targeted therapy and controlled delivery [7]. The use of chitosan due to its bio-compatibility has made

TABLE-1
ADVANTAGES AND DISADVANTAGES OF DIFFERENT ROUTES FOR ADMINISTRATION OF CHITOSAN

Chitosan drug delivery systems	Advantages	Disadvantages	Ref.
Oral drug delivery	Improves patient comfort level, compliances and provides flexibility of accommodating various formulations.	Difficulty in surviving different gastro-intestinal tract secretions such as various degrading enzymes and different pH conditions.	[11]
Gastric-specific drug delivery	Shows resistance to acidic environments and does not cause irritation in the stomach.	Poor absorption due to physiological adversities such as short gastric residence time and unpredictable gastric emptying time.	[12]
Colon-specific drug delivery	Near neutral pH, poor activity of digestive enzyme and extended transit time	Susceptible to bacterial enzymes present in colon which breaks the glycosidic linkage of chitosan	[13]
Buccal drug delivery	High bioavailability because the area is extremely vascularized and avoidance of first-pass metabolism, lesser enzymatic activity and mucosa layer is comparatively immobile.	Barrier property of buccal mucosa, accidental swallowing of delivery system, restriction of eating and drinking and salivary scavenging due to enzymes.	[14]
Ocular drug delivery	Prolong the retention, improve biodistribution and therapeutic efficacy applied onto the eye surface.	Achieve desired drug concentration in both anterior and posterior segments of eye because of many factors including nasolacrimal drainage, tear turnover, poor permeation across the cornea and reflex blinking	[15]
Nasal drug delivery	Thin and well-vascularized mucosa, elimination of needle phobia attached with parenteral route and possible self-administration leading patient convenience	Nasal obstacles such as low membrane permeability, a short local residence time and high turnover rate of a secretion in nasal cavities, the bioavailability of nasally administered drugs is often comparatively poor	[16]
Vaginal drug delivery	Avoidance of presystemic metabolism, rich vascularization, poor enzymatic activity, obvious large surface area and absence of gastrointestinal irritation and side effects. In addition, it provides scope for self-administration.	Limited acceptance, limited to specific gender, irritation and hygiene problem in patient and lesser bioavailability.	[17]
Rectal drug delivery	Effective in cases where the patient cannot take oral route, nausea, irritation and vomiting	Lesser motility while sitting and sleeping makes absorption difficult. Absence of villi or microvilli decreases surface area for absorption.	[18]

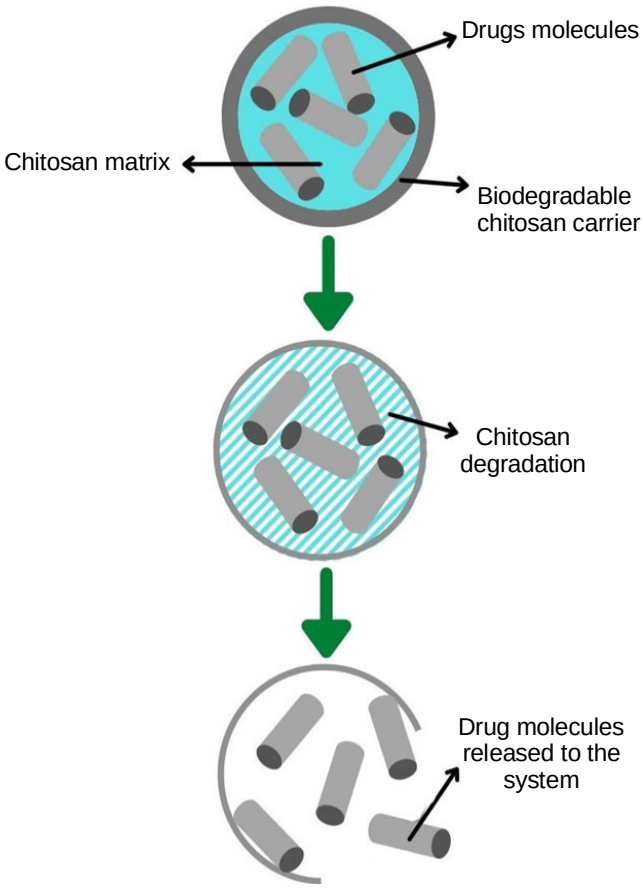

Fig. 1. Drug delivery using chitosan as carrier polymer

it an ideal substance for cancer therapy treatments. Studies have been conducted for evaluating the efficiency of chitosan-based systems for controlled drug release in cancer and tumor therapy [8,9]. Chitosan is highly modified based on its use and requirement in order to make it more compatible for specific gene delivery. Boonthum *et al.* [10] showed the use of modified chitosan for its application as a gene delivery agent to gonadotropin-releasing hormone receptor (GnRHR)-expressing cells. The GnRH-conjugated chitosan (GnRH-CS) showed positive results of higher transfection activity in the cells.

