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Review Article

Chitosan in Drug Delivery Systems and Tissue Engineering: Versatile Prospects

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ABSTRACT

Chitosan (CS), derived from the deacetylation of chitin-the second-most abundant natural polymer after cellulose-is a versatile biopolymer known for its remarkable properties. It is biocompatible, biodegradable, non-toxic, hydrophilic, and chemically resistant, with high bioavailability and ease of modification. These attributes enable the formation of diverse structures such as films, gels, nanoparticles, microparticles, and beads. Importantly, chitosan is broken down by the body into non-toxic amino sugars, making it a suitable candidate for biomedical applications. The unique properties of CS arise from the presence of amine groups along its polymer backbone, which impart functionality for various biological uses. It is especially relevant in tissue engineering due to its ability to support cell attachment and growth. Its applications include use in 2D and 3D scaffolds like sponges, gels, and films for wound healing, where it aids in drug delivery and bioactive compound absorption. Recently, chitosan has gained attention in orthopaedic tissue engineering for its antibacterial nature, low immunogenicity, and adaptability into various shapes and porous structures conducive to osteoconduction and cellular ingrowth. These properties support the regeneration and repair of tissue, highlighting its therapeutic potential. This summary explores chitosan's critical role in biomedical fields, focusing on its structural applications in scaffolds and its utility in controlled drug delivery systems. Furthermore, molecular modifications of CS are discussed for tailoring it to specific biological functions, underlining its growing importance in modern tissue engineering and pharmaceutical formulations.

Keywords: CS, Natural Polymers, Tissue Engineering, Drug Delivery.

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INTRODUCTION

Polymers are extensively used for the delivery of active pharmaceutical ingredients. Aside from these uses like slimming and wound dressing, including tissue engineering, chitin shows potential characteristics as an auxiliary reagent in drug administration. They can form a matrix or membrane that can control the release of a drug over a prolonged period, thus avoiding repetitive dosing. They can also be used to form nanocarriers to deliver drugs, in particular poorly soluble drugs or biotechnology-based drugs. Both systems can protect the drug from degradation.[1] There are numerous varieties of polymers, both natural and synthetic. Natural polymers like proteins (such as elastin, silk, and keratin) or carbohydrates (such as starch and glycogen) are frequently used as components in both traditional and cutting-edge drug formulations. Such polymers are broadly accessible, inexpensive, biodegradable, non-toxic, and chemically resistant.[2] They are also considerably inexpensive. CS cannot be simply mined from natural resources because it is not extensively distributed in nature.[3] Chitin and its derivatives are highly prevalent natural polymers because CS and its derivatives are inexhaustible sources of natural biopolymers. Chitin turns into CS when the level of acetylation is less than 60 mol%. Chitin is a natural substance found in all living things, although it is highly prevalent in insects and crustaceans, where it makes up a large portion of their exoskeleton. Many mushrooms' cell walls also contain chitin.[4] Usually speaking, CS derived from mushrooms has a narrower molecular weight dispersion than CS derived from crustaceans, making it better for biomedical and therapeutic applications (Figure 1). Controlled, sustained, targeted, as well as pulsatile drug administration systems in the GI tract, oral cavity, including the rectum administer medications more efficiently than standard dose formulations. Natural polymers such as CS, which

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are biocompatible, biodegradable, and highly nontoxic, have gained favour for this purpose. The Characteristics of CS make it a preferred option for colon-specific medication administration of medicines, peptides, and proteins, as well as genes, for a variety of medicinal purposes. Since Chitosan based preparations protect therapeutic molecules from the harsh environment of the gastrointestinal tract while also delivering functional medications, particularly to the colon, they are commonly used for colonic medication distribution.[5] CS is no longer merely a trash residue from the preparation of seafood. Marine goods have led the way in the utilisation of Natural substances for medicinal purposes towards modern ailments.[6] However, interest in using the marine biopolymer chitin, the second-most prevalent polymer in the environment and extracted from crustaceans, for medical purposes has surged.

In highly alkaline situations, 50% deacetylation of chitin converts it into CS. CS has been utilised in medical products for a long time, including pharyngeal permeation enhancers of peptide drugs as well as 3D or 2D scaffold arrangements for wound healing. CS is made up of (1-4)-2-acetamido-2-deoxy-ß-D-glucan (N-acetyl D-glucosamine) and (1-4)-2-amino-2-deoxy-ß-D-glucan (D-glucosamine).[7] CS has also been successfully employed as a non-viral delivery vector for bio-macromolecules, including low-molecular-weight medications. CS has significant levels of biocompatibility but also biodegradability, which are desirable qualities for the development of a safe and reliable medication system of administration. CS is cationic by nature, and while it is poorly soluble in water, it is accessible in solutions with a low pH. CS has also been widely used as a potential immunological booster for tumour vaccinations.[8] Additionally, using CS for mucosal drug delivery is non-invasive, effortless, and offers several advantages over parenteral administration, including easy availability, quick action, removal elimination of the gastrointestinal first pass effect, as well as high bioavailability, inexpensive self-management, and high consumer adherence. Mucosal delivery also offers various absorptive surfaces, such as the buccal, nasal, ocular, vaginal, and rectal mucosa's, creating excellent opportunities for both systemic and local administration of a wide range of drugs. This allows for the potential of This opens the door to the possibility of addressing a particular tissue as well as obtaining significant "*in situ*" medication dosages with fewer systemic adverse effects in the regional therapy of a range of illnesses. and obtaining high "*in situ*" drug concentrations with fewer systemic side effects in the local treatment of a variety of conditions.[9] The current study outlines several notable recent advances involving the application of CS in cancer immunotherapy, drug delivery, tissue engineering, and chemotherapy. CS can form gels at acidic pH levels because it's hydrophilic by nature. These gels are suitable to be utilised as a slow-release medication delivery mechanism. Healing as well as regeneration of tissue that has been injured represent significant concerns in medical situations at the moment. The development of novel and more resilient polymers is required for the speedy growth of tissue engineering. CS is one of numerous natural as well as man-made substances. For a long time, chitin-based reactive polymers have been utilised in the field of tissue engineering. The most well-known applications of using CS in tissue engineering for the production of biomaterials include those involving cartilage, bone, intervertebral discs, blood vessels, corneal regrowth, skin, tissue fixation, and periodontal tissue.[10] This review aims to provide a thorough analysis of bioactive compounds derived from CS that have been employed in tissue engineering processes over the past ten years, taking into account the constantly expanding interest in this sector. The inherent antibacterial activity of CS is another intriguing characteristic. Widespread migration through the cell membrane is interfered with by CS, hastening bacterial demise. It has been used with other polymers because of its antibacterial activity. CS is also a preferred carrier for drug delivery, thus combining its intrinsic antibacterial activity with that of the bound antibiotic. It is mostly used as a tablet ingredient, in controlled-delivery formulations, in gel, as a penetration enhancer, for drug dissolution, in medicines for treating wounds, and in the creation of micro/nanoparticles.[11] The two most important characteristics of CS that influence its usage as a matrix molecule for drug administration are its molecular mass and level of acetylation. These characteristics influence its hydrophobicity and water solubility, which change the efficacy of its drug encapsulation.[12] The accessibility of functional groups, which makes it easier to make molecular alterations, is an additional characteristic of CS that makes it useful as a drug carrier and coating molecule. By using lysozyme and chitinase, the CS molecule can be broken down. Although lysozyme is produced spontaneously by a number of surfaces of the mouth, chitinase production has been related to the intestinal microbes, which increase lysozyme breakdown.[13]

CS and chitin structure

A linear, uniformly dispersed heteropolymer called CS is made up of 2-acetamido-2-deoxy-D-glucopyranose plus 2-amino-2-deoxy-D-glucopyranose units that are paired by (1-4) units (Figure 2). Chitin, a linear polymer of (1-4) linked N-acetyl-D-glucosamine units made of mucopolysaccharides as well as amino sugars, is deacetylated to create it.[14,15] Because the acetyl molecules of the amine functional groups have been removed, CS is easily soluble in an alkaline water solution. In acidic conditions, the polysaccharide is transformed into polycation via the protonation of amino groups on the C-2 position of the D-glucosamine residue.

Physicochemical Properties

CS is the generic name for a group of polysaccharides that have been deacetylated from chitin. Chitin and CS can be distinguished by their levels of deacetylation. In reality, the reaction of deacetylating chitin in an alkaline solution still cannot be entirely completed, despite harsh treatment. The level

