Review

Mucoadhesive Hydrogel Nanoparticles as Smart Biomedical Drug Delivery System

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Abstract: Hydrogels are widely used materials which have many medical applications. Their ability to absorb aqueous solutions and biological fluids gives them innovative characterizations resulting in increased compatibility with biological activity. In this sense, they are used extensively for encapsulation of several targets such as biomolecules, viruses, bacteria, and mammalian cells. Indeed, many methods have been published which are used in hydrogel formulation and biomedical encapsulations involving several cross-linkers. This system is still rich with the potential of undiscovered features. The physicochemical properties of polymers, distinguished by their interactions with biological systems into mucoadhesive, gastro-adhesive, and stimuli responsive polymers. Hydrogel systems may be assembled as tablets, patches, gels, ointments, and films. Their potential to be co-formulated as nanoparticles extends the limits of their assembly and application. In this review, mucoadhesive nanoparticles and their importance for biomedical applications are highlighted with a focus on mechanisms of overcoming mucosal resistance.

Keywords: hydrogel system; bioadhesion; mucoadhesion; mucus

1. Introduction

Hydrogels can be defined as network structures configured at two or three dimensions which have the ability to imbibe high amounts of aqueous solutions or biological fluids [1]. This affinity for water adsorption is mostly related to the presence of hydrophilic (e.g., amide, amino, carboxyl, and hydroxyl) groups on their molecular chains [2]. Due to the presence of these groups, several physicochemical properties have contributed to the classification of hydrogel systems such as porosity, structure softness, swelling capacity, and elasticity [3]. These properties are more similar to biological system than those of any other synthetic biomaterial [4]. To reinforce their structure and strengthen their mechanical force, hydrogels are cross-linked either by using physical mechanisms or chemical cross-linkers [5]. The cross-linkers in the polymer network are formulated by covalent bonds, hydrogen binding, van der Waals interactions, or by physical entanglements [6]. Furthermore, the hydrogel system becomes more solid and insoluble in any solvent, once its polymer chains are cross-linked [7]. Such hydrogels can be assembled with cryo-gelation [8], gas foaming [9], micro-emulsion formation [10], freeze-drying [11], and porogen leaching [12]. It is reported that the porous capacity and swelling behavior of ahydrogel mostly depend uponcross-linkers [13], chemical structure of the repeating unit or chemical composition [14], network structure [15], solvent concentration, surrounding medium, polymer molecular weight, quality of solvent, and the specific stimulation. Hydrophilic hydrogels show distinctive properties due to adsorption of high amounts of water in their structures compared to hydrophobic polymericnetworks. Hence, hydrophobic groups

may collapse in the presence of water. The collapsed chains minimize their exposure to water molecules, ultimately resulting in lower swelling ratios [16].