In several diseases, gene therapy is a promising way of treatment and prevention over the conventional methods of treatment [19]. Gene therapy is effective in cancer treatment, cardiovascular, viral infections and genetic diseases, as they targets to repair or replace the direct origin of genetic diseases through insertion of nucleic acid polymers to patient cells aiming to cure the cause of the disease instead of curing the symptoms [20]. Gene replacement therapy, gene editing, genetically engineered T cell therapies and gene modulation through the use of RNA interference (RNAi) are the 4 major categories of gene therapy [21]. Gene replacement therapy was first discovered in the 1970's [22]. For delivering the genetic material viral vectors are one of the most effective means as they are naturally evolved for such purposes and some viruses such as herpes simplex virus (HSV), retro- or lenti-virus, adenovirus and adeno associated virus (AAV) are engineered for the goal of therapeutic gene delivery [23]. Even though viral vectors

are a highly efficient method of gene delivery, physiological barriers and safety in clinical application is still a major concern. High immunogenicity, acute inflammation, cost of treatment and effect of some drugs such as anticoagulants on the inhibition of viral vectors are also some of its limitations in clinical implementation [24]. The limitation in package size is also a drawback of viral vectors, as they can deliver genetic material with a certain number of base pairs only [25].

The challenges and safety concerns raised by the viral vectors highlight the importance of bringing up a non-viral vector system or the development of an alternative gene delivery system for safe and promising nucleic acid delivery while mitigating cost concerns. Alternative gene delivery systems include lipid-based vectors and cationic polymers which use similar mechanisms of viruses to enter the cell but with improved safety, immunogenicity and cost [26]. Alternative gene delivery systems have various benefits over viral vectors such as greater biocompatibility, better payload capacity, the potential to engineer their surfaces *via* functionalization and transfection of a wide variety of cell types[27]. One of the most widely studied systemic gene delivery carriers are lipid based vectors. Toxicity of permanently charged cationic lipids, unstable systems which result in low half-life stability on storage and the low transfection efficiency of lipid-based vectors are their major drawbacks in clinical application [28]. Chitosan, which is a cationic polymers exhibits better ability to select target tissues, enhanced large-scale production, lesser cell toxicity and immunogenicity [29]. This review article discusses the recent updates on applications of chitosan in gene and drug delivery with special emphasis on cancer therapy.

Properties of chitosan for drug delivery: The potential use of chitosan for medical application and drug delivery has been widely explored by various researchers. Chitosan as a biopolymer has shown different properties for its successful applications in the field of medicine, food technology and marine. The characteristic properties of the compound incorporate features like biodegradability, adsorption, antimicrobial activities, non-toxicity and more [30,31]. Chitosan has been studied for its property of drug delivery and release, researchers studied the drug dissolution, diffusion and other factors by releasing anionic drug naproxen using chitosan as the matrix system and the results were found to be more potent and stable [32]. The chitosan has also been explored for its adhesive properties. The structure of the chitosan in specific to its cationic substructures make it compatible for being a mucoadhesive compound [33]. Although chitosan has cationic charges, when compared to other molecules that are used for transfection enhancing properties it has less toxic cation polymers. This factor makes chitosan a promising applicant in chitosan-DNA based drug complexes. But the effectiveness of chitosan for transfection has been shown to work and improve only with modified polysaccharide in chitosan [34]. Foger *et al.* [35] in their studies have worked on discovering the efflux pump inhibitory properties that chitosan beholds. The study positively demonstrated the mechanism of efflux pump inhibition [36]. In addition to these properties chitosan also inherits properties of *in situ* gelling, Gupta [36] combinedly used polyacrylic acid and chitosan as a gelling delivery system. Chitosan also has permeation characteristic which makes it interact with the cell membrane, to prove the permeability feature. Kast & Bernkop-Schnürch [37] demonstrated the structural functionality of chitosan including molecular mass 181 and degree of deacetylation, which in order was responsible for increase in epithelial permeability which further could be increase by increasing molecular mass of chitosan.