of deacetylation normally ranges from 70% to 95%, depending on the method.[16] The DD in CS is one of its more important structural characteristics, and it determines how many free amino groups there are in the polysaccharide. But the polymer is referred to as chitin when the quantity is greater than 50% in industrial products. Because low molecular weight chitosan is well suited for usage as an antibacterial, antioxidant, and anti-tumor material, its molecular weight is one criterion that may be utilised as a benchmark of quality. Depending on its molecular weight range, chitosan can be categorised as high, medium, or low molecular weight.[6,9] As the molecular weight rises, chitosan becomes less soluble and viscous, which is undesirable for a number of industrial uses. Low molecular weight chitosan is recommended for usage in biological and commercial applications due to its superior solubility and stability. The amount of chitosan that has been deacetylated is crucial because it affects the material's physical, chemical, and biological properties, including its acid-base and electrostatic properties, biodegradability, self-aggregation, sorption capabilities, and capacity to chelate metal ions. The amount of free amino groups present in the polysaccharide is mostly influenced by the deacetylation level. Reduced molecular weight of chitosan results in a decrease in viscosity, which is dependent on the polymer's molecular weight and degree of deacetylation (DD). As a matter of fact, because of polymer breakdown during storage, viscosity may be used to gauge a polymer's durability in solution.[11,14] When deacetylation is more severe and the molecular weight is less, viscosity rises. The amount of time the chitosan has been stored and the size of its particles may also play a role. When compared to a standard chitosan solution at the same concentration, nanochitosan has a viscosity that is roughly 30% lower. The viscosity of regular chitosan decreases by around 10% over the course of 24 hours of storage, whereas nanocolloids see a 17% decrease over the same period of time. In contrast to neutral or alkaline solvents, chitosan is soluble in acids. Deacetylating chitin produces soluble chitosan, which possesses primary amino groups and a pKa value of 6.5, despite the fact that chitin is typically insoluble in solvents. Chitosan becomes soluble when it is combined with acidic solutions, which protonate the amine and make it positively charged. However, they lose their charge and become insoluble as the pH increases to 6 or higher. In addition to pH, chitosan's DD at the molecular level, temperature, and polymer crystallinity all have an impact on its solubility. Naturally produced polymers with neutral or acidic origins include cellulose and carrageenan, which include biopolymers like dextran, pectin, and many others.[17-21] Chitin and CS are very basic polysaccharides: therefore, modifying the DD or whether the major amino group is present in the molecule can change both their pKa and solubility, where are the main components that could affect but also alter its physical properties. The specific properties of the material, such as the level of DD or cationic, and its molecular weight affect the features of the CS.[18] The primary technique utilised to create commercially available CS is deacetylating chitin, produced from seashell material. The quality of CS products and their viscosity, deacetylation, molecular weight, and polymorphous structure can vary significantly according to a variety of factors throughout the manufacturing procedure.[19] As the fraction of CS rises, its molecular mass decreases and is subsequently influenced by viscosity, solubility, temperature, and the degree of tearing.

Biological Properties of CS

Since then, increasing attention has been given to pharmaceutical delivery methods like nanoparticles, micelles, polysaccharides, and liposomes.[20] These methods disclose a number of advantages, including increased therapeutic safety and efficacy. These devices can include both hydrophobic and hydrophilic active molecules, depending on the carrier type. They can also provide treatments that are more resistant to chemical and enzymatic degradation, prolong therapeutic actions in the target tissue, have a higher bioavailability, or target specific drugs by using specific ligands. The physical or biochemical characteristics of the polysaccharide core of CS, which is physically comparable to glycosaminoglycans, the main constituents of the extracellular matrix of bone and cartilage, are associated with the capacity of CS to facilitate cellular attachment and proliferation. From the perspective of tissue engineering, the extremely porosity as well as interconnected pore structure, osteoconductivity, and capacity to promote the development of bones are further benefits of CS.[21] This overview will discuss one of the most current pharmaceutical requirements for CS in many different kinds of biological uses, such as wound healing, tissue engineering, drug/gene delivery, protein binding, cell encapsulation, implant and contact lens preparation, biosensing, dietary supplements, food containers, and antibacterial fabrics. Chitin possesses outstanding biological properties, allowing it to be used in the pharmaceutical and biological sectors to develop novel drug delivery systems or as a scaffold for tissue engineering.[22] CS has been used as a safe excipient in pharmaceutical formulations for over 20 years. A Positive charge of CS leads to its outstanding biocompatibility. Due to its biocompatibility, it promotes adherence to the epithelium and, as a result, the time of contact for medicine administration. Because of its hemostatic properties, CS is an effective wound treatment agent.[23] In addition, the antibacterial action of CS minimises the risk of infection. It adheres to both the mucous layer that protects the surface of the tissues and the epithelial tissues. It also has anticancer, anticholesterol, fungicidal, and bactericidal properties. Clinical trials to promote CS-based biocomposites have shown no inflammation or deleterious effects after implantation, infusion, transdermal, or intake in humans.[24,25] The carrier concentration of the polycation CS is affected by the degree of acetylation and the pH. Electrostatic interaction allows CS chains to react with negatively charged substances. It is possible to make nanoparticles by ionic gelation using polyphosphates and nucleic acids.[26,27]

Remarkable properties of CS as biomaterial

As previously stated, the exceptional properties of CS provide unique opportunities for the development of biomedical applications.[28] Indeed, the presence of negatively charged moieties (sialic acid) in mucin, the glycoprotein that makes up mucus, can be utilised to define mucoadhesion of CS. CS amino groups can interact with mucin; they have a positive charge in an acidic environment. This mucoadhesion is significantly connected with the DD of CS; in fact, as the DD of CS increases, more positive ions are formed, which improves the muco-adhesive properties.[29] The presence of positive charges on the backbone of CS may further affect its hemostatic effect.[30] Because it is positively charged, CS can also link with the negative component of a cell's membrane. This action can allow tight junction proteins to reorganize as well as open, explaining how this polysaccharide increases penetration. In terms of muco-adhesion, increasing CS deacetylation enhances penetration capacity.[31] The polycationic nature of CS also explains why it has analgesic effects. The amino groups of the D-glucosamine residues may protonate in the form of proton ions formed in the inflammatory area, providing an analgesic effect.[32]

CS based Drug Delivery systems

The Molecular weight and degree of acetylation of CS are two of the most crucial properties that affect how it is used as a drug delivery matrix. These features alter its solubility in water as well as its hydrophobicity, which alters the effectiveness of its drug encapsulation.[33] CS is rarely soluble in neutral as well as basic aqueous solutions. However, it becomes more soluble when used in an acidic environment, primarily as a result of the protonation of the amino groups. Two key aspects of CS that are important for medicine delivery are its muco-adhesive qualities and its ability to temporarily open epithelial tight junctions. A number of well-organised epithelia, including the nasal,[34,35] intestinal, ophthalmic, buccal, and pulmonary,[36] have been employed to transport drugs using CS. By using lysozyme and chitinase, the CS polymer can be broken down.[37,38] Although many different mucosal surfaces spontaneously produce lysozyme, it has been discovered that the intestinal flora releases chitinase, which encourages lysozyme breakdown.[39,40] Moreover, the acidic environment of the stomach after oral delivery encourages the breakdown of CS.[41,42] It is well documented how to make drug-encapsulated CS nanoparticles utilising various techniques (emulsifier-based, coacervation/precipitation-based, electrostatic gelation, self-assembling, reverse micellar method, and other methods).[43,44] CS has a limited solubility in naturally occurring fluids (pH 7.4), which limits its application as a drug delivery method. CS is used in a variety of ways, including oral, vaginal, buccal, ocular, intestinal, and parental distribution (Table 1). Except for capsules, tablets are perhaps the most efficient dose type. Numerous medication delivery systems, including CS, are based on tablets because they give an exact dosage and are simple to make or handle.[45,46] CS, like many other polysaccharides, is destroyed in the colon. CS has been discovered as a beneficial coating to ensure site-specific distribution by utilising this colon-specific breakdown. The bioavailability of nasally delivered medications is frequently inadequate. A limited regional residency duration is caused by nasal barriers such as limited membrane permeability, or a fast turnover rate of secretion in nasal cavities.[47] The combination of permeability enhancers and mucoadhesive nasal administration devices appears to be the most effective strategy for improving nasal medication absorption. The buccal route is an alternative method for delivering medications to the application site because it prevents hepatic first-pass metabolism as well as gastric degradation as well as the small intestine. Furthermore, this approach is well-accepted by patients. A good buccal delivery system should linger in the mouth cavity for several hours and release the medicine gradually.[48]

Mucoadhesive polymers extend the residence time of drugs in the oral cavity. CS is a useful material for the creation of ocular drug delivery systems due to its benign nature, permeability-boosting qualities, and physicochemical features. Hydrogels, nanoparticles, and coated colloidal systems are CS-based formulations employed for ocular drug delivery, and they can improve the retention and biodistribution of medications administered topically to the eye.[49]

CS in Colon Targeted Drug Delivery

If an unmodified medicine is intended to enter the intestines, important considerations must be made since the medication must be protected against gastric pH levels as well as disintegrating enzymes. CS is a well-accepted and prospective polymer delivery through mucosal surfaces that is garnering a lot of attention as an alternate route of systemic biodegradation by the microorganisms prevalent in human administration.[50] Many studies show that the colon is an appropriate site for the absorption of peptides as well as protein drugs because (i) enzymes are less reactive towards drugs, (ii) mucosa in the colon is soft on peptide drugs, colon targeted drug delivery protects the drug from enzymatic hydrolysis and reduces degradation in the duodenum and jejunum, resulting in higher systemic bioavailability[51] and (iii) prolonged drug residence duration in the colon is associated with a synergistic effect of absorption enhancers on medication absorption. It is useful in the formulation of bioadhesive. CS HCl was directly crushed with medication dosage forms that can be administered by (ocular, nasal, 5-aminosalicylic acid (5- ASA)) into matrices.[52] Aside from the formulation type, the individual polymers utilised in these formulations, which are alginate, chitin, guar gum, Dextran, and others, are known to have an important impact in all aspects of the success of the tailored medication method of delivery for the colon. CS, one of the various polymers utilized for colon medication delivery, plays an important function and is frequently employed. An Oral Colon-Specific Delivery System (OCDS) is a delivery method that, after oral administration, can

avoid encapsulant premature release in the stomach and small intestine while achieving release onset upon passage into the colon (Figure 3). As a result, the production of CS-based oral colon-specific delivery is important for polyphenol administration as it helps boost the biological activity of these polyphenols. CS-based positively charged nanoparticles can easily connect to the negatively charged cell membrane as well as subsequently enter the cell primarily via endocytosis, promoting encapsulated chemical absorption. Eudragit S100, the coating agent, was involved in the intact passage of CS-based microparticles and microspheres through the gut and small intestine. While there have been few publications on the CS-based hydrogel as a carrier in the OCDS, the design of the hydrogel coating for other carriers is well-developed. Additionally, polysaccharide-based hydrogels

