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Potentials of Alginates as Capping Agent for Oral Colon Delivery of Radiosynthesized Silver Nanoparticles: A Review

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ABSTRACT

Radiosynthesized silver nanoparticles (AgNPs) offer benefits for treatment of chronic colon inflammation due to their anti-inflammatory activity. Targeted delivery of AgNPs to the colon allows topical treatment at high concentration but at reduced systemic side effects. Meanwhile, related to drug administration, oral route is a common method. However, the physiology of the gastrointestinal (GI) tract limits the AgNPs ability to achieve their therapeutic level. This is specifically related to the acidic environment of the stomach and mucus layer of the GI tract. Concurently, alginates are one of the most extensively explored biomaterial classes for drug delivery system due to its biocompatibility, gel-forming ability at mild condition, anionic nature, sensitivity, and mucoadhesiveness. In this review we provide an overview of appropriate features of alginates as capping agent for oral delivery of radiosynthesized AgNPs to the colon. As capping agents, alginates play multiple roles specific to its processing stages, i.e., radiosynthesis, stabilization of nanoparticle system, and oral colon delivery devices of AgNPs. Additionally, we describe outstanding features of alginates as capping agents for drug delivery device as well as the positive contributions of radiation processing on improving the functional effects of alginate.

INTRODUCTION

Silver nanoparticles (AgNPs) are one of the metal nanoparticle types viewed as potential topical anti-inflammatory agent for treatment of inflammatory bowel diseases (IBDs) such as Crohn's disease, ulcerative colitis, and colonic cancer [1-3]. The intestinal inflammation disorder mediated by the immune system is a typical feature of IBD. Inflammation is a natural body's response to tissue damage. However, it can cause improper tissue repair and contribute to the development of cancer through genotoxic mechanism in the long-term [1]. *In-vitro* studies reported that AgNPs show exceptional anti-inflammatory properties [4,5]. Similarly, *in-vivo* studies reported that the presence of AgNP layer on glass beads was responsible for the reduction of inflammation response on TNBS-induced colitis in a mice model [2]. Sutures coated with AgNPs showed anti-inflammatory effect with faster intestinal tissue healing after injury as compared to antibiotic-coated sutures. Meanwhile, the concentration of several pro-inflammatory cytokines (IL-1, IL-6, IL-10, and TNF-α) were found significantly lower [3]. The matrix metalloproteinase 9 (MMP-9) is up-regulated due to infiltration of macrophages into the intestine. However, Bhol and Schechter [6] reported that AgNPs treatment significantly suppressed expression of MMP-9 in colitis model in rats.

Both topical and systemic drug delivery of

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drugs can take place in colon. Among them, targeted AgNPs delivery to colon for topical treatment is highly advantageous in order to minimize the systemic side effects. However, the delivery device should be capable of protecting AgNPs from absorption and dissolution en route to the colon [7,8]. While oral delivery is the most preferred route for drug adminsitration, rectal delivery offers the shortest course for targeting the colon, though there is limitation in reaching the proximal part of the colon [9]. Rectal delivery can also be uncomfortable; thus, patients' compliance may be reduced [7].

For targeted drug delivery to colon, biopolymer polysaccharides have attracted a great deal of attention because of their high abundance, low cost, and high variety of structures [7]. Alginates are among the more attractive polysaccharides because they can form pH-sensitive hydrogels under exposure to multivalent cations [10]. Alginates are soluble in water at room temperature, and therefore they are preferable to other anionic marine polysaccharides from marine sources, such as agar and carrageenan. Also, gel formation of alginate does not required freeze-thaw cycles [8]. Overall, these properties render alginates as the most applied biomaterials in the polymeric drug delivery systems.

Previous reviews [8,10] discussing alginates as oral colon drug delivery devices have been published. However, those reviews are limited in providing specific information and covered a broader scope. Herein, we provide an overview focusing on the application of alginate as capping agent for oral colon delivery of radiosynthesized AgNPs. In this context, alginates as a capping agent have three main roles: (1) limiting the growth of zerovalent metallic silver core during radiosynthesis; (2) controlling the agglomeration of colloidal AgNPs during storage; and: (3) determining its interaction with biological systems so that the AgNPs are protected from absorption and dissolution en route to the colon. Further, the effects of gamma irradiation on alginates and their implication on alginate roles are discussed.

PROPERTIES OF ALGINATES

The term "alginate" is a collective term for linear copolymer chains of β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers. The glycosidic bond joins the C-1 of monomeric unit to C-4 of the subsequent monomeric unit at a β configuration. Alginates do not have a regular repeating pattern. They display an irregular blockwise arrangement pattern with homopolymeric regions of G residues (G-blocks) and M residues (M-blocks) interdispersed by alternating G and M heteropolymeric regions (MG-blocks) [8,10], as shown in Fig. 1.