Chitosan structural compatibility: Chitosan is a highly basic polysaccharide which is a linear copolymer of α-(1-4)-linked *N*-acetyl-2-amino-2-deoxy-D-glucose (acetylated, A-unit) and 2-amino-2-deoxy-D-glucose (deacetylated, D-units) [38]. Some specific properties shown by chitosan are viscosity, solubility in different media, mucoadhesive, polyoxy salt formation, polyelectrolyte behaviour, potential to form films, metal chelators, optical and structural characteristics [39]. One of the major reasons for chitosan showing various chemical and biological properties is due to the presence of two reactive groups ($–NH_2$ and –OH). The interaction between the chitosan and negatively charged nucleic acid by electrostatic interaction is due to the presence of several amine groups, which are protonated at mildly acidic pH. Another major factor that affects the stability of chitosan/DNA complex is the molecular weight of chitosan, which also in turn affects the cell entry mechanism, unpacking of DNA after endosomal escape and transfection efficiency. Desirable properties of chitosan for gene delivery can be enhanced by chemically modifying chitosan [40]. For better bioavailability of drugs and to overcome the mucosal delivery barriers, mucoadhesive materials are used to make up mucoadhesive drug/gene delivery systems [41]. Chitosan which is a natural mucoadhesive uses electrostatic attraction, hydrogen bonding and hydrophobic effects to interact with negatively charged mucin [42].

Chitosan modifications for gene delivery: The existing chemical and biological properties of chitosan along with some specially desired properties for specific application in gene delivery can be achieved by making modifications on the chitosan backbone. Several studies have been reported for these modifications of chitosan [43]. Chitosan is soluble in slightly acidic pH, but due to the hydrophobic effect of the chitosan backbone and the strong intermolecular hydrogen bonding formed by –OH and $–NH_2$ groups, chitosan shows weaker solubility in alkaline and neutral pH, which in turn affects its application in drug and gene delivery [44]. Reducing the molecular weight of chitosan is the most direct method to enhance its solubility in neutral water [45]. Compared to highly hydrophobic chitin and mildly soluble chitosan, low molecular weight chitosan has shown better solubility in water [46]. Another common method of increasing the solubility of chitosan is by chemical modification and the most common derivatives of chitosan with improved solubility are *N*,*N*,*N*-trimethyl chitosan (TMC), amphoteric carboxymethyl chitosan (CMC) and PEGylated chitosan [47].

The aim of gene therapy is to introduce an exogenous gene into target cells, with the goal of modifying the expression of targeted genes and thus efficient delivery of genes to the target cells plays a vital role in gene therapy. Modifying chitosan

chitosan with target ligands such as proteins, aptamers, peptides, carbohydrates and several smaller molecules have exhibited enhanced targetability [48]. As a ligand, the use of arginine-glycine-aspartate-cysteine (RGDC) peptide in gene delivery vectors fabricated by chitosan to target the αvβ3 integrin has been reported by Hu *et al*. [49]. Several studies of modifying chitosan using aptamers have been reported, where aptamers are oligonucleotides which can bind exclusively to target substances like proteins and membrane receptors. Due to the absence of Fc region and finer permeability for particles of smaller molecular weights, aptamers have lower immunogenicity than antibodies [50]. These aptamers are used for forming gene targeting delivery systems by combining with chitosan [51]. The CD44 receptors of hyaluronic acid (HA) which is a natural polysaccharide composed of glucuronic acid and *N*-acetyl glucosamine is a widely studied receptor for its application in cancer treatment [52]. Some carbohydrate-protein interactions bring out several properties in carbohydrates providing differentiation, cell recognition and promote endocytosis. In several cancer cells originating from the epithelium shows the over expression of folate receptors, which have higher affinity for folic acid (FA) and therefore making FA-based tumor targeting is a promising and potent method for cancer treatment [53]. After the cellular uptake the major step is escaping the endosomes that will combine with the lysosomes, which have low pH and hydrolytic enzymes that can degrade the nucleic acids [54]. Cationic polymers such as PEI have shown the capacity to perform endosomal escape by the "proton sponge effect" which is dependent on the buffering capacity of the polymers [55]. It is important to improve buffering capacity of chitosan for the sake of enhancing transfection efficiency. The modified chitosan is used as a promising vector in gene delivery to gonadotropin releasing hormone receptor (GnRHR) expressing cells. This gene carrier is highly needed for gene therapy to cancers related with the reproductive system, genetic disorders of sexual development and fertility [10].