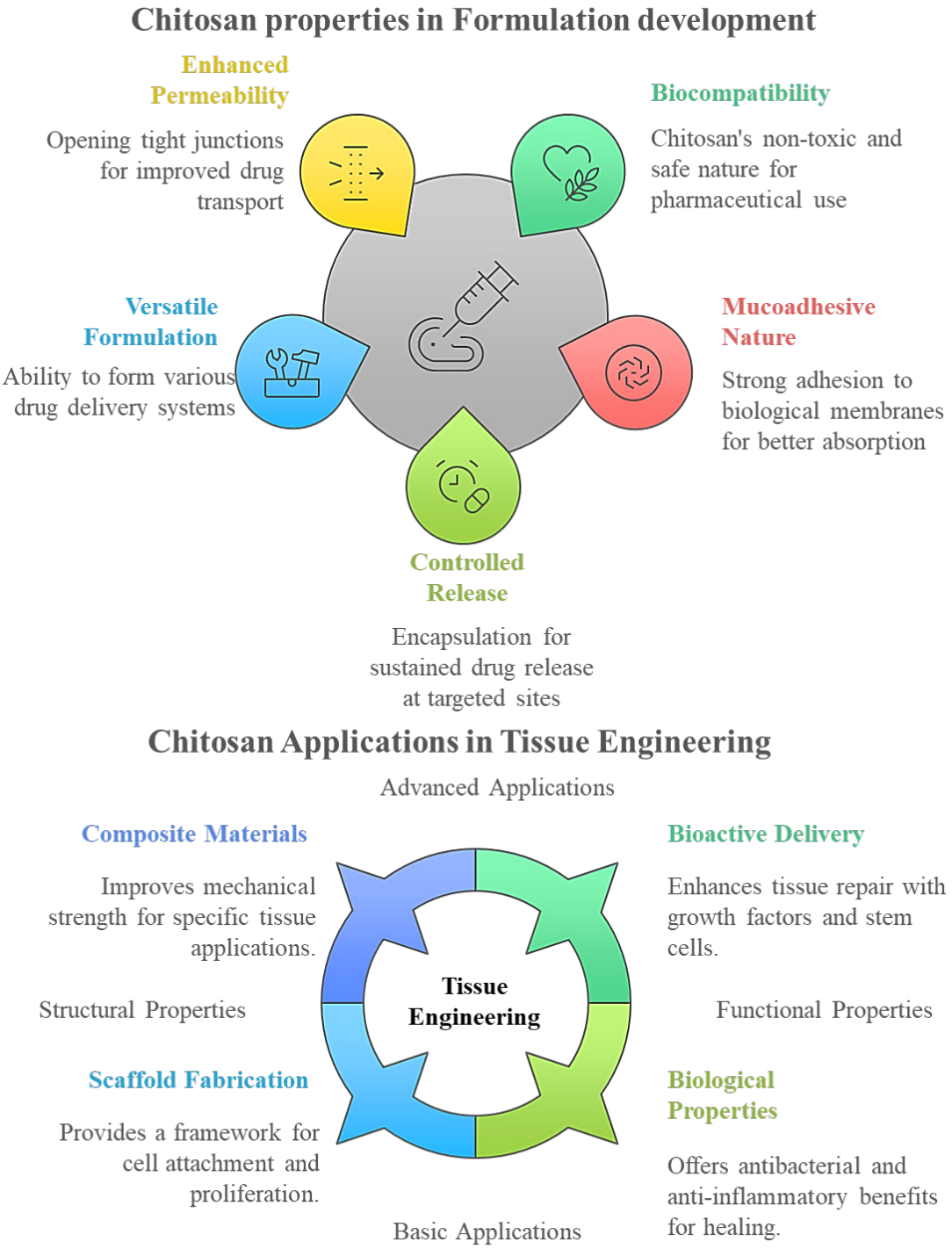

Figure 1: Key properties of chitosan in formulation development and its applications in tissue engineering.

are potential delivery systems for colon-targeted polyphenols; hence, considering the advancement of CS hydrogels for sustained drug administration, it is only a matter of time before they find wider use in OCDSs.[53,54]

Conventional colon drug delivery systems of CS

The biological mechanism's biological degradation of the medication distribution method is an important element of the drug delivery system. CS is the most common biodegradable polymer utilised in medication administration.[55] The drugs are delivered through the slow and controlled dispersion of the polymeric ingredients, which are covalently bonded to polymers like CS or dispersed in a polymeric matrix. Because of the enzymatic action of colonic microflora, particularly glycosidase, chitin can maintain its purity throughout the upper part of the gastrointestinal tract as well as discharge the encapsulated drug into the intestine. As a result, this microbiota- triggered polysaccharide-based technology has been demonstrated to be a viable technique for colon medication delivery.[46-49]

Tablets: Tablets are solid oral forms that come in a variety of shapes and sizes. The most popular of these aqueous matrix tablet forms as well as enteric-coated tablets for medication delivery in the colon. Because tablets provide a precise amount and are easy to develop as well as manage, they are used in many CS-based drug delivery systems.[56] CS has perfect qualities such as compatibility as well as compressibility, which qualifies it for use in the creation of solid oral dosage forms such as modified-release lattice tablets.[57] The medicine is disseminated in compressed polymers in matrix tablets, and these tablets retain their structure after ingestion unless the medication comes out at the site by interface degradation and transfer through the swelling matrix. In colon-targeted matrix tablets, CS is combined with other polymers to produce the tablet core. The ability of the pharmaceutical base expands whenever it gets into contact via liquid and form a gel-like obstacle results in slow drug diffusion from the core. Because of intramolecular and intermolecular hydrogen bonding, as well as its larger molecular weight, CS is known to have good filming capabilities. CS has been employed in a variety of tablet-coating activities for these qualities, either alone or in combination with another polymer.[58] Because CS is degraded in the colon by the action of enzymes generated by colon microorganisms such as cellulases and lysozyme, this sort of encapsulation promotes significant medication release.[59]

Hydrogels: A hydrogel is a cross-linked network made of a macromolecular hydrophilic polymer that can absorb a large amount of water. The liquid phase of hydrogels is water (and occasionally adjuvants). The solid phase maintains the gel's stability, allowing it to soak up and absorb huge amounts of water while staying insoluble in the liquid phase.[60] Hydrogels absorb water at rates ranging from 10% to thousands of times in their own volume. Hydrogels are biocompatible due to their high-water content, and the coherence of their mechanical properties with soft tissues aids and promotes the healing process. The mechanical compatibility of hydrogels with soft tissues also replicates the morphological as well as functional aspects of organ tissue. The viscoelastic nature of hydrogels provides minimal harm to the host's underlying tissues. Natural polymer-based hydrogels have also piqued the interest of scientists and researchers due to their increased biocompatibility, biodegradability, and significant capacity to distribute bioactive compounds in a regulated manner.[61] Three major forms of CS hydrogels have been created, with either reversible or irreversible gelation. CS can be directly connected with metal ions or irreversibly or chemically cross-linked into hydrogels. First, physically associated CS hydrogels, or "physical" hydrogels, are formed via reversible interactions among polymer chains. Second, CS can be cross-linked by coordination complexes such as Pt (II), Pd (II), and Mo (VI), resulting in the development of another type of hydrogel that is less suitable for biological applications. Third, chemically cross-linked CS hydrogels, which are formed through covalent connections among polymer chains, Because the gelation is irreversible, the resulting hydrogels are substantially more stable than the previously generated physically coupled hydrogels.[62]

Capsules: Capsules are an additional type of solid pharmaceutical dosage form that occurs when the key medicinal component and additives are encapsulated within a solid reservoir. The drug reservoirs are often comprised of collagen, chitin, as well as amylum. The primary benefit of capsules over tablets is that they subject the therapeutic constituent to severe compression pressures that could induce breakdown or make the component unstable. Chitin capsules are commonly used to transfer drugs to the intestine. The chitin capsule's enteric coating using Eudragit S-100/L-100 polymers has been used in studies to generate delayed drug release from the CS-Eudragit complex.[63] Budesonide, a corticosteroid prescribed to treat Crohn's disease, was delivered using this approach. Other medications, such as Prednisolone, which is utilised to manage inflammatory bowel disease, were subsequently administered via CS capsules. The CS capsule outperformed the standard gelatin capsule in this trial, as evidenced by a considerable elevation in prednisolone in the large intestine mucosa.[45,50]

Beads: CS is useful as a bead-forming material when combined with cross-linking agents or anionic polymers. Crosslinking compounds that are commonly employed include glutaraldehyde, glyoxal, glycol, and others. CS- alginate beads, CS-calcium-gellan gum beads, CS-gelatin beads, as well as CS-polyacrylic beads are some examples of anionic polymer-based CS-beads. Capecitabine encapsulated in CS succinate-sodium alginate beads, doxorubicin encapsulated in pectin/CS beads, and 5-fluorouracil encapsulated in dual-layered pH-sensitive alginate CS kappa- carrageenan beads are current applications of CS-based.[64]

Table 1: Applications of Chitosan via Different Routes of Administration.