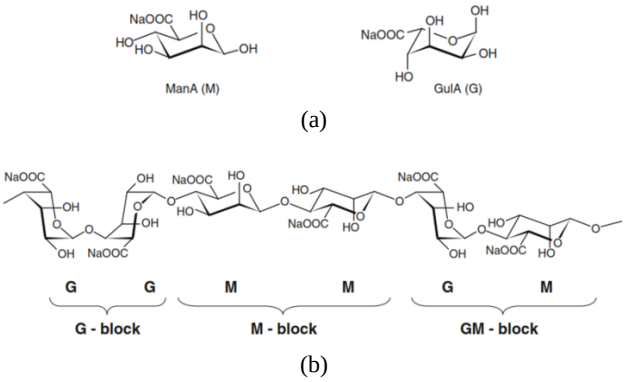

Fig. 1. Chemical structure of (a) monomers of mannuronic acid (M) and guluronic acid (G) sodium salt, respectively, and (b) alginate as a block copolymer composed of G-, M-, and MG-blocks.

Commercial alginates have been extracted mainly from brown algae (Phaeophyceae) of the following species: *Laminaria hyperborea*, *Macrocystis pyrifera*, *Laminaria digitata*, *Ascophyllum nodosum*, *Laminaria japonica*, *Ecklonia maxima*, *Lessonia nigrescens*, and *Durvillaea antarctica*. The alginates are extracted by treatment with alkali solutions. With a variety of G/M compositions and chain arrangements, they have varying molecular weights ranging from 32 to 400 kg/mol. These variations are due to different sources of extraction, age, and different parts of the algae, as well as seasonal and growth conditions [8,10,11]. Alginates with more defined molecular structures are obtained from bacterial biosynthesis. Bacterial alginates can be produced by fermentation by two bacteria genera, *Azotobacter* and *Pseudomonas*. The defining characteristic feature of bacterial alginates is the presence of *O*-acetyl groups. These acetyl groups significantly affect its physicochemical properties, such as swelling and ion binding [12,13].

Regardless of the block structure and composition of alginate chain, both mannuronic acid and guluronic acid residues are sugar acids with carboxylic functional group. The release of entropy due to counterion release makes sodium alginate a highly water soluble polymer. At neutral pH, the carboxylic moieties of alginates are negatively charged and thus alginates are polyanionic polymers. [11]. The intrinsic viscosity ([η], ml/g) of an alginate depends on its molecular weight (*M*, g/mol). The viscosity-average molecular weight relation follows the Mark-Houwink equation ($[\eta] = KM^a$). Previous

studies [11,14,15] reported that the Mark-Houwink parameters of sodium alginate are $K = 2\times10^{-3}$ and $a = 0.97$, respectively, in an 0.1 M NaCl solution at 25 °C. Consequently, a high-molecular-weight alginate forms a highly viscous solution, which is often not preferred in processing. For example, cells or enzymes dispersed in a highly viscous alginate solution risk damage from high shear forces produced during homogenization [11,15].

Alginates exhibit good biodegradability, low toxicity, and chemical versatility, but their most prominent characteristic for drugs delivery is their unique ability to form stable hydrogel in aqueous solution under room temperature by addition of multivalent cations [16]. The sol-gel transition occurs due to the formation of ionic complex between multivalent cations (such as calcium dications) and the carboxyl group of the alginate's G-blocks, as shown in Fig. 2. The junction zone of alginate gels is described through the egg-box model where four carboxyl moeties of G block interact with one divalent cation to generate a 3D network. The 3D network is macroscopically observed as the sol-gel transition of alginate solution into a hydrogel matrix. The alginate hydrogel can be employ as a drug delivery device in which therapeutic agents (such as low molecular weigh drug, macromolecular agents, metal nanoparticle, and cells) may be entrapped within its matrix [17,18].

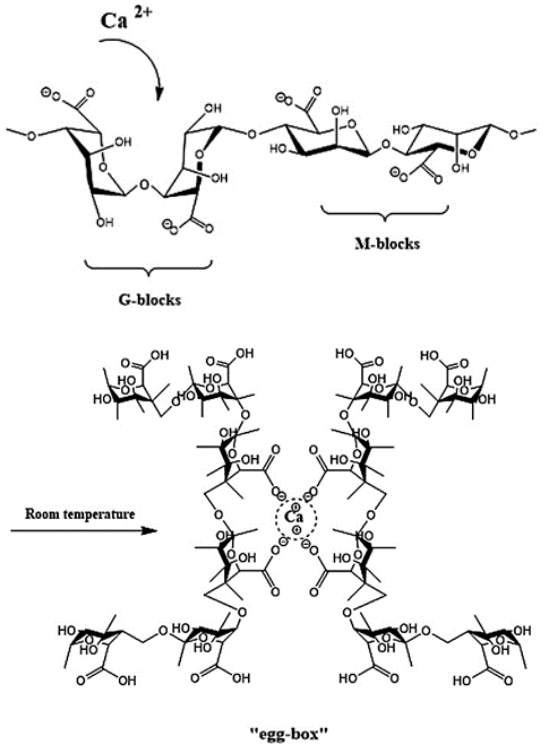

Fig. 2. Schematic representation of the egg-box structure for the ionic crosslinking of alginate by Ca^{2+} ions.