Applications in cancer treatment: Cancer is one of the most challenging diseases that we come across today and although the medical science field has overcome few important factors for the successful treatment of a few cancer types. It is a major reason for death in many people due to its complexity. Few reasons for the failure or unsuccessful attempts of cancer treatment are due to unable drug delivery methodology, painful side effects and sometimes low effectiveness [56]. With the growing demand for cost-effect cancer therapy treatment, the field of nanotechnology has introduced a better and effective way for cancer therapy treatments. With the use of polymerase such as chitosan with the combination of nanotechnology, scientists have introduced a potential carrier for cancer research [57]. The use of chitosan for drug delivery systems is growing exponentially mainly due to the structural and chemical properties of chitosan with features like high durability, eco-friendly, cost-effectiveness and more [8,58]. Chitosan has been well studied for medical application but its use for different cancer therapy processes such as drug delivery purposes, gene delivery, immune-adjuvant and as nanoparticle carrier for chemotherapeutic drugs and in immune-adjuvant therapy for cancer has grown in the past few years [59]. Chitosan nanoparticles have been already approved by FDA for their use due to their low toxicity and high biocompatibility factor. Scientists have tried different combinations and different modifications on chitosan in order to enhance its effectiveness for cancer treatment starting from increasing immune effects, enhancing antibody response to activation of immune response for immunogenicity of peptide epitope [60,61].

Shi *et al.* [62] in their research on melanoma mice was capable of designing an antitumor vaccine using chitosan nanoparticles for its application in cancer immunotherapy with its ability to produce cytotoxic T lymphocytes response. Rao *et al.* [63] on the other hand worked in developing a cancer drug consisting of encapsulated chitosan with doxorubicin encapsulated polymeric nanoparticles with specificity towards the CD44 receptor of cancer cells. The study was successful in observing a reduction in cytotoxicity and tumor size in a xenograft tumor model. Another study conducted in 2016 by Han *et al.* [64] showed effective results of using chitosan-based nanoparticles as an immune response modulatory vaccine, the study showed an increased maturation of dendritic cells for developing antigen-specific CD8+ T cell immunity. Researchers have also worked on using metals like zinc along with chitosan for creating CS-nanoparticles-linked zinc (Zn-CSNPs), which was further studied for cancer therapy and have shown a decrease in the rate of cancer cells and activate apoptotic pathway leading to an activated immune system for blood cancer therapy [65]. Specific changes on chitosan can have different effects and can be used for specific targeting on cells. Specific charges on chitosan can have different effects and used for specific targeting on cells. Studies have shown modification on chitosan covalently and non-covalently using various polymer moieties such as peptides, antibodies and more can effectively increase the process of drug delivery. Few studies have indicated that the toxicity level of drugs has been seen to decrease with the integration of chitosan with different conjugated drugs [66]. More scientific work has been carried out lately, which proves the potential of using chitosan for anticancer treatments. Active use of anticancer drugs coated with chitosan polymer has shown effectiveness along with the reduced growth of cancerous cells.

Chitosan in CRISPR/Cas9 delivery systems: The concept of gene delivery has been in the medical field for a long time now and its use has been increasing for the treatment of different diseases and infections as well. The use of chitosan as a potential gene delivery system has been studied by many researchers and has been proven to be a successful one [21]. With new discoveries and gene therapy methodologies a recent development of the use of CRISPR/Cas9 has also been highlighted by many refreshers for various cancer treatments and other related diseases [67]. The utilization of chitosan has been seen for delivering CRISPR/Cas9 complex, Srivastav *et al.* [68] have studied the potential of chitosan-coated nanoparticles for the delivery of siRNA and CRISPR/Cas9 complex and it was observed that chitosan with its biodegradable abilities successfully were able to delivery intact CRISPR complex. Another study conducted by Rabiee *et al.* [69] also studied the use of nano-chitosan

tetrazole for CRISPR delivery, the study concluded by rate of transfection efficiency of the chitosan was observed to be more than 25% that being higher than most other methodologies. The use of chitosan based nanocomplexes have been used in general for gene therapy processes and have shown great potential for its use in CRISPR delivery for anti-tumorigenic effects [70]. Thus, with the recent studies and development the prospects of gene therapy using chitosan and its derived complexes can be highly versatile in the CRISPR/Cas9 system delivery. Although there are many other ways and methods that can be explored for much more efficient gene delivery using chitosan such as by improving solubility of chitosan, use of low molecular weight chitosan derivatives, certain chitosan chemical modification, modification of its protein structures and more [21].