Route of Administration	Applications/Uses of Chitosan	Key Features
Oral	- Drug delivery for poorly soluble drugs- Controlled release tablets and capsules- Mucoadhesive formulations- Nutraceutical formulations	- Enhances drug absorption- Mucoadhesive- Non-toxic and biodegradable
Vaginal	- Anti-microbial delivery systems- Bioadhesive gels and films- Delivery of anti-HIV agents, antifungals	- Bioadhesive to vaginal mucosa- Biocompatible- Improves residence time
Buccal	- Buccal patches for sustained drug release- Delivery of peptides and proteins	- High mucoadhesion- Enhances drug permeation- Bypasses first-pass metabolism
Ocular	- Eye drops and *in situ* gels- Controlled release systems for glaucoma or anti-inflammatory drugs	- Improves ocular retention time- Enhances permeability across cornea
Intestinal	- Colon-targeted drug delivery systems- Treatment of inflammatory bowel disease	- pH-responsive delivery- Protects drugs from gastric degradation
Parenteral (Injectable)	- Nanoparticles, hydrogels for targeted drug delivery- Injectable scaffolds for tissue engineering- Gene and vaccine delivery	- Biodegradable- Can form nanoparticles and microspheres- Safe for systemic administration
Transdermal	- Skin patches for drug release- Wound healing formulations- Cosmetic delivery systems	- Film-forming ability- Promotes wound healing- Moisture retention
Nasal	- Nasal vaccines- Rapid absorption of CNS drugs- Peptide delivery	- Mucoadhesive- Enhances nasal permeation- Bypasses blood-brain barrier
Rectal	- Suppositories and gels for local drug delivery- Treatment of hemorrhoids and IBD	- Enhances mucosal adhesion- Sustained release properties

Novel colon delivery systems of CS

This method of administration aids in reducing the toxicity or off-target effects of the medicine on the physiological system. The main advantages of CS over the use of natural solvents in manufacturing are its controlled release and solubility in aqueous acidic solutions. These particulate drug carriers may protect the drug from the severe environment of the gastrointestinal tract while also increasing colonic epithelial absorption.[65] CS has hydrophobic as well as hydrophilic components grafted onto it, resulting in the formation of an amphiphilic chitin analogue. The attachment of hydrophobic & hydrophilic molecules to the CS backbone promotes the production of polymeric particles. Carboxymethylation, as well as PEGylation, are the most commonly used changes to improve water solubility.[66] Spray-drying, coacervation, and sieving procedures are used to create CS nanoparticles.[67] Many studies have demonstrated that chitin nanoparticles can be used for targeted medication administration in the colon for active components including metronidazole, 5-fluorouracil, celecoxib, and others. Liposomes in medication delivery to the colon are dependent on pH polymers to aid in acidic liposome stabilisation and site-specificity. For higher acidic stability, Colon-targeted liposomal preparations of sorafenib were the particles of liposomes using glycol CS as well as pH-dependent Eudragit® S100.[68]

Microparticles/Microspheres

Microparticle, or microsphere, are small spherical particles with diameters ranging from 1 to 1000 nm that have the benefit of having a larger surface area, allowing the medicine to have a larger contact point to ensure efficient distribution. This contributes to the drug's lower toxicity or off-target effects on the biological system. The primary benefit of CS in drug delivery is its regulated release and solubility in aqueous acidic solutions, which outperforms the application of natural solvents during production. These particulate drug carriers may protect the medicine from the severe environment of the gastrointestinal tract while also increasing colonic epithelial absorption. Its dimension or degree of disintegration is affected by the form of chitin used as well as the crosslinking methods used.[69] CS-based microparticles were used in a variety of applications. To deliver prednisolone to the colon, CS microparticles are used, and they are produced by precipitating sodium sulphate. A new investigation found that CS-alginate microparticles might be used to transport mangostin, which exhibits a strong anti-proliferative effect

on colon cancer cell lines. The ionotropic gelation procedure using tripolyphosphate as the linking agent was used to create mangostin-loaded CS microparticles. A further investigation demonstrated the use of alginate-CS-coated microparticles in the increased selective distribution of protein nanoparticles in the therapy of chronic inflammatory gastrointestinal (GI) diseases.[70]

Liposomes

Liposomes are a biodegradable, biocompatible drug delivery device made of double-layered phospholipids. They can contain both hydrophilic & hydrophobic medicines. Liposomes in intestine-focused medication delivery Liposomal systems are coated with pH-dependent polymers to aid in liposome stabilisation in acidic environments as well as location specificity. Sorafenib-targeted liposomal preparations were designed by coating the liposomes with glycol CS and pH-dependent Eudragit® S100 for increased acidic stability. CS-coated liposomes containing 5-FU were reported to have increased antitumor efficacy in the treatment of colorectal tumors.[71] Nano-liposomes: Nano-liposomes are a newer approach for drug encapsulation as well as delivery in a submicron bilayer lipid vesicle. In terms of chemical, structural, as well as thermodynamic features, liposomes and nanoliposomes are fundamentally identical. Nanoliposomes, on the other hand, are different from liposomes in terms of improved solubility, bioavailability, and precision targeting.[72] CS-coated nanoliposomes demonstrated increased bioavailability, mucoadhesive properties, storage stability, and encapsulation efficiency. The nanoliposomes made by the ethanol injection technique performed better than those prepared by the regularly used dry thin-film method.[73]

Micelles

CS has grafted both hydrophilic and hydrophobic moieties, as a result of which an amphiphilic chitin compound is formed. The attachment of hydrophobic as well as hydrophilic components aids in the production of micelles made of polymers. Carboxymethylation, as well as PEGylation, are the most commonly used changes to promote water solubility. Whereas acylation or alkylation is the most widely used process to aid in the encapsulation of hydrophobic medicines.[74] Curcumin colon-focused drug delivery via CS-based pH-sensitive polymeric micelles was described in recent works. Turmeric is a hydrophobic polyphenolic compound with cancer resistance properties that is being extensively researched in the therapy of colorectal cancer. In this study, curcumin synthesis from turmeric-loaded tiny particles in Simulated Gastric Fluid (SGF) was restricted to around 20%, but it greatly increased following interaction with Simulated Intestinal Fluid (SIF) (50-55%) and Simulated Colonic Fluid (SCF) (60-70%). In another investigation, an andrographolide analogue was transported to the colon using folic acid-functionalized amphiphilic chitin micelles made of polymers. Furthermore, due to the continuous release of 5-fluorouracil via delayed doxifluridine conversion, the self-assembled micelle displayed enhanced anticancer activity.[75]

Mucosal Drug Delivery systems of CS

CS is an intriguing biological substance that could be used in mucosal as well as transmucosal medicine administration methods. The mucosal layer is dynamic because it is constantly undergoing stages of production, breakdown, darkening, and digesting. Mucins have an important function in the attachment of cells to the mucous membrane, and they also assist in the formation of a viscous matrix that traps nanoparticles as well as pathogens. Nasal and pulmonary medication delivery via mucosal surfaces is gaining popularity as alternate routes of systemic administration.[56-59] These surfaces are utilised to administer the medicine over time at a controlled rate using a mucoadhesive agent. Because CS has mucoadhesive qualities, it can be used to create bioadhesive dosage forms that can be administered orally through the (ocular, nasal, buccal, gastro-enteric, and vaginal-uterine) routes. The gastrointestinal mucus layer is widely recognised to contain anionic substances such as sialic acid and sulfonic acid. Muco-adhesion takes place involving anions from the gel-like properties of the mucous barrier as well as multiple cations on CS amino groups. CS chains entangle in the mucus layer at the microlevel, and therapy with polyanionic medicines, polymers, and complexing agents increases mucoadhesive characteristics.[76] Trimethylation and PEGylation of CS boost mucoadhesive capability by roughly 3.4 times, owing to an increase in net cationic charge on amino groups. The nasal mucosa has high permeability and allows drugs easy access to the absorption site. Because of the availability of free amino groups in an acidic solution, CS protonates, and the resulting soluble polysaccharide is positively charged, allowing it to bind firmly to negatively charged surfaces such as cell surfaces as well as mucosa. The mucoadhesive feature of CS as well as its derivatives improves the residence time of dosage forms in the GIT - the buccal region, stomach, sections of the small intestine and colon, as well as other body regions with a mucous layer such as the vaginal and nasal mucosa.[77] This protects medicines against degrading enzymes, allowing for regulated medication administration while also increasing penetration capability. Electrostatic forces of attraction between the charged polymer as well as mucin's negative-charged glycoproteins appears to be the primary cause of CS mucin interactions, which are augmented by other variables like bonds of hydrogen as well as hydrophobic interactions.[78,79] As a result, CS composition may considerably enhance the residence time of medication on tissues and cells and demonstrate sustained release of pharmaceuticals there, improving drug bioavailability as well as reducing drug administration frequency. CS increases the permeability of medicines through the epithelial cell barrier by enhancing the paracellular transport of active compounds.[80] CS interacts with integrins, stomach tight junctions, and intestinal cell epithelial

