Review Article

Alginate-Based Nanosystems for Therapeutic Applications

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Alginate is being explored widely for an array of medicinal and diagnostic measures by way of nanosystems fabricated for techniques along the lines of drug delivery, imaging, tissue regeneration, etc. Their biocompatibility, nontoxicity, sensitivity to pH, mucosadhesiveness, and biodegradability have invited an extensive body of work in this field. Their gelling ability facilitates the encapsulation of different molecules like drugs, proteins, and genes, through the formation of multidimensional matrices. Furthermore, the feasibility of alginates for physical and chemical modification allows appropriate synthesis of nanosystems for their application. The current review highlights delivery of therapeutic compounds via alginate nanosystems as an effective, eco-friendly alternative for expediting controlled and target-specific release.

1. Introduction

Nanocarriers are currently being leveraged for their size, targeting proficiency, affinity for water, degradability, bioadhesive properties, and promising treatment capacity by healthcare-based industries [1] in cancer treatment [2], drug delivery [3], and tissue and regenerative medicine [4]. Liposomes, stimuli-responsive polymeric systems, and metal nanoparticles have been inspected prior to improve drug efficacy and minimize their antagonistic effects [2, 3]. Addition of natural polymers (alginate, gelatine, or chitosan) in the fabric of nanosystems was supported by in vitro and in vivo research studies. In due course of time, these biologically enhanced polymeric nanosystems came into use, constructed by modulating different fabrication techniques, types of polymer, and cross-linking agents used for synthesis [5].

The plethora of physicochemical and biological characteristic alginate offers makes it a favoured polymer for building nanosystems [6]. Ascophyllum nodosum, Laminaria digitata, Laminaria hyperborea, Laminaria japonica, and Macrocystis pyrifera are brown seaweeds whose cell walls and intracellular spaces have been a source of alginates now available
commercially [7, 8] Members of the alginate (Alg) family are natural unbranched polyanionic polysaccharides composed of 1→4 linked β-D-mannuronic acid (M) and α-D-guluronic acid (G) residues in chain homosequences of MMM and GGG interlaced with MGM heterosequences [7–10]. Pendant carboxylic acid moieties of G units ionically cross-link with divalent cations (Ca²⁺, Sr²⁺, Ba²⁺, etc.) to form permanent gels. This gelation mechanism, alternatively referred to as the “egg-box model,” allows for the complexation of compounds by generating a three-dimensional scaffold [10, 11].

Alginates have garnered the attention of scientists and researchers as an unrealized biomaterial for modelling nano-target sites within the host value such as drugs, hormones, peptides, and genes to make them ideal for the delivery of substances of therapeutic friendly, mucoadhesive, and sensitive to shifts in pH, which and drug delivery [15].

2.1. Alginate-Based Nanocarrier for Tuberculosis Therapy.

Mycobacterium tuberculosis (M. tb) is the source of tuberculosis (TB), a pulmonary airborne disease that can extend to the bones and joints, the circulatory, lymphatic, urogenital, and central nervous systems in man [17, 18]. M. tuberculosis will initiate infection should the airborne bacilli be inhaled and ingested, followed by multiplication in alveolar macrophages and invading neighbouring macrophages [19, 20]. Patients with active pulmonary TB can transmit the disease to other individuals. However, the infection can be avoided through innate or acquired immune responses (individual immunity). If the individual fails to fight the infection, it can develop into a latent, subclinical, or active TB infection. The ailment is curable if aided with proper medication. Isoniazid, rifampicin, pyrazinamide, and ethambutol are drugs conventionally used against TB but are affiliated with debilitating side effects such as hepatotoxicity, exanthema, and arthralgia [18, 21, 22].

Rifampicin (RIF) eliminates infection caused by M. tuberculosis bacilli residing in alveolar macrophages. By binding with the β-subunit of the bacterial RNA polymerase, RIF can inhibit transcription of genes and this phenomenon was explored by Praphakar et al. Firstly, alginate was grafted to allyl amine by atom transfer radical polymerization technique at 80°C under nitrogen, and further, mannose was linked in presence of tetrahydrofuran and sodium acetate buffer. Further, they synthesized RIF-loaded Zn²⁺-cross-linked sodium alginate-g-allylamine-mannose (SA-g-AA-M) polymeric nanoparticles (PNPs) by oil in water emulsion technique followed by ionic gelation technique for alveolar macrophage-targeted delivery. The encapsulation efficiency of drug within the Zn²⁺-cross-linked SA-g-AA-M PNPs was recorded to be 91.65% with a zeta potential of -17.59 mV. The Zn²⁺ RIF-SA-g-AA-M PNPs with spherical morphology and 268 ± 3 nm in size on average exhibited strong antimicrobial activity against M. tuberculosis in vitro. Further, in vitro release of drug at pH 5.3 showed 95% of release whereas at pH 7.4 maximum, it reached up to 45%. Furthermore, alveolar macrophage targeting of the PNPs was labelled high on account of cellular uptake by A549 cells upon conjugation with mannose, infating the intracellular concentration of RIF. Detail studies on investigation of Zn²⁺ RIF-SA-g-AA-M nanocarriers are required as a candidate for treatment in the future [23].