Conclusion

Chitosan is a widely applied biopolymer in the pharmaceutical sector, especially in drug and gene delivery, due to its various properties and structural compatibility. Biodegradability, adsorption, antimicrobial activities and non-toxicity are some major characteristics of chitosan, which makes it a major applicant in the field of medicine. The presence of $–NH_2$ group and –OH provides various chemical and biological properties for chitosan and the cell entry mechanism, unpacking of DNA after endosomal escape and transfection efficiency is also influenced by the molecular weight of chitosan. For specific application in gene delivery, some specially desired properties such as increasing the solubility, buffering capacity, *etc*. are incorporated by modifying the structure of chitosan. Chitosan with the combination of nanotechnology is a promising methodology of drug delivery for cancer treatment, which reduces painful side effects, cost-effectiveness and increases treatment efficiency. Reduction in growth of cancer cells are also widely observed by the active use of anticancer drugs coated with chitosan polymer. The emerging technologies and developments in gene therapy support the use of CRISPR/Cas9 coated with chitosan nanoparticles in cancer treatment, cardiovascular, viral infections and genetic diseases.

ACKNOWLEDGEMENTS

The authors are thankful to Center for Research Projects and Department of Life Sciences, CHRIST (Deemed to be University), Bangalore, India for providing necessary research facilities and financial support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. A. Ali and S. Ahmed, *Int. J. Biol. Macromol.*, **109**, 273 (2018); https://doi.org/10.1016/j.ijbiomac.2017.12.078
2. S.A. Agnihotri, N.N. Mallikarjuna and T.M. Aminabhavi, *J. Control. Release*, **100**, 5 (2004); https://doi.org/10.1016/j.jconrel.2004.08.010
3. L. Hu, Y. Sun and Y. Wu, *Nanoscale*, **5**, 3103 (2013); https://doi.org/10.1039/c3nr00338h
4. C. Puglia, M.R. Lauro, G.G. Tirendi, G.E. Fassari, C. Carbone, F. Bonina and G. Puglisi, *Expert Opin. Drug Deliv.*, **14**, 755 (2017); https://doi.org/10.1080/17425247.2017.1234452
5. S.S. Ameeduzzafar, S.S. Imam, S.N. Abbas Bukhari, J. Ahmad and A. Ali, *Int. J. Biol. Macromol.*, **108**, 650 (2018); https://doi.org/10.1016/j.ijbiomac.2017.11.170
6. M. Marciello, S. Rossi, C. Caramella and C. Remuñán-López, *Carbohydr. Polym.*, **170**, 43 (2017); https://doi.org/10.1016/j.carbpol.2017.04.051
7. J.A. Luckanagul, C. Pitakchatwong, P.R. Na Bhuket, C. Muangnoi, P. Rojsitthisak, S. Chirachanchai, Q. Wang and P. Rojsitthisak, *Carbohydr. Polym.*, **181**, 1119 (2018); https://doi.org/10.1016/j.carbpol.2017.11.027
8. A. Babu and R. Ramesh, *Mar. Drugs*, **15**, 96 (2017); https://doi.org/10.3390/md15040096
9. M.A. Ghaz-Jahanian, F. Abbaspour-Aghdam, N. Anarjan, A. Berenjian and H. Jafarizadeh-Malmiri, *Mol. Biotechnol.*, **57**, 201 (2015); https://doi.org/10.1007/s12033-014-9816-3