ROLE OF ALGINATE DURING GAMMA RADIOSYNTHESIS OF AgNPs

The methods for AgNP synthesis can be categorized as top-down or bottom-up approaches. The top-down approach disassembles bulk materials to generate the required structure through various physical forces, such as electrical energy (laser ablation method and electrical arch-discharge), thermal energy (vapor condensation method), and mechanical energy (crushing, grinding, and ball milling). This approach may produce AgNPs free of chemical contaminants from reducing or capping agent; however, it poses a big challenge in counteracting the agglomeration process. Also, the process requires complex equipment and huge external energy [19,20]. Hence, AgNPs are commonly synthesized by bottom-up approach which constructs single atoms and molecules into larger nanostructures to generate nanosized materials by employing nucleation and growth process. In this approach, reaction system consist of silver salt precursor, reducing agent, and capping agent [19,20]. The silver salt usually used as precursor is silver nitrate ($AgNO_3$) due to its high solubility in water [21]. Specifically, for alginate-capped AgNPs, various methods have been reported. Several such methods are presented in Table 1. Indeed, the use of chemicals additives as reducing and/or capping agent may limit their medical applications, especially if those agents are toxic, such as sodium borohydrate [19]. Therefore, the chemical reduction method can be combined with alternative energy sources or types, for instance, through photochemical processing [22], electrochemical processing [23], microwave irradiation [24], and gamma irradiation [25].

Radiosynthesis method relies on the energy of ionizing radiations, such as gamma ray, to reduce silver ion (Ag^+) to zerovalent metallic silver (Ag^0). In this context, gamma radiosynthesis offers unique advantages for the synthesis of AgNPs. First, Ag^+ ions reduced to uncharged state of Ag^0 by reducing radicals produced by radiolysis of water. Thus, there is no issue about potential toxicity of the residual reducing agent, ensuring its biocompatibility for application in biomedical field [26,27]. Additionally, gamma radiosynthesis performs under ambient conditions that allow the homogenous reduction and nucleation of AgNPs, production of AgNPs with narrow particle size distributions, and effective control of morphology. It can also be employed to synthesize alloyed and core-shell metal nanoparticles [28].

Table 1. Several reports on alginate-capped AgNPs and their method of synthesis.

Materials	Synthesis Approach	Reducing Agent	Role of Alginate	Reference
Colloidal AgNPs	Bottom-up	Ascorbic acid	Capping agent	Shao et al [84]
Colloidal AgNPs	Bottom-up	Glucose under heat treatment	Capping agent	Bhagyaraj and Krupa [85]
Colloidal AgNPs	Bottom-up	Aldehyde-modified sodium alginate	Reducing and capping agent	Xiang et al [86]
Colloidal AgNPs	Bottom-up	Gamma irradiation	Capping agent	Phu et al [25]
Colloidal AgNPs	Bottom-up	The discharge of contact non-equilibrium low-temperature plasma	Capping agent	Skiba et al [87]
Colloidal AgNPs	Bottom-up	Ultrasonic radiation	Capping agent	Faried et al [22]
Colloidal AgNPs	Bottom-up	Microwave irradiation	Capping agent	Velusami et al [24]
AgNPs/hydroxyethylacryl chitosan/alginate hydrogel film	Bottom-up	Sodium borohydrate	Capping agent	Chalitangkoon et al [88]
Poly (γ- glutamic acid) /alginate/AgNP microsphere	Bottom-up	UV irradiation and alginate	Reducing and capping agent	Tong et al [89]
Alginate nanoparticle containing AgNPs	Bottom-up	Green tea (*Camelia synensis*) extract	Capping agent	Urzedo et al [90]
Alginate/AgNPs nanocomposite	Bottom-up	Solution plasma process	Capping agent	Nam et al [91]
AgNPs/alginate hydrogel beads	Bottom up	*In situ* reduction	Capping agent	Piras et al [92]
AgNPs/alginate and AgNPs/alginate/polyvinyalcohol hydrogel microbeads	Bottom-up	Electrochemical synthesis	Capping agent	Obradovic et al [23]

The radiation processing of polysaccharide solutions containing metal ion precursors lead to generation of zerovalent silver atom through indirect effect. Most of the radiation energy is absorbed by water during irradiation of aqueous solution, while the absorption by metal ions precursor and dissolved polymer can be neglected [29,30]. The radiolysis of water generates a large number of radicals through complex physical and physicochemical reactions which are simplified as Eq. (1).

$$H_2O \xrightarrow{\gamma-\text{ray}} e_{aq}^-, H_3O^+, H^\bullet, H_2, {}^\bullet OH, H_2O_2 \quad (1)$$

In (1), e_{aq}^- is solvated electron ($E°(H_2O/e_{aq}^-)$ = -2.87 V_{NHE} at pH = 7), while $H^\bullet$ is hydrogen radicals ($E°(H^+/H^\bullet)$ = -2.3 V_{NHE}). Based on their redox potentials, both radical species are strong reducing agents [27-30]. Therefore, they can reduce silver ions into zerovalent silver atoms, following reactions given in Eqs. (2,3).

$$e_{aq}^- + Ag^+ \rightarrow Ag^0 \quad (2)$$

$$H^\bullet + Ag^+ \rightarrow Ag^0 \quad (3)$$

The gamma ray can penetrate deeply enough into aqueous solution to form radical species that are homogenously distributed throughout the reaction system. The reduction reactions in Eqs. (2,3) occur evenly throughout the reaction system. Thus, the zerovalent silver atoms are formed and distributed homogenously. Each newly-formed atom play the role as a solitary center of nucleation.