Ahmad et al. covered the pharmacokinetics of several renowned varieties of antitubercular drugs (ATDs) along the lines of isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB) encapsulated in alginate, in mice at discrete doses along with their tissue distribution. While free drugs were removed 12-24 h postadministration, alginate NPs extended the retention period of ATDs up to 7-11 days in the system. Additionally, the alginate-encapsulated drugs administered persisted in tissues for 15 days at concentrations higher than the minimal inhibitory concentration (MIC) [24]. In another report by Ahmad et al., detailed analyses on the pharmacokinetic and chemotherapeutic properties of alginate NPs enclosing INH, RIF, and PZA synthesized as inhalable drug carriers for tuberculosis therapy were conducted. Nearly 80.5% of the nanoparticles were in the respirable range of 0.4–2.1 μm with a mass median aerodynamic diameter (MMAD) of 1.1 ± 0.4 μm. Encapsulation of the drugs in aerosolized alginate nanoparticles increased their relative bioavailability and proficiency as compared to orally administered drugs as evident from in vivo studies on guinea pigs infected with Mycobacterium tuberculosis [25].

Another interesting combination of Chitosan-coated alginate nanoparticles coalesced with Tween 80 was developed for inhalable delivery of RIF with ascorbic acid (ASC) for systematized treatment of tuberculosis. The size of the nanoparticles was found in the range of 300-500 nm with zeta potential in the range of -25.6 to -32.2. The size of the nanoparticles showed reduction with increase in Tween 80 concentration. The reason can be attributed as it is a surface-active substance, it might have provided stability to
nanoparticles without forming aggregation, and the optimal concentration was maintained at 0.16% w/v. The nanoparticles were found to be crystalline in nature. Further, the release of free drug was found to be higher at pH 5.5, pH 6.8, and pH 7.4 (95% release in 5 h) as compared to the drug-loaded nanoparticles (24 h). Interestingly, free RIF acting against nine clinical strains of \textit{M. tuberculosis}, the antimicrobial activity of RIF/ASC NPs had been established as superior with remarkable redispersibility of RIF/ASC NPs freeze-dried and powdered in water, making them suitable for inhalable delivery through the pulmonary route [26]. The \textit{in vivo} and \textit{ex vivo} potential of azole antifungals in synergy with conventional antitubercular drugs for clinical implementation in humans was validated by their toxicology profile [27, 28]. The chemotherapeutic ability of econazole (an azole drug) and ATDs enclosed within alginate nanoparticles against murine tuberculosis was surveyed. After allotment, ATDs and econazole were detectable for 15 days and 8 days, respectively, as opposed to the standard 12–24 h detectability period of native drugs. Encapsulation of econazole in alginate NPs can reduce the bacterial burden in the lungs and spleens of mice infected with \textit{M. tuberculosis} by more than 90% [29].

Ethionamide is a second-generation antitubercular drug with side effects along the lines of hypothyroidism (in children) and gynecomastia over others. Controlled release of ethionamide may decrease associated toxicity. Carrageenan-stabilized nanoparticles developed with chitosan-alginate complex were furnished with ethionamide for the remediation of tuberculosis. Inclusion of Carrageenan aids in easing entrainment of drug in alginate chitosan NPs. The minimum inhibitory concentration (0.61 μg/ml) of ethionamide-loaded nanoparticles was higher and expressed regulated and sustained release when collated with free ethionamide [30]. To summarize, alginate-based nanosystems are emerging vehicles for delivering ATDs, to reduce side effects linked with higher generation ATDs and MDR.