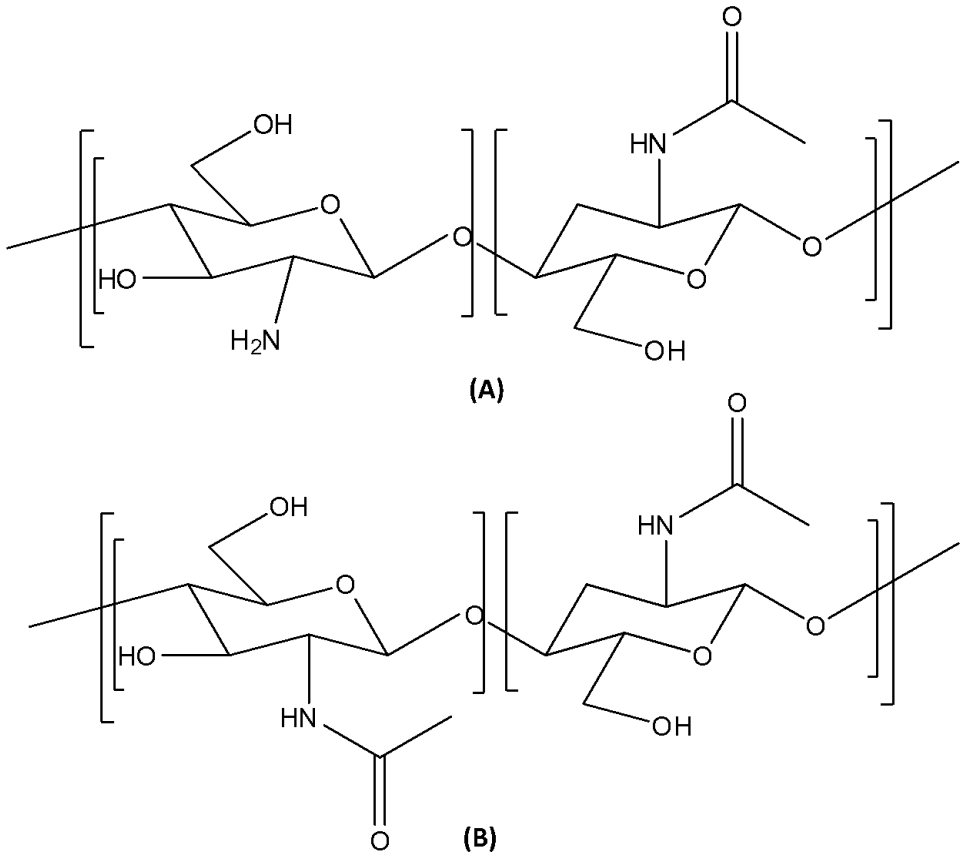

Figure 2: Chemical Structure of (A) CS; (B) Chitin.

layers, which it automatically opens, enhancing drug permeability and thus systemic absorption. CS is a particularly promising biological material in mucosal therapeutic administration, not only because of its numerous beneficial biological properties, the most important of which are addressed further below, but also because of its fundamental biological properties and capabilities, particularly wound healing, anti-inflammatory, as well as antimicrobial characteristics.[81] One of the most important advantages of CS in the mucosa is that it accomplishes its beneficial effects without eliciting an immunological response, swelling, or other substantial negative influence on the biological system. Several reviews[82] have focused on biocompatibility, including the lack of cytotoxicity of CS carriers. *In vivo*, CS is rapidly degraded by the hydrolytic actions of an enzyme known as lysozyme, a non-specific enzyme found in mucous, as well as chitinases and N-acetyl-D-glucosaminidases, enzymes produced by colon- residing microorganisms. This allows for the safe distribution as well as breakdown of locally administered CS-based mucous delivery devices. The CS permeability-boosting action can be enhanced further by appropriate chemical alterations. Thiolate-CS derivatives, in particular, in addition to better mucosal adherence, may have extra contacts involving the surface of the mucous membrane because of the formation of disulfides with mucin residues of cysteine,[83,84] which also demonstrated an increased penetration enhancer impact over unaltered CS. Mucoadhesive polymers that gel *in situ*, such as CS and its derivatives, can be particularly valuable for enhancing the efficacy of mucosal medication administration. On the one hand, they do indeed make it easier to use the liquid formulation at the different delivery sites; on the other hand, their rapid *in situ* gelation at the site of attraction causes a significant increase in viscosity, which improves the mucoadhesive polymer impact and lengthens mucosal residence time, reducing errors like rapid clearance and medication outflow from the intended area.[85] CS amlodipine besylate The external phase, which is a 1:1 mixture of heavy and light liquid paraffin, was optimised to produce microspheres via a straightforward emulsification cross-linking procedure. It was discovered that as the amount of CS was raised, so did the percentage of *in vitro* mucoadhesion. This could be because the amount of polymer boosted and increased the number of amino groups accessible for interacting with the sialic acid residues in the mucus layer, resulting in an elevation in microsphere *in vitro* mucoadhesion. CS nanoparticles on insulin bioavailability, and it was discovered that insulin-loaded CS nanoparticles improved nasal absorption of insulin and had a sustained impact. It was discovered that CS-based nanoparticles improve both mucosal and systemic immune responses.[86]

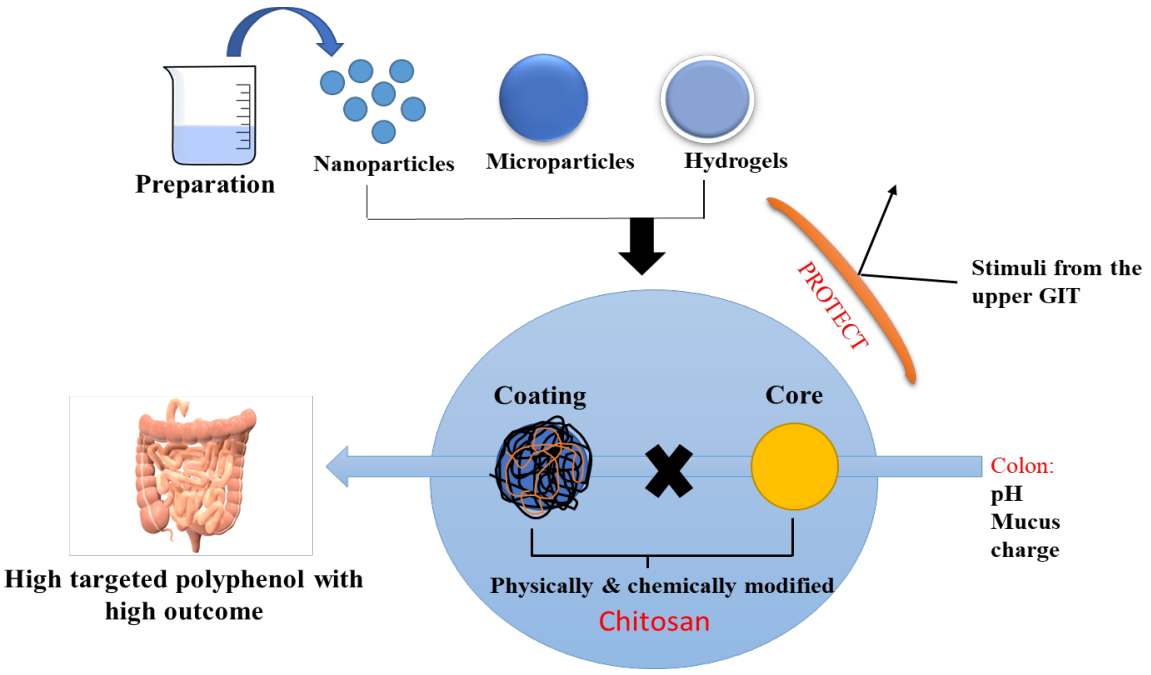

Figure 3: Schematic illustration of chitosan-based colon-targeted drug delivery system.

Ocular Drug Delivery systems of CS

It is extremely challenging to analyse the eye as a separate organ from the standpoint of drugs. This is because delicate ocular structures such as the uveal tract and retina are present. Furthermore, the presence of tissue barriers to drug penetration such as the lipophilic corneal epithelium, hydrophilic corneal or scleral stroma, conjunctival lymphatics, choroidal vasculature, and blood-ocular barriers makes ocular medication delivery even more difficult.[82] CS was chosen for ocular medication delivery because of its excellent mucoadhesive as well as absorption-boosting capabilities, as well as its superior biocompatibility with ocular structures. CS is a very attractive biomaterial in ophthalmology, not only due to its advantageous biological features but also because of its inherent biological activity, which may have an effect on ocular therapies. The use of CS-based nanostructures in the administration of ocular medicines has received extensive attention.[87] Because of the low bioavailability of topically administered ocular medicines, repeated instillation is required to achieve therapeutic effect. An extended release of the medication in the ocular region could alleviate this problem. The ability of CS-based colloidal systems to act as transmucosal drug carriers has also been found, with the possibility of speeding up drug delivery to the inner eye or accumulating drugs in the corneal epithelia. The use of CS-based colloidal solutions *in vivo* increased ocular medication bioavailability significantly. CS is a useful product for the creation of ocular drug delivery systems due to its benign nature, permeation-boosting qualities, and physicochemical features. Hydrogels, nanoparticles, and coated colloidal systems[88,89] are CS-based formulations employed for ocular drug delivery. Using its *in situ* gelling capabilities, CS can be administered or disseminated across the ocular surface in practically liquid form before changing into gel form. This results in a longer ocular residence period and increased therapeutic efficacy. Indeed, the capacity of CS to extend precorneal drug retention through its viscosity-increasing effect, capacity to bind ofloxacin, and, most likely,[24,45,53] mucoadhesive qualities. PLL (poly-L-lysine (PLL) and CS both had a positive surface charge, but only CS-coated nanocapsules improved indomethacin ocular penetration over uncoated nanocapsules. The increased absorption was caused by an unidentified feature of CS rather than the positive surface charge. Furthermore, as determined by the ocular-lesion indices, CS-coated particles demonstrated good ocular tolerance. In comparison to the pure drug, CS microspheres induced a modest decrease in the *in vitro* dissolution rate of acyclovir. Nonetheless, acyclovir-loaded CS microspheres provided considerable *in vivo* sustained ocular drug release. As a result, the scientists hypothesised that, in addition to its mucoadhesive properties, CS can slow the rate of medication release. These tested technologies are promising approaches to increasing medication's ocular bioavailability. CS has been found to have wound healing and antibacterial properties in addition to being a key component in drug delivery systems. These consequences may be extremely effective for the medical management of a variety of eye illnesses. The recognised enhancement of wound-healing activity of CS degradation products inspired the notion of utilising CS in corneal wound healing. More specifically, it was believed that CS would play an active role in enhancing keratocyte migration, resulting in faster collagen formation and enhanced corneal healing. Furthermore, the prolonged pre-corneal residence duration of CS solutions supported its use in the treatment of dry eye and keratoconjunctivitis sicca. Along with this distinct

capability, CS has other appealing properties, which are discussed in the following paragraphs, that make it a unique option for ocular drug delivery.[90,91]