10. C. Boonthum, K. Namdee, S. Boonrungsiman, K. Chatdarong, N. Saengkrit, W. Sajomsang, S. Ponglowhapan and T. Yata, *Carbohydr. Polym.*, **157**, 311 (2017); https://doi.org/10.1016/j.carbpol.2016.09.015
11. M. Morishita and N.A. Peppas, *Drug Discov. Today*, **11**, 905 (2006); https://doi.org/10.1016/j.drudis.2006.08.005
12. S. Arora, J. Ali, A. Ahuja, R.K. Khar and S. Baboota, *AAPS PharmSciTech*, **6**, E372 (2005); https://doi.org/10.1208/pt060347
13. V.R. Sinha and R. Kumria, *Drug Dev. Ind. Pharm.*, **30**, 143 (2004); https://doi.org/10.1081/DDC-120028709
14. J. Xu, S. Strandman, J.X.X. Zhu, J. Barralet and M. Cerruti, *Biomaterials*, **37**, 395 (2015); https://doi.org/10.1016/j.biomaterials.2014.10.024
15. M. de la Fuente, M. Raviña, P. Paolicelli, A. Sanchez, B. Seijo and M.J. Alonso, *Adv. Drug Deliv. Rev.*, **62**, 100 (2010); https://doi.org/10.1016/j.addr.2009.11.026
16. F.W.H.M. Merkus, J.C. Verhoef, E. Marttin, S.G. Romeijn, P.H.M. van der Kuy, W.A.J.J. Hermens and N.G.M. Schipper, *Adv. Drug Deliv. Rev.*, **36**, 41 (1999); https://doi.org/10.1016/S0169-409X(98)00054-4
17. S.A. Chore and S.J. Dighade, *Int. J. Res. Pharm. Chem.*, **10**, 4 (2020); https://doi.org/10.33289/IJRPC.10.4.2020.10(91)
18. A. Yamamoto and S. Muranishi, *Adv. Drug Deliv. Rev.*, **28**, 275 (1997); https://doi.org/10.1016/S0169-409X(97)00077-X
19. F. Yin, B. Gu, Y. Lin, N. Panwar, S.C. Tjin, J. Qu, S.P. Lau and K.-T. Yong, *Coord. Chem. Rev.*, **347**, 77 (2017); https://doi.org/10.1016/j.ccr.2017.06.024
20. Z. Shariatinia, *Adv. Colloid Interface Sci.*, **263**, 131 (2019); https://doi.org/10.1016/j.cis.2018.11.008
21. R.M. Haley, R. Gottardi, R. Langer and M.J. Mitchell, *Drug Deliv. Transl. Res.*, **10**, 661 (2020); https://doi.org/10.1007/s13346-020-00724-5
22. H.G. Terheggen, H. Haug, K.P. Hellriegel and H.E. Schaefer, *Z. Kinderheilkd.*, **119**, 123 (1975); https://doi.org/10.1007/BF00443566
23. W. Walther and U. Stein, *Drugs*, **60**, 249 (2000); https://doi.org/10.2165/00003495-200060020-00002
24. A. Rey-Rico and M. Cucchiarini, *Polymers*, **11**, 514 (2019); https://doi.org/10.3390/polym11030514
25. W.T. Godbey, K.K. Wu and A.G. Mikos, *J. Control. Release*, **60**, 149 (1999); https://doi.org/10.1016/S0168-3659(99)00090-5
26. S.E. Lawler, Y. Saeki, E.A. Chiocca and R. Wade-Martins, Eds. P.R. Lowenstein and M.G. Castro, iBAC Technologies for Neurological Disease, In: Gene Therapy for Neurological Disorders, CRC Press, Ed.: 1, p. 115 (2006).
27. L. De Laporte, J. Cruz Rea and L.D. Shea, *Biomaterials*, **27**, 947 (2006); https://doi.org/10.1016/j.biomaterials.2005.09.036
28. J. Buck, P. Grossen, P.R. Cullis, J. Huwyler, D. Witzigmann and D.N.A. Lipid-Based, *ACS Nano*, **13**, 3754 (2019); https://doi.org/10.1021/acsnano.8b07858
29. D. Chuan, T. Jin, R. Fan, L. Zhou and G. Guo, *Adv. Colloid Interface Sci.*, **268**, 25 (2019); https://doi.org/10.1016/j.cis.2019.03.007