Multidrug-resistant (MDR) tuberculosis is challenging to treat as the pathogen becomes resistant to isoniazid and rifampicin, two popular first-line anti-TB drugs [31]. Amikacin is an injectable used to treat MDR-TB, but its prolonged exposure may result in nephrotoxicity, neurotoxicity, and irreversible cytotoxicity [32]. Consequently, moxifloxacin is also an option to treat MDR-TB. However, extended use of moxifloxacin can result in extensively drug-resistant tuberculosis (XDR-TB) [33, 34]. The combined use of both drugs has shown significant synergistic impact on MDR-TB. Enclosure of these drugs within polymeric nanoparticles can reduce their side effects and toxicity. Alg modified -PGLA nanoparticles were formulated using w/o/w emulsion for capturing amikacin and moxifloxacin as a treatment protocol for MDR-TB. Alg modified-PLGA nanoparticles revealed a significantly steady release pattern of antibiotics when compared with uncoated nanoparticles. Individual loading of nanoparticles with amikacin and moxifloxacin accelerated growth by 34.9% and 3.27%, respectively, but only by 1.75% when collectively loaded at the same concentration. Therefore, we can infer that dual-drug nanoparticle formulations aptly regulate the release of second line antitubercular drugs [35].

Chen et al. reported the antimycobacterial properties on active and latent forms of TB using nontoxic, stable, and highly biocompatible AgNPs overlayed with alginate. Formation of TEM-approved spherical, well dispersed, and sharp AgNPs was reaffirmed by SPR screening at 410 nm; their mean particle size (70 nm) and zeta potential (-47 Mv) were noted as well. XPS analysis confirms the release of 3D photoelectrons as peaks were obtained at binding energies of 372.8 Ev and 378.8 Ev. Dose-dependent
inhibition of M. tb was examined to check for MIC values employing the microplate Alamar blue assay protocol in the process (MABA). H37Rv, W6 (Beijing strain), CHCH005 (Beijing strain), and CHCH029 (EAI strain) were the drug-sensitive strains selected as test, and their MICs were calculated in the order of $4.17 \pm 1.04 \mu g/ml$, $7.29 \pm 2.76 \mu g/ml$, $41.67 \pm 8.33 \mu g/ml$, and $2.60 \pm 0.52 \mu g/ml$. $1.04 \pm 0.26 \mu g/ml$ and $16.67 \pm 4.17 \mu g/ml$ were the values noted for MDR strains KVGH376 and KVGH264. Lastly, TCHL002 and TCHL017 were chosen as XDR-TB sample strains, and their MIC values were $7.29 \pm 2.76 \mu g/ml$ and $8.33 \pm 2.08 \mu g/ml$. CFU analysis was the route considered for estimation of bactericidal activity, and affirmative results were obtained when cultures of H37Rv, Beijing strain W6, KVGH064, and TCHL017 were enumerated. In contrast with control, colony formation was reduced significantly when reviewed after 7 days of incubation as compared to massive growth in the absence of ALG-AgNPs. For latent TB, oxygen-limiting conditions were imposed upon the H37Rc strain, inoculated with $25 \mu g/ml$, $50 \mu g/ml$, and $100 \mu g/ml$ of the nanocomposites. Under a persistent nonreplicating state, colonial growth was prevented as the cells were coerced into becoming dormant, whereas this observation was repeated when compared to the PBS-treated hypoxic environment [36].

2.2. Alginate-Based Nanocarrier for Cancer Therapy. The term “cancer” denotes the unregulated growth of cells forming a mass or tumour with numerous categories observed in the population such as cancers of the liver, breast, prostate, and blood vessels [37]. Chemotherapy is a preferred strategy of treatment against cancer. However, resistance against the drugs used coupled with the side effects of their accumulation in normal tissues can develop subsequently [38]. Chemotherapeutic drugs encapsulated in Alg-based nanocarriers can improve their efficacy, reduce the drawbacks, and assist in precise yet targeted release.

Curcuma longa (Turmeric) yields a phenolic compound called Curcumin (CUR) that inhibits the proliferation of cancerous cells and is a cancer-specific ligand. Doxorubicin-loaded Alg/Cs nanoparticles (FPA NPs) were synthesized by electrostatic complexation with w/o emulsion of Alg/Cs. Fluorescence spectroscopy was ideal for observing the uptake of the Alg/Cs NPs by 4T1 cells where reduced cell viability significantly with an IC50 value (0.15 μg/ml) comparable to free DOX (0.13 μg/ml) within 72 h [45].