The binding energy between Ag^0 and the solvent is weaker than between two Ag^0 dimers or between Ag^0 and Ag^+ [27-30]. Therefore, once silver atoms are synthesized, they tend to interact to form dimers, following Eqs. (4,5).

$$Ag^0 + Ag^0 \rightarrow Ag_2 \quad (4)$$

$$Ag^0 + Ag^+ \rightarrow Ag_2^+ \quad (5)$$

Reduction of charged silver dimers follows the reaction in Eqs. (2,3). These zerovalent and charged silver dimers play the role as the centers of cluster nucleation. During the radiosynthesis, free metal ions undergo either reduction or absorption to form dimers/clusters. The competition between these processes could be tuned by controlling the rate of formation of reducing agents [27-30].

Reduction of ions which settle on the clusters leads to the growth of cluster. Between cluster with unreduced ions or two charged cluster, the binding energy is strong. Therefore, the association process is fast, following Eqs. (6,7).

$$Ag_2^+ + Ag_2^+ \rightarrow Ag_4^{2+} \quad (6)$$

$$Ag_m + Ag^+ \rightarrow Ag_{m+1}^+ \quad (7)$$

Finally, monodisperse AgNPs are generated by coalescence process, as given in Eq. (8).

$$Ag_{m+x}^{x+} + Ag_{p+y}^{y+} \rightarrow Ag_{n+z}^{z+} \quad (8)$$

In (8), nuclearities are represented by *m*, *n*, and *p*, while *x*, *y*, and *z* are the numbers of associated silver ions. To obtain the particles with final sizes at nano scale, the coalescence process beyond certain nuclearity must be strictly limited [27-30].

Appropriate capping agents play critical roles in controlling the growth of nucleation center into free clusters in solution to obtain nanosize particles [27-30]. Alginates are one of the beneficial capping agents for colloidal AgNPs due to the occurrence of carboxylate pendant groups. The carboxyl groups have a high affinity to metal ions. Prior to

radiosynthesis, anionic carboxyl group interacts electrostatically with Ag^+ cation within the reaction solution. During the radiosynthesis, pendant carboxyl groups along the alginate chains act as templates for nucleating points, in which the reduction of immobilized Ag^+ cations to zerovalent Ag take place. Consequently, the immobilization avoids coalescence of nucleating points, thus limiting the growth of metal core during synthesis [25,31].

The radiolytic products also react with alginate molecules. Macroradicals are formed upon the contact of alginate with hydrogen or hydroxyl radicals. These radicals are localized at multiple carbon atoms within alginate's monomeric building block, as shown in Eq. (9).

$$^{\bullet}OH(H^{\bullet}) + RH \rightarrow R^{\bullet}(C_1 - C_6) + H_2O(H_2) \quad (9)$$

Only radicals formed at C_1 and C_4 in β(1→4) linkage can undergo rearrangement involving breakage of glycosidic bonds, as shown in Eqs. (10,11).

$$R^{\bullet}(C_1, C_4) \xrightarrow{H_2O} F_1^{\bullet} + F_2 \quad \text{(hydrolysis)} \quad (10)$$

$$R^{\bullet}(C_1, C_4) \rightarrow F_3^{\bullet} + F_4 \quad \text{(scission)} \quad (11)$$

After the main chain scission, the carbonyl group is formed at C_1 and C_4, while stabilization of macroradicals can also occurs via disproportionation reaction, as simplified as Eqs. (12,13), respectively:

$$F_1^{\bullet}(F_3^{\bullet}) \rightarrow R(C=O) \text{ or } R(COOH) \quad (12)$$

$$2R^{\bullet}(F^{\bullet}) \rightarrow 2R(F) \text{ (disproportionation)} \quad (13)$$

In the reactions given in (12) and (13), degradation of alginate is increased in monotonic mode following the increase in radiation dose function [32,33].

Following the Mark-Houwink relationship, decreasing molecular weight is accompanied with decreasing viscosity of the alginate solution. It is reported that minimum viscosity is observed after irradiated at dose of 10 kGy, while still maintaining its ionotropic gelling ability [34]. Visually, the alginate solution becomes less viscous and more transparent [32,33,35]. However, the solution turns brownish in color due to formation of double bond when irradiated under inert gas atmosphere [35].