Nasal Drug Delivery systems of CS

The bioavailability of nasally delivered medications is frequently limited because of respiratory challenges like minimal permeability of the membrane, a short local residence period, and a rapid conversion rate of secretion in respiratory cavities. Several medications, particularly peptides and proteins, are gaining interest in the development of nasal administration methods. CS nanocarriers were created using CS and CS derivatives with varying molecular weights and degrees of deacetylation and derivatization. In this case, the medication may be discharged from the nanocarrier in the nasal cavity,[92] or the nanocarrier might be transferred with the encapsulated drug across the nasal mucosa, potentially transcellular via the M-like cells found in the lymphoid tissues at the back of the nose. Various absorption enhancers, including mucoadhesive nasal administration devices, have been studied to enhance the bioavailability of these drugs. They may offer longer interaction between medication formulation and absorptive sites in the nasal cavity by delaying mucociliary formulation clearance. Mucoadhesive systems can take the form of powders, liquids, or liquid *in situ* gelling systems. However, the majority of these are related to negative side effects, such as irreversible alterations in the nasal mucosa. Several investigations have reported the use of CS as a safe protein delivery technology via the nasal cavity.[93] CS has been studied as an auxiliary agent in nasal medication delivery systems, primarily due to its mucoadhesive as well as penetration-increasing qualities. It was discovered that insulin-loaded nanoparticles of CS improve nasal medication absorption more than equivalent CS solutions. CS dramatically improved the uptake of both polar small-molecular-weight medicines and bigger peptides as well as proteins over the nasal epithelial membrane. The impact of diverse CS molecular masses, degrees of acetylation, salt forms, derivatives, and formulations such as solutions, freeze-dried powders, gels, nanoparticles, or microspheres on absorption.[94] An additional intriguing approach is to use CS as a gel, such as thermosensitive CS or derivatized CS hydrogel, which, when utilised as drops or a spray into the nose at 35-37°C, forms a gel capable of decreasing nasal mucociliary elimination rate and, to some extent, allowing sustained drug release. The potential of such thermosensitive gels to improve residence time in comparison to nongelling control systems, while the presence of CS boosted permeability throughout the membrane, allowing for improved drug bioavailability. In contrast, a mucoadhesive gel spray based on CS and polyethylene glycol 400 was found to increase nasal absorption of 9-tetrahydrocannabinol (THC), whose bioavailability is reduced by first-pass metabolism following oral administration. The idea of a temperature-sensitive gelling CS formulation for nasal drug delivery is very interesting because the product would beneficially effects from being able to be utilized as a liquid formulation with added the advantage of enhanced mucoadhesive ness after gelling, resulting in prolonged residence in the nasal cavity and potential for increased absorption. A nasal gelling substance with a low molecular weight. Furthermore, CS microspheres have been investigated as an alternate nasal delivery route for several medications. The microspheres were created using various ways such as spray drying or emulsification followed by crosslinking and were generally given to the nasal cavity as a dry formulation that demonstrated the capacity to absorb water as well as form a mucoadhesive gel.[95,96] Nano or microemulsions are one of the delivery systems incorporating CS as a mucoadhesive/absorption enhancer that has been intensively investigated for nose-to-brain augmentation of various medications. The use of Nano emulsions for improved nose-to-brain drug delivery has resulted in significantly increased drug uptake into the brain. The introduction of CS as a mucoadhesive ingredient in the formulation may enhance this outcome. There has been little, if any, effort in these investigations to explicitly target the olfactory region.[97]

Nanocarriers in Tumor-Targeted Drug Delivery of CS

The targeting of chemotherapeutic medications in a particular area will have two effects. To begin, optimum site delivery reduces the overall dosage required, resulting in more effective treatment. Second, by reducing the overall drug quantity, drug-induced adverse effects will be avoided or greatly reduced.[98] Tumour-targeted medication delivery capitalises on the distinctions between malignant and healthy tissues. The tumour's surroundings alter as the tumour grows. Because of increased metabolism and growth rates, oxygen supply becomes inadequate and glucose turns to lactate, lowering the interstitial pH of tumour tissue. Hypoxia as well as glucose deficiency, when combined, cause the development of new blood vessels (angiogenesis), which is essential for tumour growth, movement, and survival.[99] The high toxicity of most anticancer medicines is a key bottleneck for conventional cancer chemotherapeutics. Furthermore, anticancer medications frequently have poor water solubility, necessitating the use of natural solvents or detergents in clinical applications, leading to adverse reactions such as venous irritation and respiratory distress. Among the numerous techniques, nanocarriers (especially those with sizes ranging from 10 to 100 nm) have several distinguishing characteristics, such as a high surface area-to-volume ratio. The primary benefits of nanocarriers involve their elevated solubilization potential, superior encapsulation, changed absorption pathways, avoiding metabolic degradation within the gastrointestinal tract, chemical versatility of compounds suitable for nanomedicines, flexibility in surface functionalization, drug and disease-specific tailor-made design capability, targeting potential, and the capacity to carry a wide range of drug compounds (Figure 4). Nanocarriers can also be created to transport medicinal chemicals that set them apart

from other cancer therapies. Nanoparticles have lately gained popularity as a means of addressing the toxicity issues associated with conventional cancer therapies.[100]

Micelles, liposomes, dendrimers, carbon nanotubes, nanocrystals, polymeric nanoparticles, and nanogels have all been tested for cancer chemotherapy.[101] Because of their physicochemical as well as biological features, nanoparticles have significantly improved medication solubility, stability, biocompatibility, release profile, and non-specific toxicity. Nanotechnology technology provides NPs with unique active or passive targeting qualities that allow medications to be delivered to precise target sites.[102] The capacity to tailor therapy to extremely specific cancer cells also take advantage of a disease's framework, as many tumours excessively express specific antigens, even on their surface. As long as the targets for a certain cancer cell type can be identified with certainty and do not exist in considerable amounts elsewhere in the body, they are suitable targets for drug administration.[103]

CS based tissue engineering

Tissue engineering is a new topic in regenerative medicine that integrates biology with engineering expertise to create alternatives for damaged tissues.[104] Tissue engineering is primarily concerned with healing damaged or sick tissues and organs by manipulating the biological microenvironment. Tissue engineering entails numerous processes, including cell proliferation, differentiation, and Extracellular Matrix (ECM) formation. Because of their distinct physicochemical features, CS and its derivatives provide numerous advantages as a cell and tissue-supportive biomaterial. Therefore, CS looks to be a promising choice for the fabrication of such biomaterials, which could substitute for missing or damaged tissue and organs and permit cell adhesion as well as proliferation if 3D scaffolds are formed. An excellent tissue engineering scaffold is a template utilised for 3D tissue growth that offers a porous structure for tissue growth, oxygen diffusion, as well as nutrition distribution to imitate the natural tissue microenvironment.[105] Tissue engineering scaffolds need biological and mechanical qualities similar to those of the natural ECM to function. The following characteristics have been recognized as important when producing scaffolds for tissue engineering: 1) the presence of pores that are linked together with appropriate proportions to encourage tissue incorporation as well as vascularization; 2) regulated biological degradation as well as bio-restorability to correspond with tissue regeneration; 3) the existence of advantageous interfacial chemistry for alterations as well as attachment of cells and differentiation, including proliferative progress; and 4) the best kinematic characteristics that are similar to tissue replacement.[106] Body reliability, mechanical characteristics, recovery, as tissue regeneration ability, along with scaffold shape & porosity, should all be considered during the construction of implantable scaffolds. It may communicate with neighbouring cells, preserving the phenotype of the rejuvenated tissue. A great scaffold must be biodegradable, biocompatible, and encourage cell adhesion, proliferation, and retention of cell metabolic activity.

Furthermore, scaffolds with suitable pluripotent stem cells, angiogenic impact, and sustained nutritional resources will achieve tissue regeneration in a variety of ways. An implanted scaffold must be compatible with the body and have appropriate mechanical properties, shape, porosity, healing, and tissue replacement capabilities. CS is an effective option for tissue engineering applications due to its naturally occurring antibacterial characteristics, biodegradability, as well as biocompatibility. CS-based scaffolds are frequently employed in engineering applications. Skin tissue engineering is used to create a scaffold that helps with skin healing. Furthermore, it shouldn't jeopardise the skin's physical characteristics and regular functions, such as obstacle activities, thermal regulation, exposure to Ultraviolet (UV) defence, and cosmetic functions. The standard treatment method for serious skin lesions and injuries is to use skin grafts. These transplants, on the other hand, provide both functions as dressing components as well as skin replacements, providing a good healing stimulus.[107] Additionally, there have been numerous studies on the use of chitin as a biological material for skin tissue repair. A synthetic graft, on the other hand, must meet a few basic parameters in order to effectively encourage bone growth. Among these are osteoconductive, porosity, biocompatibility, and biodegradability. Synthetic scaffolds have the benefit of being able to be coupled by combining cells or development factors to increase osteoconductive activity, thereby boosting tissue renewal.[108]