In another study, doxorubicin-loaded alginate nanoparticles modified with glycyrrhetinic acid (DOX/GA-Alg NPs) were applied for situ liver cancer therapy. Kunming mice were used as test subjects to study the hepatic biodistribution of DOX/GA-Alg NPs and their antitumour activity. The DOX-loaded nanoparticles were successful in inhibiting the growth of liver tumour cells by passive targeting via the EPR effect and active targeting of liver tumours from GA at the same time with some side effects. The DOX/GA-Alg nanoparticles, while the neighbouring liver and heart cells, were nearly unaffected in the meantime [46]. Folate-mediated core-shell phytosterol-Alg nanoparticles (FPA NPs) were developed which targeted intracellular anticancer drug delivery. Folate permits specialized targeting of cancer cells overexpressing the folate receptor as it is a cancer-specific ligand. DOX was loaded into FPA NPs via dialysis with 75% encapsulation proficiency. Furthermore, 52.4% of KB cells were determined as viable in the presence of free DOX (0.13 μg/ml) within 72 h [45].
release and reduced side effects. Nanoemulsified polymer cross-linking of alginate nanoparticles loaded with paclitaxel (PTX-ALG) arrested the cell cycle to accelerate apoptosis in human primary patient breast cancer cells. They were more hydrophobic and soluble than free PTX [48]. Initially, paclitaxel- (PTX-) loaded alginate nanoparticles with mean dimensional value of 200 ± 21 nm were synthesized. A novel double emulsion cross-linking electrostatic attraction method was tested to coat the Alg-PTX nanoparticles with chitosan (CS) and folate chitosan (FA-CS). The CS/Alg-PTX and FA-CS/Alg-PTX NPs had higher drug loading and entrapment ratios than Alg-PTX NPs and prolonged the drug release time. Further, in vitro studies confirmed that all the three PTX-loaded NPs effectively inhibited the growth of HepG2 cells. FA-CS-conjugated NPs could be better internalized due to folate-mediated targeting [49]. Alginate nanoparticles coated with egg whites (EW) were prepared for the delivery of PTX to cancer cells by electron spray. The morphology of PTX-loaded Alg-EW NPs was recorded to be 200 ± 18 nm with a zeta potential of −5.85 ± 0.43 mV. The encapsulation efficiency and loading capacity for the PTX-loaded Alg-EW NPs were found 12% and 5.2%, respectively. Further increased in cell death was observed in cell viability study as concentration increased from 0.05 μg/mL to 10 μg/mL. The PTX-loaded Alg-EW NPs (PTX/Alg-EW) had good biocompatibility as they exhibited minimal haemolytic activity and induced higher apoptosis of 6% in CT26 colorectal cancer cells as they exhibited minimal haemolytic activity and induced higher apoptosis of 6% in CT26 colorectal cancer cells compared to free PTX, implying their pronounced targeting ability [50].

Fabrication of chitosan/alginate hydrogel hybridized Cu nanoparticles in situ was proposed as a green, facile, and sustainable solution for prohibiting the proliferation of breast cancer cell lines. Backed by SEM-imaging, the nanoparticles had quasi-spherical morphology with a double-shell-core structure which stabilizes the nanoparticle and prevents oxidation or self-aggregation whereas TEM results argued that the NPs were perfectly spherical and well-dispersed with no aggregates discovered. Furthermore, they were estimated to be around 10-20 nm in size on average. EDX profiling uncovered the presence of C, N, O, Na and Cu as elements essential to the fabrication of the biocomposite while XRD patterns support authentic crystallinity of the CuNPs as diffraction peaks were obtained at 2θ = 43.2°, 50.3°, and 74.1°. The growth of cancer cells is inhibited by CuNPs through various channels such as diminishing cell metabolic activity, escalating oxidative stress levels, changes in cell morphology, and DNA damage by synthesizing reactive oxygen species (ROS). In addition, secretion of LDH, activation of autophagy genes and apoptosis, promoting caspase, and uncalibrated mitochondrial activity are extended anticancer mechanisms. MTT assay successfully computed the IC_{50} values of the CuNPs@CS/Alg nanocomposite at 297 μg/mL, 386 μg/mL, and 359 μg/mL, respectively, for KYSE-270, OE33, and ESO26 breast cancer cell lines. IC_{50} for normal line HUVEC was null [51].

2.3. Alginate-Based Nanocarrier for Insulin Delivery. Defective insulin secretion, insulin action, or both can result in the inception of a chronic metabolic disease called diabetes mellitus characterized by hyperglycaemia [52]. Type I and type II diabetes mellitus are observed in people as the most prominent types. In type I, the insulin-producing cells residing in the pancreas are being attacked by the host’s immune system and are virtually incapable of producing insulin whereas in type II, patients are unable to use insulin efficiently as the host’s cells develop resistance to the hormone [53]. An estimate of 640 million cases of diabetes mellitus worldwide by 2040 has been projected following the rate of occurrence, with 5-10% of all incidences being type I [54, 55].