ROLE OF ALGINATE IN STABILIZATION OF COLLOIDAL AgNPs

Preventing agglomeration is one of the great challenges in the synthesis of colloidal nanomaterials. Due to their surface energy, all nanostructured materials are thermodynamically metastable. The surface energy is sourced from the huge ratio of surface area to volume. At close distances, colloidal nanoparticles are attracted to each other by the van der Waals force. This interaction leads to particles aggregation and subsequently destabilizes the colloidal system [29,36]. For that reason, counteracting force is needed to overcome the large surface energy of colloidal nanoparticles. Modifying the surface charge of AgNPs using charged ions, commonly anionic citrate, provides electrostatic repulsion among them. However, electrostatic repulsion can prevent formation of colloidal AgNPs only at low concentrations and in environments with low electrolyte concentration [36-38].

Alginate solution can limit the agglomeration of colloidal AgNPs during storage due to the electrosterical phenomenon. The immobilization of AgNPs at carboxyl group by covalent interaction prevent interparticles contact, while free anionic carboxyl groups facing the solution provide electrostatic repulsion [36,37]. Electrosteric repulsion provides a better stabilization than electrostatic repulsion because of its ability to stabilize high-concentration colloidal AgNPs. Also, this stabilization is stronger at high electrolyte concentrations, such as in biological environment [37,38]. In addition, AgNPs aggregation is restricted by polymer chain through steric hindrance of particle movements. However, electrosterical stabilization can easily be flocculated via surface charge neutralization or bridging mechanism [39].

Alternatively, alginates can be ionically crosslinked into alginate particles to improve its steric stabilization. Schematic representation of steric and electrosteric stabilization is shown in Fig. 3. AgNPs are immobilized within the matrix of alginate particles. Semenov [39] reported that these steric stabilization relatively insensitive to the present of electrolytes. The degree of steric stabilization can be explained in terms of energy change that occurs upon interaction of the adsorbed layers. If it is considered that the adsorbed layer is impenetrable by polymer chains attached to another surface, thus the adsorbed layer is compressed upon the contact. Also, the configurational entropy of polymer segments occuring in the contact interface is lost [39].

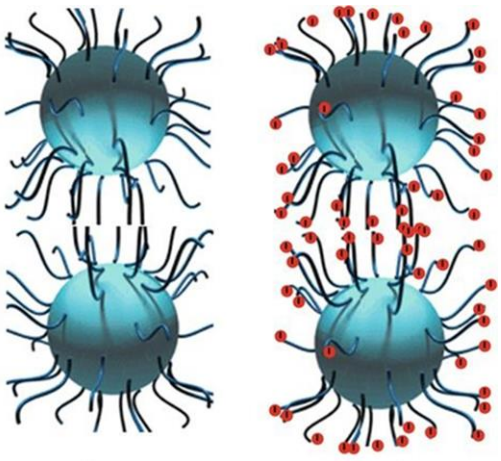

Fig. 3. Schematic mechanism of steric and electrosteric stabilization of nanoparticles.

Nanometer-sized alginate particles are required to maintain the stability of colloidal AgNPs. Otherwise, micrometer-sized particle is formed which would undergo sedimentation by gravitational force. Therefore, ionic crosslinking must be conducted under controlled environment to obtain small-size alginate gel. The methods for synthesis of nanosized alginate nanoparticles have been conclusively reviewed by Paques *et al.* [40]. Briefly, alginate nanoparticles can be formed through four methods: (i) self-assembly and complex formation to form a nanoaggregate; (ii) complexation on the the interface of emulsion droplets to forming a nanocapsule; (iii) water-in-oil emulsion to forming a nanosphere; and, (iv) nanocapsule with structured interior [40]. Schematic representations of the four alginate nanoparticle types resulting are shown in Fig. 4. As previously described, degradation of alginates is accompanied with decreasing solution viscosity, while still maintaining its ionotropic gelling ability [34]. For synthesis of nanoparticles, low molecular weight and low viscosity provide technical advantage in controlling the rate of crosslinking to obtain desired nanosized gel.

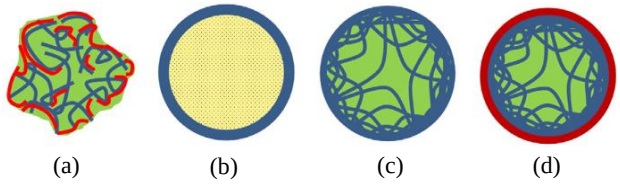

Fig. 4. Schematic structure of (a) nano-aggregate, (b) nanocapsule, (c) nanosphere and (d) nanocapsule with structured interior, respectively. Blue line represents alginate chain, red line represents the polymer that is able to form complexes with alginate, yellow matrix represents hydrophobic liquid, while green matrix represents aqueous liquid [40].

ROLE OF ALGINATE AS ORAL COLON DRUG DELIVERY DEVICE

Localized inflammation-targetting drug delivery is an approach to develop safer and more efficacious therapy. Targeting drug selectively to inflamed region allows locally-high drug concentration with minimal exposure to healthy or distant tissues. The colon is the site in gastrointestinal (GI) tract that can be effectively treated by topical drug therapy for several reasons. As compared to the stomach and the small intestine, this region presents a less hostile environment, a better responsiveness to drug enhancer, and a lengthier residence time [7,8]. Meanwhile, oral administration is the most common route of drug delivery because of its non-invasive nature and simplicity [9,10].