3D-Scaffolds for Tissue Engineering

Hybridising biomaterials yields 3D-printed scaffolds with acceptable mechanical strength for implantation in high-load areas. Body compatibility, mechanical characteristics, scaffold morphology, as the porosity, in addition to healing and tissue replacement ability, should all be considered throughout the construction of implantable scaffolds. As a result of the biomimetic element of the customised scaffold, bone repair is accelerated. The biocompatible, bio absorbable, osteoconductive, osteoinductive, and osteointegrative, including biodegradable, features of the scaffolds promote osteogenic cell adhesion, growth, and regulation. 3D-printed scaffolds use a wide variety of polymers, with natural polymers having greater biocompatibility and bioactivity. CS has been widely employed as a biopolymer in biomaterials for bone tissue regeneration.[109] According to the literature, the primary requirements for the development of tissue engineering scaffolds involve the following: the scaffold should not induce acute or chronic reactions; it should be renewable so that the healed tissue can replace the biomaterial; it should have surface characteristics that encourage cell attachment, differentiation, as well as proliferation; it should have suitable mechanical properties for handling and mimicking the injured tissue; and it should be manufactured in a biocompatible

manner. Because of its extraordinary qualities, CS looks to be a viable candidate for the fabrication of such biomaterials, which might replace missing or damaged tissue and organs and allow cell attachment and proliferation if 3D scaffolds are formed. As a result, approaches for precisely shaping CS hydrogels and foams (or sponges) as relevant 3D scaffolds for tissue engineering have been established. Before neovascularization, these scaffolds also induce vascular invasion and distribution, which leads to stimulation of cell recruitment and adhesion, which enables cellular permeation, proliferation, and differentiation.[110,111]

CS in cartilage tissue engineering

Articular cartilage is a connective tissue in the joints that is non-vascularized and has an inadequate cellular density. It is frequently injured as a result of an accident or natural wear and strain. Articular cartilage supports the underlying subchondral bone by resisting compressive stresses. It also serves as a low-friction surface on which to pivot a diarthrodial joint. Osteoarthritis, which affects roughly 300 million people globally and is the largest cause of impairment among the aged, is one of the most frequent joint diseases affecting articular cartilage. Scaffolds in three dimensions (3D) are required for the formation of synthetic articular cartilage. Ideal scaffolds are biocompatible and bio absorbable, with predictable porosity and degradation rate. They offer to scaffold for new tissue ingrowth, and their mechanical properties are similar to those of native tissue, increasing the likelihood that the repair procedure will be compatible with the host's tissue physiology.[112] Because of its structural closeness to numerous GAGs found in articular cartilage, CS has been used as a scaffolding material in articular cartilage engineering. This is significant since GAGs are thought to play a key role in modifying chondrocyte shape, development, and functioning. Alginate is another biomaterial possibility for cartilage engineering, although it has poor cell adhesion. Iwasaki *et al.*,[113] described an alginate-based CS hybrid polymer fiber that demonstrated greater cell adhesion as well as proliferation *in vitro* than alginate. These hybrid polymer fibers demonstrated improved tensile strength, hinting that they could be used to create a 3D load-bearing scaffold for cartilage regeneration. CS as well as gelatin were used to create the protein-based hydrogels for cartilage tissue creation. A freeze-drying approach was used to create cellulose, the collagen, with hydroxyapatite hydrogel. Collagen is an important component of the cartilage ECM of joint cartilage and regulates chondrocyte expression as well as chondrogenesis. However, key issues with collagen involve quick breakdown & poor mechanical characteristics. Collagen was therefore mixed with cellulose to enhance its physical properties, decrease its biological degradation rate, and boost cell adhesion and proliferation.[109-111]

The hydrogel was made up of separate interconnecting macropore (30-75 μm) or tiny pores (<2 μm), allowing it to transfer biologic fluids via convection.[114] It also discusses transplantation approaches for cartilage restoration, namely synthetic grafts, which have aroused a lot of interest. Under

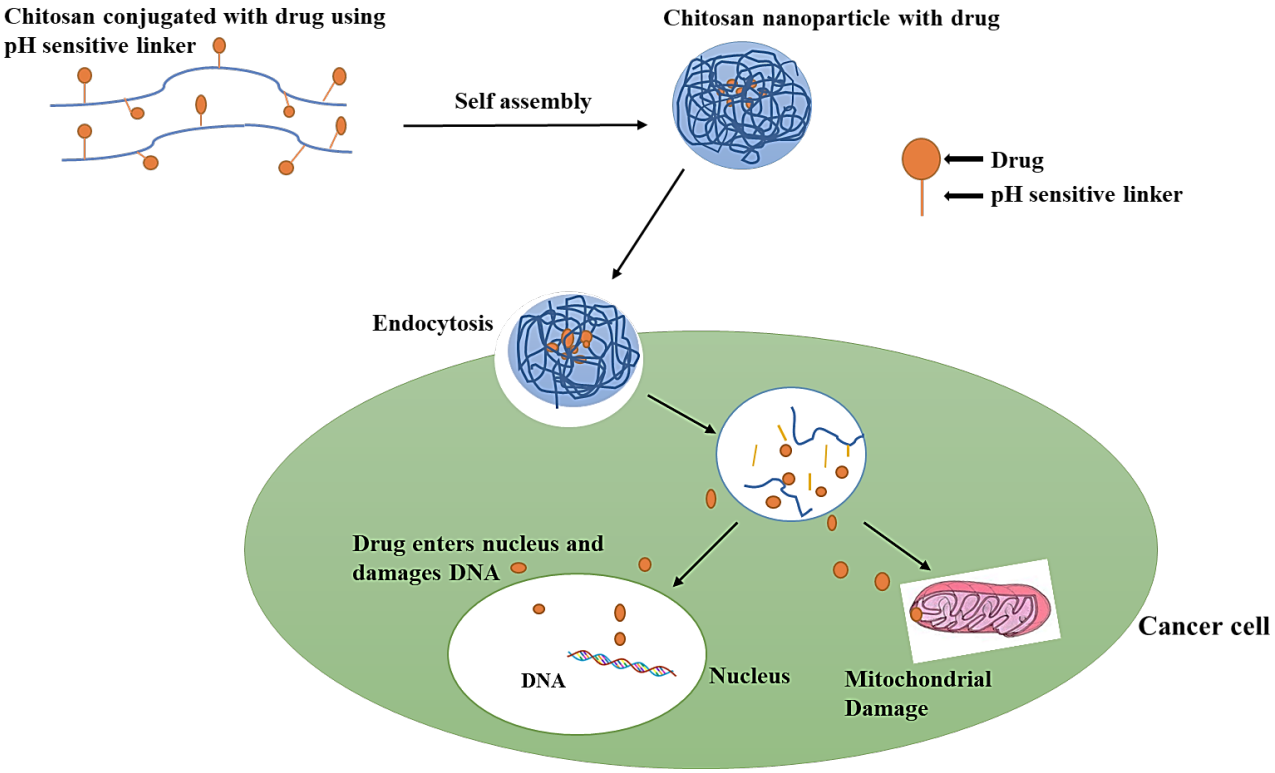

Figure 4: Schematic representation of drug release from chitosan-based nanocarriers and intracellular drug action.

normal monolayer culture circumstances, chondrocytes (cells that make up healthy cartilage) have an unstable phenotype. The ECM significantly stabilizes their phenotype. The ECM has also been linked to chondrocyte development, metabolism, and activity. The abilities of CS as a scaffold component for adhesion of cells, transformation, and multiplication are well established. CS is found to maintain indistinguishable chondrocytes and to keep chondrocytes secreting cell-specific ECM. As a result, CS combinations with various polymers have been produced as well as used in cartilage engineering. Compounds that include Bone Morphogenetic protein (BMP7) and Transforming Growth Factor-1 are encapsulated (TGF-1), and other techniques have been discovered to improve the chondrogenic characteristics of CS-based polymers. BMP7 inclusion has been demonstrated to increase chondrocyte cell proliferation in the articular environment as well as partial articular surface healing.[115,116] CS has been widely mixed with another protein-based biopolymer, silk fibroin. Silk fibroin, which is often generated from Bombyx mori cocoons, has received interest in tissue engineering of cartilage because of its biological compatibility, superior mechanical characteristics, delayed disintegration, and cell attachment or proliferation. CS derivatives were combined with silk fibroin to create hydrogels for uses in cartilage tissue, and carboxymethyl CS as well as silk fibroin hydrogel was created by chemical cross-linking with Horseradish Peroxidase & Hydrogen Peroxide (HRP/H_2O_2). Carboxymethyl CS (CMC) is one of the most widely utilized CS derivatives. CMC derivatives may communicate with tissues, leading to proliferation of cells as well as regrowth of tissue.[117]