Subcutaneous insulin injections are offered to maintain blood glucose levels but allergy, itching, and poor compliance may lead to complications such as infections and renal hypertrophy. These issues can be resolved through oral delivery of insulin. Nanosystems composed of alginites can increase the bioavailability of the product and confer other benefits due to their unique features. Alginate is a suitable candidate for developing oral delivery mechanisms due to its distinct characteristics [56]. Alginate-based nanoparticles were concocted by emulsion or internal gelation using Ca²⁺ as a crosslinker for loading insulin. The influence of varying concentrations of calcium salt on gel integrity and particle size was investigated. At calcium and alginate mass relation of 7% (w/w), nanoparticles with the smallest mean diameter of 2.604 ± 2.141 μm were obtained. At this formulation, insulin encapsulation was over 71% with truncated release at gastric pH ranges. Thus, oral insulin provision is the most ideal route when combined with a mild encapsulation mode of synthesis and high entrapment efficiency [57]. Alg nanoparticles with a polyelectrolyte complex coating of chitosan were prepared using ionotropic pregelation technique. Alg and chitosan concentrations of 0.05 and 0.012% (w/w), respectively, were optimal to produce nanosized particles. Insulin was successfully encapsulated within the nanoparticles without any significant influence on particle size. Insulin association capacity was improved by decreasing alginate: chitosan mass ratio by incorporation of higher chitosan coating material; however, it also resulted in larger particle size [58]. In another report, diabetic rats were administered insulin orally through Alg/Cs NPs synthesized using ionotropic pregelation. The streptozotocin-induced diabetic rats witnessed a sharp decline in serum glucose levels up to 59% and 55% of their basal glucose level, respectively, at insulin doses of 50 and 100 IU/kg [59].

In another interesting combination, alginate/dextran sulphate (ADS) nanoparticles loaded with insulin and coated with chitosan (Cs) and albumin (ALB) were studied for increasing the efficacy and bioavailability of oral insulin drug delivery. Cs and ALB enhanced the interaction of nanoparticles with intestinal cells while the mucosal activity and pH sensitivity of the nanoparticles allowed sustained release of insulin in simulated intestinal conditions. Dual coating ratified higher insulin permeability in Caco-2/HT29-MTX/Raji B cell monolayers as compared to uncoated nanosystems. Dual coated ADS nanoparticles showed potential for insulin therapy in type 1 diabetes mellitus [60]. Mukhopadhyay et al. aimed to develop a method of preparation of Cs/Alg nanoparticles without using harsh
chemicals to increase in vivo bioefficacy of insulin. In in vivo models, relative bioavailability of insulin was ~8.11%, indicating improved efficacy of these nanoparticles. After administration, no significant toxicity was observed, suggesting potential vehicles for insulin delivery [61].

Cs/Alg in combination with polyurethane has also been explored to for polyurethane (PU)-alginate/chitosan (Alg/Cs) core-shell nanoparticles for the oral delivery of insulin. Insulin-loaded PU-Alg/Cs nanoparticle remarkably lowered the blood glucose levels in diabetic mice, enhanced the bioavailability of insulin in diabetic mice, and were deemed safe [62]. Similarly, calcium alginate nanocarriers also synthesized by emulsion crosslinking, increased percent loading of insulin from 11.7 to 38.9 positively corresponded to the augmented 60% released from the initial 18%. Stringent acidic environments mimicking gastric conditions (pH 1.2) did not alter the chemical stability of the loaded insulin. The percent haemolysis and protein adsorption of the insulin-loaded nanocarriers were also very low [63].

Glucose-responsive nanoparticles are receiving attention by the scientific community due to their potential in the remediation of diabetes. Phenylboronic acid-based materials are popularly studied and used to invent glucose-responsive remediation of diabetes. Phenylboronic acid-based materials were also very low [63].

Insulin-alginate nanoparticles modified with octa arginine (INS-SA/R8 NPs) were conceived to refine insulin permeability and investigate the mechanism of penetration. The INS-SA/R8 NPs considerably improved intestinal perfusion and villi uptake of insulin because of their appreciable stability in simulated in vitro gastric (SGF, pH 1.2) and intestinal fluids (SIF, pH 6.8). Most importantly, INS-SA/R8 NPs induced the production of nitric oxide (NO), an endogenous signalling molecule, which additionally elevated insulin uptake [67].

The foundation for the synthesis of oral delivery vehicles of insulin nanoemulsions comprises of homogenized water in oil in water (w/o/w) arrangement and castor oil emulsions coated with calcium alginate and aloe vera (AV) gel by employing the ionic gelation method. The nanopolymeric systems were characterized by SEM imaging where the nanoeumulsions were estimated to be ~400 nm in footage. The results were deemed valid on the basis of DLS results (~500.02 nm). For uncoated and coated nanoemulsions, the PDI were 0.400 and 0.406, respectively, as the alginate/ aloe vera (A/AV) extract reduces the size and leaves the impression of a rough, irregular quasi-circular particle with an entrapment efficiency of 47.3%. Caco-2 cell line is assayed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide to verify the feasibility of insulin delivery. The experiment proved to act as a substantial approach to the treatment of insulin-related disorders as an increment of up to 20-25% was noted in insulin transmembrane movement [68].