However, the physiology of the gastrointestinal track including the acidic gastric environment as well as mucus barrier prevent the clinical translation of oral formulation [9]. In the case of AgNPs (commonly electrostatically stabilized by citrate capping), the acidic gastric environment can replace its anionic coating and dissolve the AgNPs as silver ions [41,42]. Of important note, silver ions are more cytotoxic and genotoxic to healthy cells as compared to AgNPs [43,44]. Moreover, mucus barrier prevents AgNPs penetration and subsequent contact with and absorption by epithelial cells. To overcome these limitations, alginate nanoparticles are potential candidate to be developed for encapsulation, protection, and delivery of AgNPs to inflamed colon sites. The biocompatibility, pH-sensitiveness, mucoadhesiveness, and negatively-charged alginate nanoparticles are among key features of alginate for oral delivery of AgNPs to the colon [8].

Biocompatibility

Alginates' biocompatibility allows them to be extensively studied in various biomedical applications. Oral administration of alginates has not induced a severe immune response. From the regulatory perspective, alginates are recognized as a "generally recognized as safe" (GRAS) material by the US Food and Drug Administration (US-FDA) [11]. However, many studies observed the immunogenic response after injection or implantation of alginate-based materials [45-47]. The immunogenic responses were attributed to various impurities since the alginates were obtained from natural sources. Further, they reported that the main contaminants responsible to immune response were heavy metals, endotoxins, and polyphenols.

Importantly, multiple-step extraction procedures have been reported to removing heavy metals, endotoxin, and polyphenols. When implanted into animals, the high-purity alginate from these procedures did not induce significant foreign body reaction [45,48,49].

In relation to radiation-induced degradation, oligoalginates interestingly showed beneficial biological activity, such as anti-inflammatory and antioxidant activities. Oral administration of oligoalginate improved intestinal morphology and barrier function and inhibit enterocyte death through reducing apoptosis via mitochondria-dependent pathway [50]. It also showed anti-inflammatory activity which repressed aneurysm recurrence by indirectly affecting TLR signaling via miR-29b [51] as well as preventing development of MCT-induced hypertension and ventricular hypertrophy via inhibition of the TGF-β1/p-Smad2 signaling pathway [52]. It inhibits neuroinflammation and promotes microglial phagocytosis of β-amyloid [53], and also promotes the *ex-vivo* expression of genes important for spermatogenesis [54].

pH sensitivity

Alginates are pH-sensitive biopolymers because of their carboxylic pendant group. The carboxylic acid functional groups are in nonionized (-COOH) state under pH condition of below its pKa (pH > 3.4), in which alginate is in insoluble structure. Conversely, the carboxylic groups are at ionized ($-COO^-$) form at pH above pKa and fully ionized at pH > 4.4. Ionization of carboxylic group leading to polymer chain's expansion and swelling due to increasing electrostatic repulsion of these negative charges [8,55,56]. The pH responsiveness of alginates is schematically presented in Fig. 5. A variation of pH occurs along gastrointestinal tract. It has been reported that the intragastric pH is within the interval of 1 to 2 during fasting, which increases after ingestion of food. In the small intestine, the pH is about 6.5 and 7.5 in the proximal and distal regions, respectively. The pH decreases to 6.4 at cecum and 5.7 in the ascending part of the colon. Then, the pH increases again to 6.6 and 7.0 in the transverse and descending part of the colon, respectively [8,56]. Based on pH variation along GI tract, the pH-responsive feature of alginates allows them to play role as a carrier for oral colon drug delivery system [56,57]. For oral colon delivery of AgNPs, alginate particles may protecting AgNPs in the stomach from dissolution by gastric acid. After then, the pH condition in the intestine and colon allows for diffusion release of AgNPs.

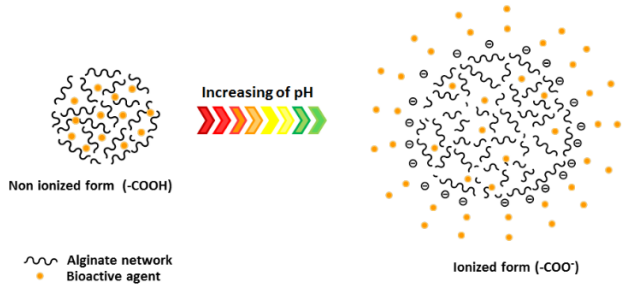

Fig. 5. Schematic representation of sensitiveness of alginate particles to pH stimuli [8].

Mucoadhesiveness

The epithelial cells of GI tract is protected by the mucus layer. This physical barrier protects epithelial cells against acidic and proteolytic environment of the gastrointestinal tract. It also protects the epithelial cells by trapping and promoting clearance of foreign material and pathogens to distal region of GI tract [58,59]. The mucus is a complex of viscoelastic hydrogels which is primarily composed of branched glicoproteins. The main protein components of the mucus are mucins. Mucins are mostly O-linkages to oligosaccharides that protect them against breakdown by digestive enzymes of the digestive system. The glycosylated regions are interspersed by naked protein regions. The naked regions are hydrophobic because they adsorb and are linked to lipids moieties through covalent bond. These lipid-coated domains can adhesively trap hydrophobic particles via hydrophobic bonds. Mucin-mucin interaction also occurs in these region, providing the viscoelastic properties of the mucus [9,60].