30. H. Kaczmarek and J. Zawadzki, *Carbohydr. Res.*, **345**, 941 (2010); https://doi.org/10.1016/j.carres.2010.02.024
31. P. Sahariah, M.Á. Hjálmarsdóttir and M. Másson, *Marine Glycobiol.*, **345**, 345 (2016); https://doi.org/10.1201/9781315371399-26
32. K.S. Bhise, R.S. Dhumal, A.R. Paradkar and S.S. Kadam, *AAPS PharmSciTech*, **9**, 1 (2008); https://doi.org/10.1208/s12249-007-9001-0
33. H.L. Lueßen, B.J. de Leeuw, D. Pérard, C.-M. Lehr, A.B.G. de Boer, J.C. Verhoef and H.E. Junginger, *Eur. J. Pharm. Sci.*, **4**, 117 (1996); https://doi.org/10.1016/0928-0987(95)00042-9
34. D. Lee and S.S. Mohapatra, *Methods Mol. Biol.*, **127**, 433 (2008); https://doi.org/10.1007/978-1-59745-237-3_8
35. F. Föger, T. Schmitz and A. Bernkop-Schnürch, *Biomaterials*, **27**, 4250 (2006); https://doi.org/10.1016/j.biomaterials.2006.03.033
36. S. Gupta, *Sci. Pharm.*, **78**, 959 (2010); https://doi.org/10.3797/scipharm.1001-06
37. C.E. Kast and A. Bernkop-Schnürch, *STP Pharma Sci*., **12**, 351 (2002).
38. S. Kaur and G.S. Dhillon, *Crit. Rev. Microbiol.*, **40**, 155 (2014); https://doi.org/10.3109/1040841X.2013.770385
39. S.K. Shukla, A.K. Mishra, O.A. Arotiba and B.B. Mamba, *Int. J. Biol. Macromol.*, **59**, 46 (2013); https://doi.org/10.1016/j.ijbiomac.2013.04.043
40. M. Ahmad, K. Manzoor and S. Ikram, *Appl. Nanocomp. Mater. Drug Deliv.*, **27**, 27 (2018); https://doi.org/10.1016/B978-0-12-813741-3.00002-9
41. A. Sosnik, J. das Neves and B. Sarmento, *Prog. Polym. Sci.*, **39**, 2030 (2014); https://doi.org/10.1016/j.progpolymsci.2014.07.010
42. T. M. Ways, W. Lau and V. Khutoryanskiy, *Polymers*, **10**, 267 (2018); https://doi.org/10.3390/polym10030267
43. M. Prabaharan and A. Tiwaria, Eds.: S.-K. Kim, Chemical Modifications of Chitosan Intended for Biomedical Applications, In: Chitin, Chitosan, Oligosaccharides and Their Derivatives, CRC Press, p. 173 (2010).
44. S.B. da Silva, M. Krolicka, L.A.M. van den Broek, A.E. Frissen and C.G. Boeriu, *Carbohydr. Polym.*, **186**, 299 (2018); https://doi.org/10.1016/j.carbpol.2018.01.050
45. Y. Fu and C. Xiao, *Int. J. Biol. Macromol.*, **103**, 575 (2017); https://doi.org/10.1016/j.ijbiomac.2017.05.066
46. R.A. Krishnan, P. Deshmukh, S. Agarwal, P. Purohit, D. Dhoble, P. Waske, D. Khandekar, R. Jain and P. Dandekar, *Carbohydr. Polym.*, **151**, 417 (2016); https://doi.org/10.1016/j.carbpol.2016.05.082
47. P.K. Panda, J.-M. Yang, Y.-H. Chang and W.-W. Su, *Int. J. Biol. Macromol.*, **136**, 661 (2019); https://doi.org/10.1016/j.ijbiomac.2019.06.082
48. C. Corbet, H. Ragelle, V. Pourcelle, K. Vanvarenberg, J. Marchand-Brynaert, V. Préat and O. Feron, *J. Control. Release*, **223**, 53 (2016); https://doi.org/10.1016/j.jconrel.2015.12.020
49. J. Hu, M. Zhu, K. Liu, H. Fan, W. Zhao, Y. Mao and Y. Zhang, *PLoS One*, **11**, e0166673 (2016); https://doi.org/10.1371/journal.pone.0166673
50. K.-T. Guo, X.-R. Yan, G.-J. Huang, C.-X. Xu, Y.-S. Chai and Z.-Q. Zhang, *Sheng Wu Gong Cheng Xue Bao*, **19**, 730 (2003).
51. S. Yang, Z. Ren, M. Chen, Y. Wang, B. You, W. Chen, C. Qu, Y. Liu and X. Zhang, *Mol. Pharm.*, **15**, 314 (2018); https://doi.org/10.1021/acs.molpharmaceut.7b01093