Another example of ionotrophic gelation was employed in the synthesis of alginate/chitosan nanoparticles incorporated with insulin. Further, it was complexed with Cp1-11 peptide (C-insulin) for improving oral bioactivity, release profile, and retention period of the hormone as compared to pure insulin or even insulin-loaded chitosan/alginate NPs. DLS results conveyed important data pertaining to the size of the nanoparticles, >500 nm in size if blank ALG/CS NPs are used but would compress to 310.1 nm or 237.2 nm if loaded with insulin (ILN) or C-insulin (CILN), respectively, due to ionic/electrostatic affinity between the hormone and substrate. TEM visuals commented on the spherical, smooth, and uniform margins of the nanoparticles and smaller size which confers a greater surface-area-to-volume ratio for CILN. Association or entrapment efficiency and loading capacity for ILN and CILN stand at 85.64% and 90.43% and 24.43% and 28.06%, respectively, in tandem with the interpretation of TEM images. 4.58% and 7.42% of total insulin concentration were released from CILN and ILN in the first 2 h, respectively, but increased when the simulated gastric fluid (SGF, pH 1.2) was replaced by simulated intestinal fluid (SIF, pH 7.4). Over the next 2h, 30% cumulative insulin content was released. As the NPs were immersed in this fluid for 10 h undisrupted, an improvement in the release pattern of insulin for CILN was noted (63.5% over 50.15% for ILN). Pharmacological and pharmacokinetics accounts were drawn for understanding their effect on blood glucose levels (BGLs) in diabetic rats. When injected subcutaneously, a 30% discount from the initial reading was observed after 1.5 h but was around 80% restored after 4 h. Pharmacological availability of insulin from ILN was 5.36%. 2 h postadministration, BGLs dropped to 55.1% of the initial concentration which is a significant improvement over free insulin. Furthermore, 60% of the total BGL after 8 h was retained. For CILN, the bioavailability stood at 9.83% which is the highest value out of the lot. Bioavailability of ILN and CILN relative to the amount of solution injected subcutaneously was 14.1% and 15.62%, respectively, much higher than free insulin’s [69].

2.4. Alginate-Based Nanocarrier for Gene Delivery. Intentional regulation of gene expression achieved by introducing mRNA, small interfering RNA (siRNA), microRNA (miRNA), antisense oligonucleotides, or other exogenous nucleic acids in specific cells for the treatment of pathological conditions is defined under gene therapy [70]. For delivering genes to targeted sites, vectors derived from viruses are most preferred due to their high specificity. However, their application has been hindered owing to their immunogenicity. Polymeric nanosystems allow site-specific delivery of the genes without inducing any immune responses. Alginate is one of the many biopolymers used within domains under gene therapy.

Intracellular delivery of plasmid DNA (pDNA) was accomplished by employing coassembled Ca++ alginate/sulphate nanoparticles. The AlgS-Ca++- pDNA nanoparticles had an average size of 270 nm with a slightly anionic surface charge (ζ = -14 mV). Clathrin-triggered endocytosis facilitated the entry of AlgS-Ca++ into human breast cancer cell
line, human hepatocellular carcinoma cells, and primary rat neonatal cardiac fibroblasts *interalia* other cell types. Suppression of the activation of T-lymphocytes in human peripheral blood mononuclear cells speaks to their cytocompatibility [71].

In another study, alginate nanoparticles were synthesized by w/o microemulsion followed by crosslinking with Ca$^{2+}$ as gene carriers. The as-synthesized Ca-alginate nanoparticles with 80 nm in measurement efficiently encapsulated green fluorescent protein- (GFP-) encoding plasmids. The Ca-alginate nanoparticles were internalized in NIH-3T3 cells via the endocytic pathway. The GFP gene was successfully transfected in the cells, expressed and observed using fluorescence microscopy [72].

Abbreviated to CRISPR, clustered regularly interspaced short palindromic repeats is emerging as a new technique for gene editing, with appealing merits in the biomedical field. CRISPR plasmids were placed into alginate nanoparticles via electrospraying to edit the green fluorescent protein (GFP) gene present in another plasmid. The CRISPR plasmids were enveloped within the nanoparticles with 99% encapsulation ability while preserving payload integrity. The alginate nanoparticles effortlessly introduced the CRISPR plasmids into HepG2 cells. Successful transcription of the plasmids was visualized by red fluorescence due to expression of RFP reporter gene. The CRISPR ribonucleoproteins were able to edit the GFP gene by introducing double-strand breaks, which resulted in decreased green fluorescence [73].