The mucus is the major barrier to penetration of nanoparticles. Most of orally administrated nanoparticles undergo direct transit through the GI tract. In the case of inflammatory bowel disease (IBD), diarrhea is a prevalent symptom, lowering the oral drugs efficacy due to increased elimination from GI tract [61]. Therefore, mucoadhesion is a commonly applied strategy to prolong the residence time of nanoparticles. Mucoadhesion is defined as the material's ability to adhere to the mucus layer through various interactions, such as hydrogen bonding, ligand-receptor interaction, electrostatic interaction, and hydrophobic interaction [62].

Alginates have been reported to have good mucoadhesive properties resulting from the occurrence of free carboxyl and hydroxyl groups distributed along the polymer chain backbone [62-65]. However, the electrostatic repulsion force takes place between alginate and mucin in

physiological environment. Mucin has a significant negative charge from its sialic acid and sulphate residues, while alginates performed anionic nature from carboxylic groups. Haugstad *et al.* [64] suggested that the interaction between alginate and mucin prevalently occurred by means of hydrogen bonding. They stated that it is important to reckon the mucus layer as a highly hydrated system, in which over 95 % (w/w) of system is water, while alginate contains a large number of hydrophilic groups that provide numerous interaction points for intramolecular and intermolecular hydrogen bond. Overall, it result in a strong cumulative adhesive force [66]. Additionally, Carvalho *et al.* [63] suggest that the wetting and swelling of alginate is the early step responsible for intimate contact with mucus. The contact allowed for interpenetration between alginate and mucin as well as entanglement between them. Subsequently, these interactions promote the formation of hydrogen bonds.

RECENT DEVELOPMENT IN ALGINATE-BASED ORAL COLON DELIVERY DEVICE

Development of alginate-based drug delivery device for oral colon delivery still attracts much attention until the present time. Several reports in the interval of 2015 to mid-2020 are shown in Table 2. It can be seen that the oral colon delivery of AgNPs is not much explored yet. The anti-inflammatory property of AgNPs have been reported [3-5] with *in-vivo* study have reported promising efficacy of AgNPs for treatment of colitis-induced mice [2,66]. At the same time, several studies have reported the adverse effect of AgNPs on gastrointestinal homeostasis that was driven by inflammation and oxidation [67,68], but the mechanisms of action are poorly understood. It is still in debate whether the toxic effects are caused by silver in form of nanoparticulates [69,70], or whether solely by released silver ions after oxidation of the nanometal [71-73]. It seems important to quantify dissolved silver ions in AgNPs suspensions for investigations of nanoparticle toxicity.

Table 2. Reports on development of colon targeted alginate-based drug delivery device since 2015.

Delivery device	Smart Properties	Active Compound	Bioactivity and Biocompatibility	Research stage
AgNPs/N,N,N-trimethyl chitosan and sodium alginate hydrogel beads	-	AgNPs	Cytotoxic for the Caco-2 cells; not cytotoxic on healthy VERO cells	Pre-clinical *in vitro* [79]
Portulaca oleracea polysaccharide-alginate-borax	pH responsive; Slow release up to 16h	5-Fluorouracil	not studied	Pre-clinical *in vitro* [80]
Alginate-chitosan microspheres	pH responsive; High retention up to 12h	Icariin	Anti-inflammatory	Pre-clinical *in vivo* in rat [81]
alginate microbeads incorporating folic acid-grafted solid lipid nanoparticles	pH responsive	Irinotecan	Cytotoxic effect against COLO-205 cells	Pre-clinical *in vitro* [82]
High mannuronic acid Ca-alginate capsules dried at 45 °C or freeze dried, hydrated Ba-alginate, and hydrated Zn-alginate	pH responsive	Indomethacin	Not studied	Pre-clinical *in vitro* [74]
Alginate-chitosan coated layered double hydroxide nanoparticles nanocomposites	pH responsive	Protein vaccine	Enhanced protein internalization of proteins by Caco2 and macrophage cells.	Pre-clinical *in vitro* [75]
Mucopenetrating polymer – lipid hybrid nanovesicles as subunits in alginate beads	Mucoadhesive; Mucopenetrating properties	-	Mucoadhesive and mucopenetrated to mucus layer on Caco-2/HT29-MTX-E12 cell culture	Pre-clinical *in vitro* [93]
Alginate modificated graphene oxide	Controlled-release	5-fluorouracil	Lower toxicity, inhibited tumour growth and liver metastasis, and prolonged the survival time of mice	Pre-clinical *in vivo* in mice [94]
Sodium dodecylsulfate modified calcium alginate bead	pH responsive, controlled-release	Bovine serum albumin	Not studied	Pre-clinical *in vitro* [76]
Calcium alginate - carboxymethyl cellulose beads	pH responsive; Mucoadhesivity	5-Fluorouracil	Colonic microflora-catered biodegradability	Pre-clinical *in vitro* [95]
Barium crosslinked guar gum succinate-sodium alginate beads	pH responsive	Ibuprofen	No cytotoxic effect against C3H10T1/2 mouse mesenchymal stem cells	Pre-clinical *in vitro* [78]
Chitosan/alginate/ Eudragit S multilayers	pH responsive; Controlled-release	Dexametha-sone	Significant therapeutic activity	Pre-clinical *in vitro* [83]
Chitosan/alginate core-shell nanoparticles	pH responseive; Mucoadhesive; Controlled-release	Naringenin	Significant antidiabetic responses, no systemic toxicity	Pre-clinical *in vivo* in rat [96]
Alginate/chitosan microparticles	pH responseive	AvrA, a *Salmonella* effector enzyme	Reduced clinical symptoms in a murine DSS-induced colitis pre/co-treatment model.	Pre-clinical *in vitro* [77]