52. D. Naor, *Front. Immunol.*, **7**, 39 (2016); https://doi.org/10.3389/fimmu.2016.00039
53. H. Li, Y. Li, H. Ao, D. Bi, M. Han, Y. Guo and X. Wang, *Drug Deliv.*, **25**, 880 (2018); https://doi.org/10.1080/10717544.2018.1455761
54. P. Garg, S. Kumar, S. Pandey, H. Seonwoo, P.-H. Choung, J. Koh and J.H. Chung, *J. Mater. Chem. B Mater. Biol. Med.*, **1**, 6053 (2013); https://doi.org/10.1039/c3tb20939c
55. L.M.P. Vermeulen, T. Brans, S.K. Samal, P. Dubruel, J. Demeester, S.C. De Smedt, K. Remaut and K. Braeckmans, *ACS Nano*, **12**, 2332 (2018); https://doi.org/10.1021/acsnano.7b07583
56. A.C. Fonseca, A.C. Serra and J.F.J. Coelho, *EPMA J.*, **6**, 22 (2015); https://doi.org/10.1186/s13167-015-0045-z
57. D.P. Potdar and U.A. Shetti, *MOJ Cell Sci. Rep.*, **3**, 39 (2016); https://doi.org/10.15406/mojcsr.2016.03.00049
58. M. Lee, J.W. Nah, Y. Kwon, J.J. Koh, K.S. Ko and S.W. Kim, *Pharm. Res.*, **18**, 427 (2001); https://doi.org/10.1023/A:1011037807261
59. L. Alizadeh, A. Zarebkohan, R. Salehi, A. Ajjoolabady and M. Rahmati-Yamchi, *J. Drug Target.*, **27**, 839 (2019); https://doi.org/10.1080/1061186X.2018.1564923
60. T. Kean and M. Thanou, *Adv. Drug Deliv. Rev.*, **62**, 3 (2010); https://doi.org/10.1016/j.addr.2009.09.004
61. P.-G. Chen, Z.-H. Huang, Z.-Y. Sun, Y. Gao, Y.-F. Liu, L. Shi, Y.-X. Chen, Y.-F. Zhao and Y.-M. Li, *Pure Appl. Chem*., **89**, 931 (2017); https://doi.org/10.1515/pac-2016-0913
62. G.-N. Shi, C.-N. Zhang, R. Xu, J.-F. Niu, H.-J. Song, X.-Y. Zhang, W.-W. Wang, Y.-M. Wang, C. Li, X.-Q. Wei and D.-L. Kong, *Biomaterials*, **113**, 191 (2017); https://doi.org/10.1016/j.biomaterials.2016.10.047
63. W. Rao, H. Wang, J. Han, S. Zhao, J. Dumbleton, P. Agarwal, W. Zhang, G. Zhao, J. Yu, D.L. Zynger, X. Lu and X. He, *ACS Nano*, **9**, 5725 (2015); https://doi.org/10.1021/nn506928p
64. H.D. Han, Y. Byeon, J.-H. Jang, H.N. Jeon, G.H. Kim, M.G. Kim, C.-G. Pack, T.H. Kang, I.D. Jung, Y.T. Lim, Y.J. Lee, J.-W. Lee, B.C. Shin, H.J. Ahn, A.K. Sood and Y.-M. Park, *Sci. Rep.*, **6**, 38348 (2016); https://doi.org/10.1038/srep38348
65. K. Saravanakumar, E. Jeevithan, R. Chelliah, K. Kathiresan, W. Wen-Hui, D.-H. Oh and M.-H. Wang, *Int. J. Biol. Macromol.*, **119**, 1144 (2018); https://doi.org/10.1016/j.ijbiomac.2018.08.017
66. Y. Xu and Y. Du, *Int. J. Pharm.*, **250**, 215 (2003); https://doi.org/10.1016/S0378-5173(02)00548-3
67. A. Ghaemi, E. Bagheri, K. Abnous, S.M. Taghdisi, M. Ramezani and M. Alibolandi, *Life Sci.*, **267**, 118969 (2021); https://doi.org/10.1016/j.lfs.2020.118969
68. A. Srivastav, K. Gupta, D. Chakraborty, P. Dandekar and R. Jain, *J. Pharm. Innov.*, (2020); https://doi.org/10.1007/s12247-020-09496-4
69. N. Rabiee, M. Bagherzadeh, M. Tavakolizadeh, A. Pourjavadi, M. Atarod and T.J. Webster, *Int. J. Polym. Mater. Polym. Biomater.*, **71**, 116 (2022); https://doi.org/10.1080/00914037.2020.1809405
70. B.-C. Zhang, P.-Y. Wu, J.-J. Zou, J.-L. Jiang, R.-R. Zhao, B.-Y. Luo, Y.-Q. Liao and J.-W. Shao, *Chem. Eng. J.*, **393**, 124688 (2020); https://doi.org/10.1016/j.cej.2020.124688