Efficient delivery systems have been developed based upon on the understanding of their interactions with the biological environment, target cell population, target cell surface receptors, changes in cell receptors that occur with progression of disease, mechanism and site of drug action, drug retention, multiple drug administration, molecular mechanisms, and pathology [17]. For these reasons, many reports have referred to bio-adhesive polymers as promising materials that should be integrated into the moieties of nanoparticles [18]. This is in part due to their reactive potential and good penetration into stomach and mucosal layers. Bio-adhesive polymers are classified according to their interactions with biological systems into subgroups: mucoadhesive polymers which can penetrate mucus layers (e.g., ocular, nasal, vaginal, and small intestinal); gastro-adhesive polymers which can adhere onto stomach lining; and stimuli responsive polymers which can react to biological stimulants (e.g. glucose, enzymes, pH, and inflammation) (Figure 1) [19]. In addition to tablets, patches, gels, ointments, and film formulations; mucoadhesive nanoparticles have emerged recently to improve the controlled delivery of drugs. For nasal, vaginal, ocular, and oral administration, many nanoparticles have been optimized to possess mucoadhesive properties [20–22].
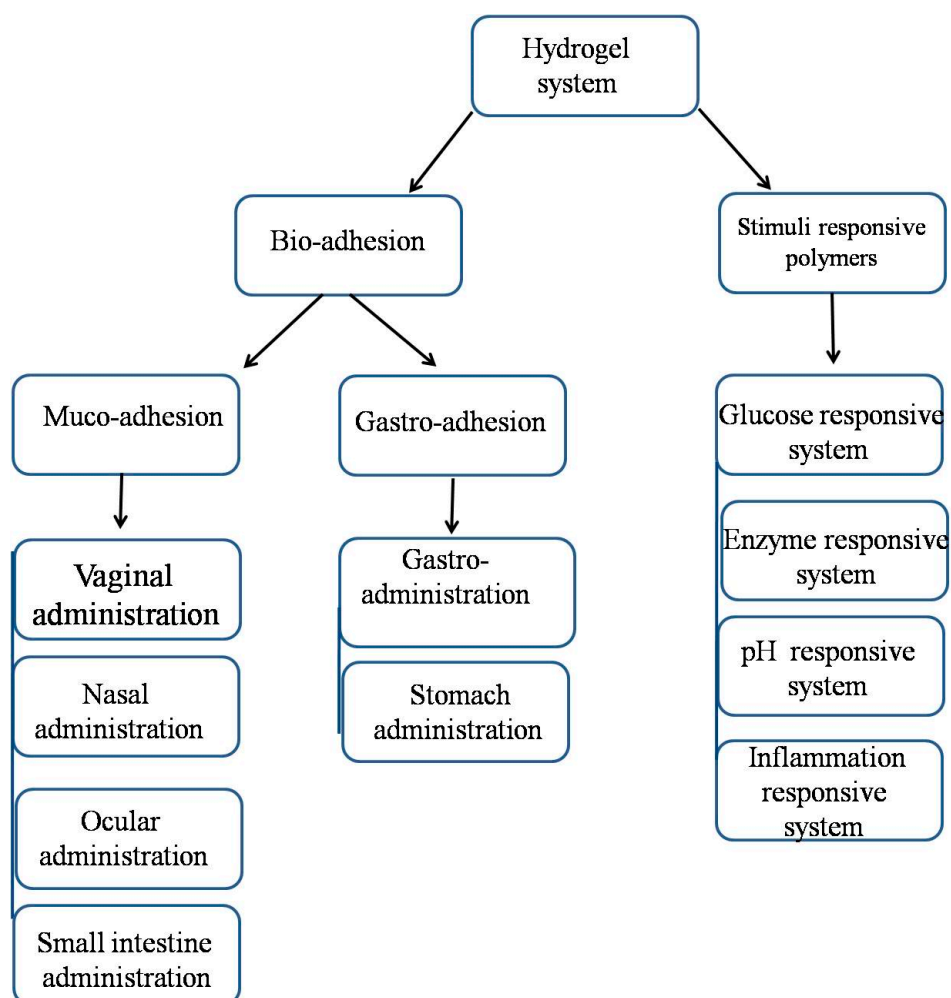

Figure 1. Classification of hydrogel systems according to their response to biological systems.

2. Structure of Mucus Layers

Mucosal layers mainly composed of viscoelastic fluid are secreted by glandular columnar epithelial cells and contain around 99% mucin and water, with trace amounts of lipids, other proteins,

and muco-polysaccharides [23]. They line the walls of various bodily cavities such as gastrointestinal and respiratory tracts, as well as vaginal and ocular surfaces. They consist of a connective tissue layer (the lamina propria) above that is an epithelial layer, the surface of which is made moist usually by the presence of a mucus layer. The epithelia may be composed of a single layer (e.g., in the bronchi, stomach, and small intestine) or be multilayered/stratified (e.g., in the esophagus, vagina and cornea). The former contain goblet cells that secrete mucus directly onto the epithelial surfaces and the latter contain (or are adjacent to tissues containing) specialized glands such as salivary glands that secrete mucus onto the epithelial surface. Mucus is a visco-elastic gel which contains mucin as the main dry weight component, typically at 2–5% (w/v), with smaller proportions of lipids and other proteins [24].

Mucins consist of high molecular weight glycoproteins encoded for by more than 20 different MUC genes. Mucins are either secreted or membrane-bound, and their expression pattern varies with their physiological site. In the gastrointestinal tract the membrane-bound MUC1, MUC3, MUC4, MUC12, MUC13, MUC16, and MUC17 proteins are expressed in addition to the secreted MUC2, MUC5AC, MUC5B, MUC6, and MUC7 [25]. MUC2 and MUC5A are mucins secreted in the small intestine and the stomach, respectively. The protein structure of mucin is rich in repeats of serine, threonine, and proline. These domains are heavily glycosylated with fucose, galactose, sialicacids, N-acetylglucosamine (GlcNAc), and N-acetylgalactosamine (GalNAc) [26]. The oligosaccharides account for 70–80% of the molecular weight of mucin and are believed to protect the protein core from degradation. The carbohydrate-bound ester sulphate residues and carboxyl groups of sialic acids provide mucus with a net negative charge [27]. Thus, the charge of the attached oligosaccharides causes intestinal mucus to bear a more negative charge, as compared to gastric mucus. The glycosylated regions of mucins are hydrophilic, whereas the non-glycosylated protein domains are hydrophobic. These naked domains are rich in cysteine, and disulphide bonds link the mucin monomers. On average, mucins are composed of four monomers with each monomer having four to five glycosylated domains [28]. Some of the lipids in mucus are covalently linked to the non-glycosylated domains of mucin, while most phospholipids adsorb and form a hydrophobic luminal lining of the mucus. The anatomic structures of mucosal layers found in different organs are shown in Table 1 [29].