Ahn et al. devised a new procedure for the preparation of alginate–chitosan nanoparticles. Modulation in the pH, alginate/chitosan ratio, and polymer molecular weight influenced the formation of the nanoparticles. Low molecular weight chitosan and low viscosity alginate (1.5:1) were combined to forge nanoparticles with a mean diameter of 314 nm. Maximum mass loading of 60 μg DNA per mg of nanoparticles was observed alongside 60% DNA loading efficiency. Calcium phosphate-alginate (CaP-Alg) core-shell nanoparticles (NPs) were synthesized via w/o emulsification and precipitation. pH values can be manipulated during synthesis to obtain different crystalline structures of CaP in the core region, i.e., brushite (Bru) and hydroxyapatite (HA). HA-Alg NPs can be adopted for intracellular delivery for their environment-appropriate release rates and higher loading capacity. However, Bru-Alg NPs were better capable of extracellular distribution. Therefore, CaP-Alg nanoparticles could contribute to biomolecule and intracellular/extracellular gene delivery [74].

Another aspect of using Cs-Alg-DNA nanoparticles was derived by complexation of chitosan-alginate at different charge ratios and the pAcGFP1-C1 plasmid with an average size of 600–650 nm. The prepared nanoparticles exhibited a loading efficiency > 90%, prevented digestion of the transgene by DNase I, and improved the efficacy of gene transfection of 293T and HeLa cells by an impressive margin. Exposure to ultrasound can curtail cell viability but the concomitance of gene transfection elevated consequently, and tumour infection could be used for cancer gene therapy [75]. pEGFP-N2 pDNA-loaded Cs/Alg-dextran sulphate nanoparticles were fabricated through modified ionotropic gelation. The chitosan/alginate-dextran sulphate nanoparticles were nontoxic to HEK298 cells and also amplified their proliferation. The encapsulated plasmid DNA was protected from degradation by BamHI nuclease and efficiently transfected the plasmid DNA into HEK293 cells [76].

Interestingly, Alg/Cs nanoparticles have also been studied and tested as vectors for the freighting of the epidermal growth factor receptor (EGFR). The alginate–chitosan nanoparticles with a zeta potential of about ±30 mV were notably stable. Ratios of 0.2% (CaCl$_2$/Alg), 1 (Alg/Cs), and 5 (N/P at Alg/Cs nanoparticles which were synthesized using the pregel preparation method, measured 194 nm in size, and exhibited a loading efficacy of 95.6% [77]. In another report, optimized Cs/Alg nanoparticles were synthesized yet again using pregelation with a N/P ratio of 5 and alginate/chitosan mass ratio of 1:1. Cs/Alg nanoparticles which were loaded with EGFR antisense oligonucleotide were able to downregulate the expression of EGFR in T47D breast cancer cells. Furthermore, RT-PCR and immunocytochemistry analyses proved that nanoparticles with N/P ratio of 5 downregulate the expression of EGFR more competently than naked antisense by inflicting their cellular uptake and stability [78].

Belguessa et al. isolated antimicrobial peptides (bacteriocins) adsorbed onto alginate nanoparticles (Alg-NPs) as vehicular systems for delivery from *Lactocaseibacillus para-casei* CNCM I-5369. Based on DLS data, the nanoparticles possess an average size of 119 nm at pH 5 before loading and expand up to 124 nm after loading the 12 wt.% E20 bacteriocin fraction. Similarly, the zeta potential values at pH 7 (-32 mV) and pH 5 (-12 mV) for blank Alg-NPs contrast significantly from the loaded NPs which are effectively neutral in charge at pH 5. SEM photographs assist in visualizing the rectangular and rod-shaped nanoparticles 100–108 nm when scaled. When used for antimicrobial action studies, the Alg-NPs/E20 exhibited striking results after 1 h of incubation with target strains such as *E. coli* 184, *E. coli* 289, *E. coli* ATCC8739, *E. coli* SBS36, *E. coli* TOP10, *E. coli* E4A4, and *E. coli* E5A16 which carry the mcr-1 gene. When the E20 fraction is used independently, MIC values can vary anywhere from 250 to 2000 μg/ml but can decline up to 2-4 μg/ml when loaded with Alg-NPs. Furthermore, these nanoparticles will induce topological changes in bacterial cells such as raised cytosol density, thickening and invasion of the periplasmic space, formation of lesions on the inner and outer cell membranes, and leakage of intracellular material but exhibit no toxicity towards human (HT29) or animal (IPEC-1) cells [79].