Regarding the delivery device, most reports in Table 2 focused on improving alginate stability in small intestine due to rapid dissolution of calcium alginate beads in intestinal fluid [74]. It is important to prevent degradation of peptide drug (modelled by protein vaccine [75], bovine serum albumin [76], or AvrA [77]) by digestive enzyme in the intestine. Improvement in delivery device stability is approached either by crosslinking with various divalent cation [74,78], blending with other polymers [79-81], formation of layer-by-layer shell-core composite [75,82], or modification with surfactants [76,83]. It must be noted that most of research activities are at *in-vitro* stage. More effort is needed to accelerate translation of this technology from bench to the bedside.

CONCLUSION AND PERSPECTIVE

Alginates have attracted high interest for use as a pharmaceutical excipient for development of oral delivery for local colon therapy. This condition is supported by regulatory perspective in which alginate is approved by US FDA as a GRAS material. Concurently, there is a growing interest in the therapeutic revolutions driven by nanotechnology. This situation, together with alginate's great versatility in ters of its modifiable physicochemical properties, has steadily increased research activities at the interface of alginate and nanomaterials. At the same time, high purity alginates must be required to ensure low concentration of immunogenic impurities.

This review highlights the outstanding versatility of alginates in drug delivery of radiosynthesized nanoparticle system. Alginates as a capping agent can play multiple roles: radiosynthesis, stabilization of nanoparticle system, and oral colon delivery devices of AgNPs. Interestingly, radiation-induced degradation gives benefits not only by providing technical advantages during synthesis on nanosized alginate particles, but also by showing anti-inflammatory activity. The most outstanding characteristics are pH-responsiveness of alginate particles for protection and selective release of AgNPs, as well as mucoadhesiveness to prolong residence time in the GI tract. These characteristics allow targeted delivery of AgNPs into the colon, thus minimizing the drug uptake into circulatory system. Ultimately, alginate-based drug delivery devices are expected to improve drug efficacy while reducing the systemic side effects.

However, there are still many barriers and challenges that need to be overcome. Recent studies on alginate nanoparticles seem to be more focused on the production methods, ranging from simple ionotropic crosslinking to more complex production systems. Indeed, it is important to make a stable nanosystem under both processing and biological environment to allow effective delivery to the target tissue and efficient uptake by cells. This effort can alternatively be approached through modification of alginate molecules. The versatility of alginate chemistry is well known, and it allows the modification of alginate backbone chains into novel polymers with desired properties. The modification can be designed to improve drug bioavailability, reduce non-specific toxicity, and control drug release profiles of alginate-based drug delivery system.

In relation to nanomedicine, research on alginate nanoparticles has been limited to drugs and at *in-vitro* scale. Meanwhile, there has been a growing interest in theranostic nanomedicine in which therapy and diagnosis are integrated in a single platform using nanomaterials. The theranostic nanomedicine has been exemplified in the oncology field. Interestingly, AgNPs are known for their selective toxicity to cancer cells. Also, their nanosize and quantum confinement provide superior optical properties for biosensing and bioimaging. However, uncertainties regarding the causes and mechanism of AgNPs toxicity are major issues when assessing the effects of AgNPs. Furthermore, the fate and biological activity of AgNPs within living systems vary and are dependent on cell types, uptake mechanism, and the functionalities presented by the particles, *i.e.*, concentration and exposure time, shape, size, and nature of stabilizing agent. Therefore, toxicology evaluation is required for the particular system of colloidal AgNPs.

Moreover, novel targeting and nanoparticle stabilization strategies are needed to achieve site-specific drug delivery. Due to the chemical versatility of alginates, specific ligands can be conjugated on the surface of alginate-based nanoparticles for targeting a specific overexpressed receptor on cancer cells. Simultaneously, this ligand-based targeted therapy critically depends on novel ligand discovery for efficacious cancer treatment. Overall, alginate-based platforms have a great translational potential for targeted nanometal delivery system which may drive the development and implementation of innovative delivery systems for novel metal-based nanomedicine.

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AUTHOR CONTRIBUTION

All authors equally contributed as the main contributors of this paper. All authors read and approved the final version of the paper.

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