Table 1. Comparison of mucus membranes in different organs.

Mucus Membrane	Mucus Surface Area	Thickness of Mucus Layer	Layers	Turnover Time
Buccal	30 cm^2	0.1–0.7 mm	Epithelium, basement membrane, and connective tissues	5–6 days
Nasal	160 cm^2	5–20 μm	Both keratinized and nonkeratinized epithelial cells	10–15 min
Ocular		3–10 μm	Pithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium	15–20 h
Vaginal	6–10 cm^2	10–15 layers	The epithelial layer consists of the lamina propia and stratified squamous epithelium	7 days
Rectal	300 cm^2	50 μm	Single layer of cylindrical cells and goblet cells secreting mucus	90 h

3. Mucus Barrier Resistance to Drugs and Delivery Systems

The viscous state of mucus structure allows poor penetration for drugs and drug delivery systems, in most cases causing high limitations for hydrophobic drug compounds [30,31]. Upon a drug's interaction with mucosal layers, it may suffer from one or more obstructions such as dynamic, steric, and interactive barriers.

3.1. The Dynamic Barrier

Mucus secretion occurs mostly in a continuous state and can be increased or modified by disease. Additionally, peristalsis contributes to the horizontal flow along mucosal surfaces. Thus, drugs or

drug delivery systems that adhere and bind to the outermost layer of mucus might be more successful in penetrating the mucosal surface.

3.2. The Steric Barrier

It is reported that mucin can form network properties exhibiting a size exclusion filter. Thus, larger compounds maybe blocked. The viscosity of the mucus and the unstirred water layer also contribute to the steric properties of the mucus barrier.

3.3. The Interactive Barrier

Mucus moieties can interact with drugs or drug delivery systems forming multiple low affinity interactions. The carbohydrates of mucin provide numerous hydrogen bond acceptors and donors as well as the potential for ionic interactions. Thus, hydrophobic interactions between the naked protein core of mucin or the lipids of mucus with diffusing compounds can be observed [32].

4. Mucoadhesive System

Mucoadhesion describes the ability of hydrogel nanoparticles to adhere to and penetrate the surface of mucosal layers [33]. Indeed, nanoparticles produced by mucoadhesive polymers have the advantage of being immobilized at the mucosal surface by an adhesion mechanism. The mucoadhesive action depends on the physical reaction of hydrogel moieties with the structure of mucosal content, and results in the swelling and expanding of the hydrogel. The swelling state perhaps facilitates and supports the adhesion of the hydrogel along mucosal layers. This stage is followed by a chemical interaction between hydrogels and mucosal contents allowing for cellular penetration and internalization of nanoparticles (Figure 2) [34]. Many factors can affect mucosal–hydrogel interactions, such as average molecular weight of polymers, chain flexibility, hydration, hydrogen bonding capacity, charge, and biological environmental factors (e.g., pH, ionic strength, and mucins) [35].
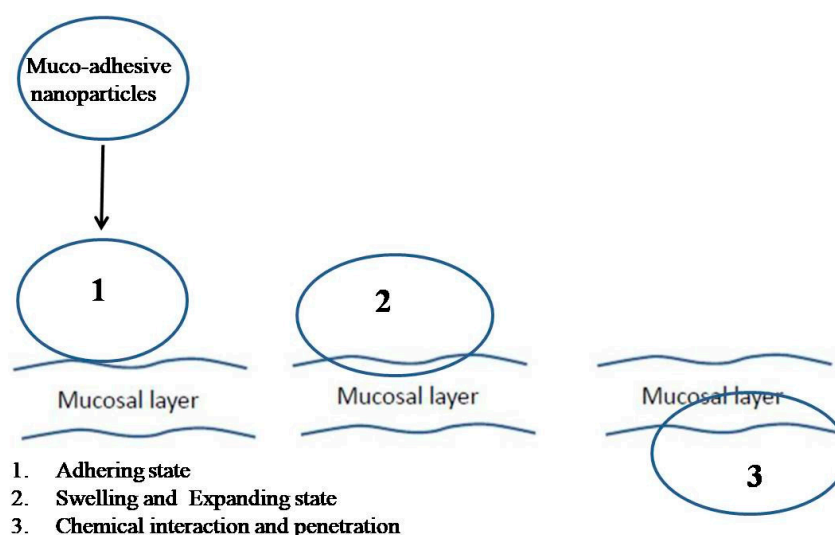