### 3. Conclusion

Alginates are being spotlighted for developing nanosystems capable of delivering diverse therapeutic molecules. Alginate-based nanosystems enhance the bioavailability of loaded molecules due to their novel characteristics like biocompatibility and biodegradability. Additionally, the numerous functional groups present on alginates allow successful loading of the molecule without affecting their native
structure. They facilitate bioresponsive and site-specific transport of therapeutic molecules such as insulin, genes, and drugs. This enhances their competency while reducing the side effects associated with them. Also, therapeutic molecules can be administered less invasively through alginate nanosystems, which also improves patient compliance.

4. Future Perspectives

Green approaches are considered to be more viable and sustainable due to a plethora of benefits with minimal risks endowed by plant-mediated sources. Alginates with their nontoxicity, biodegradable potential, biocompatibility, and bioavailability can replace nonrenewable or polluting synthetic resources. An eminent fragment of tissue engineering, diagnostic, imaging, drug delivery, and therapeutic studies is being performed on in vitro samples such as microbial or animal cells/cell lines. Clinical testing sanctions in therapeutics can introduce a cost-effective, convenient, and effective range of medications that can be administered noninvasively with minimal practice or domain expertise. Innovation of CRISPR and other modern technologies permit experts to edit or rework genomic sequencing for gene therapy. Application of alginate-based nanosystems for therapeutics in industries can be a new discipline to expand into, which can inspire a variety of entrepreneurial or scientific ventures.

5. Executive Summary

5.1. Alginate Nanosystems for Drug Delivery. Role of alginate-based nanosystems in drug delivery can be best explained through the management of two widespread diseases: tuberculosis (TB) and cancer. Treatment of tuberculosis incorporates understanding the types of TB infection caused by Mycobacterium tuberculosis, namely, active, latent, and subclinical, along with popular prescription-based medication through noninvasive and nontoxic procedures. Green approach-oriented nanomaterials confer biocompatibility, bioavailability, biodegradability, nontoxic, and a nontoxic alternative that can complement the release profile and retention period of the drugs. In cases of multidrug resistant or extensively drug-resistant strains, neoeantitubercular drugs such asazole antifungals, moxifloxacin, and amikacin can be used as the risk of developing side effects (nephrotoxicity, neurotoxicity, irreversible cytotoxicity, etc.) can be partly mitigated by enclosing them in biopolymeric NPs. Admission of chemotherapeutic drugs for cancer treatment can be modified to reduce drug resistance and cytotoxicity while improving their efficacy and uptake. Curcumin and other bioactive compounds retrieved from plant-based or microbe-based sources possess numerous health benefits but are not in practical use due to their chemical stability and cellular uptake. Doxorubicin and paclitaxel are two commonly used synthetic drugs whose effects were enhanced by NPs with high sa/vol distributions which streamline uptake and retention for minimal consumption but maximum effect.

5.2. Alginate Nanosystems for Insulin Delivery. Insulin therapy is a principal line of treatment for both juvenile and adult-onset diabetes mellitus as well as chronic metabolic syndrome, insulinoma, polycystic ovary syndrome, or PCOS among others. Alginate-based nanosystems have revolutionized the mode of treatment as oral delivery has now become a choice for those who are uncomfortable with subcutaneous injections due to existing medical conditions or individual preferences. By refining the bioavailability, efficacy, resistance to simulated gastric or intestinal conditions, and pharmacokinetic parameters of insulin, controlled and sustained release can be easily achieved. Several studies have employed polyurethane, PAAPBA, R8, AAV extract, and even C- insulin (Cp1-11 peptide) for the intake of insulin which offer multifold benefits.

5.3. Alginate Nanosystems for Gene Delivery. Manipulation of gene expression and regulation mechanisms in order to correct congenital or acquired genetic defects is the principle of gene therapy. Short nucleic acid sequences synthesized in vitro are delivered to target sites using vectors. Alginate-based nanocarriers are now successfully replacing conventional viral/bacterial vectors on account of their negligible immunogenicity. Chitosan/alginate or calcium alginate-based nanocomposites have been used in most cases to transfect mRNA, siRNA, miRNA, or even plasmid DNA which are internalized by cells to ameliorate functionality or for fluorescence studies. Cancer cells can be modified to induce immunogenic responses to protect the host from harm, bacterial growth can be inhibited through the use of bacteriocins, and any pathogenic activity can be arrested without harming the host via antisense technology as seen in T47D breast cancer cells and E. coli strains.

Data Availability

All data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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