CS in intervertebral disc tissue-engineering

The Intervertebral Disc (IVD), the biggest non-vascular tissue in the body of an individual, is part of the "three-joint complex" made up of fibrous cartilage. The discs in these joints allow for motion, weight bearing, and flexibility while also safeguarding the neuronal circuitry of the spine as a whole. IVD is mostly composed of fibre-content-filled hydrogels and is divided into three tissue zones. The three areas are the Nucleus Pulposus (NP), Annulus Fibrosus (AF), as well as Vertebral Endplates (VEPs). Spine tissue engineering applications for CS include three distinct fields: spine fusion, gene therapy, and nucleus pulposus regeneration. Many local biomechanical aspects are evaluated when a bone graft alternative is used during spinal fusion surgeries, based on the type and position of the chosen graft. The most significant avascular tissue in the human body,[118] the Intervertebral Disc (IVD), is part of the "three-joint complex" formed of fibrous cartilage. These joints support motion, weight-bearing, and flexibility while protecting the neural architecture of the entire spine. Spine tissue engineering applications for CS include three distinct fields: spine fusion, gene therapy, and nucleus pulposus regeneration. Many local biomechanical aspects are evaluated when a bone graft alternative is used during spinal fusion surgeries, based on the type and position of the chosen graft. A potential use for CS might be the creation of a composite graft material with a predictable degradation rate and a microporous structure that would enable the linkage of growth agents as well as osteogenic cells for spinal fusion. A degeneration cycle causes proteoglycan reduction, disorganisation of the ECM, rips in the IVD, ejection fragment formation, and height of the disc decline.[119,120] Self-regeneration in IVD is unimpressive due to its non-vascular nature; in particular, atrial tissues have very little ability to repair themselves following injury or ripping. Suture fixation was a prominent therapy for a torn AF, as was disc resection and union of the neighbouring Vertebral Bodies (VBs). However, because of the changed segmental motion, this leads to additional deterioration. As a result, the medications used to combat IVD issues have been ineffective. CS-based polymers may be a viable method for treating IVD illnesses because of their biodegradability, low toxicity, cytocompatibility, or hemostatic effect. An intravenous solution composed of chitin and nanofibers of cellulose was produced by NP augmentation for IVD healing and regrowth. The implant was implanted at the injection site, restoring the elastomeric properties of the discs, which had been associated with the restoration of disc kinematics. Furthermore, restoring and increasing the level of the disc lessened or eliminated back pain by eliminating nerve root compression. A potential transparent injectable treatment for IVD regrowth was found without requiring invasive surgery. CS-based gels and temperature-responsive hydroxybutyl CS are two further IVD tissue engineering projects.[121,122]

CS in bone tissue engineering

When a bone is broken, it has an amazing capacity to mend itself. In most situations, the immobilisation of bones at broken sites results in recovery over time. Major bone deformities, on the other hand, may necessitate intervention in the form of grafts to aid the physiological healing process. Grafts for this procedure can be derived from a secondary site in the patient's body (autograft) or cadaver bones (allograft). Synthetic graft-based bone tissue engineering technologies offer intriguing possibilities for autografts and allografts. A synthetic graft, on the other hand, must meet a few basic parameters to effectively encourage bone growth. Since osteoblasts in culture were demonstrated to develop and deposit mineral-rich matrix, CS has been widely used in bone tissue engineering. CS is also biocompatible (minimises extra local inflammation), biodegradable, and can be shaped into porous shapes (allowing for osteoconduction). Several studies on the usage of CS-Calcium Phosphate (CP) composites for these reasons, have been conducted.[123] A 3D macroporous CP bioceramic with porous CS sponges inserted. A nested CS sponge in this scaffold increased the mechanical strength of the ceramic phase via matrix reinforcement while preserving the osteoblast phenotype. Similarly, for bone engineering, gentamycin-conjugated macroporous CS scaffolds

reinforced with beta-tricalcium phosphate ßTCP and CP have been produced. A freeze-drying procedure is used to create a macroporous CS-gel/b-TCP composite scaffold for bone tissue engineering. The impact of composite suspension concentration as well as freezing temperature on its capacity to withstand compression by the scaffold was studied in this study. CS is utilized as an auxiliary in bone cement to promote injectability while maintaining surgically acceptable chemophysical characteristics. When Calcium Phosphate Cement (CPC) is mixed with CS, octocalcium phosphate is formed, which has been demonstrated to increase injectability as well as strength. Many of these CS gel composites are primarily intended to treat non-load-bearing bone deformities. The capacity of CS to bind growth factors makes it a good candidate for bone tissue engineering. CS-based materials bind growth factors and release them in a controlled manner due to their cationic character and predictable breakdown rate. CS has been utilized to change the surface characteristics of prosthetic components to improve osteoblast adhesion. Enhanced osteoblast adhesion as well as proliferation were observed on a Titanium (Ti) surface coated with CS using silane- glutaraldehyde chemistry.[124] The bond strength of the CS coating with titanium was 1.5-1.8 MPa, and it deteriorated slowly over 8 weeks. While CS bond strength was found to be less than that of CP coatings, these findings supported the notion that CS coatings could increase the osseointegration of Ti implant devices routinely used for orthopaedic and craniofacial implants. Cellulose & Hydroxyapatite (HA) are both biocompatible as well as biodegradable compounds with osteoconductivity. As a result, they have been employed alone or in conjunction with bone tissue engineering applications. CS and alginate-biodegradable porous scaffolds were found to have significantly improved physical and physiological characteristics. The scaffolds increased osteoblast adhesion and cell growth through the formation of a calcium matrix. CS was also discovered to strengthen the fibre network of collagen and prevent distortion during fluid flow. CS collagen composite films were demonstrated to improve osteoblast proliferation, differentiation, as well as matrix mineralization in a different investigation.[125,126]

FUTURE DIRECTIONS

In particular, the possibility to generate structures with predictable pore sizes and degradation rates make CS a suitable material as a bone graft alternative in orthopaedic procedures. However, efforts to improve the mechanical properties of CS-based composite biomaterials are essential for this type of application. Of great importance is the ability of CS to bind anionic.[127] The exact arrangement of biological components, cells, and macromolecules in the scaffolds is made possible by a revolutionary production technique called 3D scaffold. By adjusting the fabrication conditions, it is possible to simulate natural tissues with specific biological and mechanical characteristics useful for tissue restoration.[128] According to recent papers, 3D-printed chitosan structures can be used for medication administration, hepatic, vascular, skin, and even cartilage regeneration. However, further research is being done on these useful uses. Furthermore, 3D printing has only recently been used to create a small number of chitosan and other substances. Consequently, future research should investigate a number of innovative chemicals.[129,130]

CONCLUSION

CS, a naturally biodegradable and non-toxic polymer, has shown potential as a biomedical material. This structure confers not just very desirable physicochemical features on this polysaccharide but also specific interactions with proteins, cells, and biological beings. Furthermore, after protonation in acidic circumstances, this polycation exhibits enhanced solubility in aqueous media, allowing for the production of this substance in extremely mild circumstances in a wide range of geometries. CS has a wide range of uses, involving tissue engineering and drug delivery. Therefore, this polymer is very attractive for biomedical uses, notably in tissue engineering. CS is now one of the most potential biopolymers for tissue engineering, including orthopaedic applications. The ability to create structures with predictable pore sizes and degradation rates, in particular, makes CS a viable bone transplant option in orthopaedic treatments. Attempts to enhance the mechanical properties of CS-based composite biomaterials, on the other hand, are critical for this type of application.

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ABBREVIATIONS

ALT: Alanine aminotransferase; **A-ß:** Amyloid beta-peptide; **Bax:** BCL2-Associated X Protein; **Bcl-2:** B-cell lymphoma 2; **BHT:** Butylated hydroxytoluene; **CCL_4:** Carbon tetrachloride; **COX:** Cyclooxygenase; **c-Src:** c-sarcoma; **DLD:** Dibutyl Lead Diacetate; **DM:** Diabetes Mellitus; **DNA:** Deoxyribonucleic acid; **DPPH:** 1,1-diphenyl-2-picrylhydrazyl; **EGFR:** estimated glomerular filtration rate; **EMT:** Emergency medical technicians; **ERK1/2:** Extracellular signal-regulated kinase ½; **FL:** fibroblast-like synoviocytes; GI: Gastrointestinal; **HeLa:** Henrietta Lacks; IL: Interleukin; **INOS:** Inducible nitric oxide synthase; **Iκßα:** I-kappa ß alpha; **JAK-2:** Janus kinase 2; **MAPK:** Mitogen-activated protein kinase; **MCF-7:** Michigan Cancer Foundation-7; **MCP-1:** Monocyte chemo-attractant Protein-1; **MDA:** Malondialdehyde; **MMP:** Matrix metalloproteinases; **MMPs:** Matrix metalloproteinases; **NADPH:** Nicotinamide Adenine Dinucleotide Phosphate Hydrogen; **NF-kß:** Nuclear factor-kß; **Nrf2:** Nuclear factor erythroid 2-related factor 2; **p53:** Tumor

protein p53; **PAK:** Pancreas after kidney; **PGE2:** Prostaglandin E2; **PTU:** Propyl-thiouracil; **ROS:** Reactive Oxygen Species; **STAT3:** Signal transducer and activator of transcription 3; **STZ:** Streptozotocin; **TLR:** Toll-like Receptor; **TNF-α:** Tumor Necrosis Factor-α; **TWIST:** Twist-related protein; **WBC:** White Blood Cell; **XIAP:** X-linked inhibitor of apoptosis protein.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUMMARY

Chitosan, a naturally derived polysaccharide obtained by the deacetylation of chitin, has gained significant attention in biomedical research due to its exceptional biocompatibility, biodegradability, mucoadhesiveness, and non-toxic nature. This review article provides a comprehensive overview of the versatile applications of chitosan in drug delivery systems and tissue engineering. In drug delivery, chitosan serves as a multifunctional carrier for various routes of administration, including oral, nasal, buccal, ocular, vaginal, and parenteral. Its mucoadhesive property enhances drug absorption, while its ability to form nanoparticles, microspheres, and hydrogels enables controlled and targeted drug release. Additionally, chitosan derivatives are being developed to overcome its limited solubility under neutral and alkaline conditions, further expanding its applicability. In tissue engineering, chitosan-based scaffolds and hydrogels have shown promising potential in bone, cartilage, nerve, and skin regeneration. Its structural similarity to glycosaminoglycans in the extracellular matrix makes it suitable for supporting cell adhesion, proliferation, and differentiation. Furthermore, blending chitosan with other biomaterials and incorporating growth factors or bioactive agents can enhance its mechanical properties and biological performance. Overall, this review highlights chitosan as a versatile and promising biomaterial for future advancements in drug delivery and regenerative medicine. Ongoing research focused on its chemical modification, hybrid formulations, and clinical translation is expected to further accelerate its application in personalized and targeted therapies.

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