Figure 2. Scheme illustrating a possible penetration mechanism of synthetic mucoadhesive nanoparticles on mucosal structure.

5. The Theories of Mucoadhesion

Mucoadhesion is thought to be caused by two mechanisms: the contact stage and the consolidation stage. Mucoadhesive nanoparticles which attach to a mucus membrane may result in swelling of the formation. This condition leads to the spread of the formation along the surface of the mucosal layer. This may facilitate penetration of nanoparticles inside mucosal layers [36]. In both ocular and vaginal administration, a delivery system is mechanically attached to the membrane, while in the nasal route

an aerodynamic mechanism enables the delivery system to be attached. In the consolidation step, moieties of nanoparticles initiate their interaction with the moisture of mucus with weak van der Waals forces, followed by the formation of hydrogen bonds in most cases of penetration by nanoparticles into deeper layers of mucus. For instance, the glycoproteins of the mucus contribute to interpenetration of delivery systems by building secondary bonds [30]. Several theories describe physical, chemical, and mechanical attachment of a mucoadhesive system to mucous layers. These theories are shown in Table 2.

Table 2. Theories of mucoadhesion.

Theory	Chemical and Physical Reaction	Biological Reactive System	Result
Electronic theory	Electron transfer reaction	Mucus and the mucoadhesive system	Electrical double layer of charges at the mucus and mucoadhesiveinterface
Adsorption theory	Hydrogen bonding reaction	Mucus and the mucoadhesive system	Adhesive interaction betweenthe substrate surfaces
Diffusion theory	Adhesive force	Mucus and the mucoadhesive system	Interpenetration of both polymer and mucin chains to a sufficient depth
Wetting theory	Spreading property	Mucus and the mucoadhesive system	Attachment regarding to contact angle
Fracture theory	Detachment of polymer moieties	Mucus and polymer moieties	Relates the force for polymer detachment

6. Mucoadhesion and Its Relation with Certain Polymers

Several approaches have been designed to optimize hydrogel with bio-adhesive properties to overcome the issue of mucosal penetration and improve gastro-intestinal tract uptake [37]. Such properties can be obtained by using marine mussels incorporating the catechol moiety 3,4-dihydroxy-L-phenylalanine (DOPA) in hydrogels to promote bio-adhesion [38]. Bio-adhesion may also be improved by the interconnection of polymers having mucoadhesive properties such as chitosan, hyaluronic acid, polygalacturonic acid (pectin), alginate, and poly(acrylic acid) (Table 3) [39].

Additionally, lectin proteins canfurtherincrease the adherence of microparticles to the intestinal epithelium and enhance the penetration of drugs. Recently, lectins have been incorporated into nanoparticle moieties as a targetedtherapy that can bind to carbohydrate moieties attached to glycoproteins expressed on the surface of cancer cells [40]. Thiolated polymers have also emerged as a promising approach that can be used for mucoadhesion and muco-penetration in drug delivery [41].

The advantage of thiolated polymers is the ability of their free thiol groups to bind with cysteine domains in mucin [42]. The inter- and intramolecular disulfide bonds that form between mucin and thiol groups, can lead to relatively improved stability, prolonged residence and disintegration time in the mucus, and more sustained drug release [43]. Various thiolated polymers include chitosan–iminothiolane, poly(acrylic acid)–cysteine, poly(acrylic acid)–homocysteine, chitosan–thioglycolic acid, chitosan–thioethylamidine, alginate–cysteine, poly(methacrylic acid)–cysteine, and sodium carboxymethylcellulose–cysteine [44].

It is demonstrated that chitosan adheres to mucosal surfaces within the body [45] and canopen tight junctions between epithelial cells even through well organized epithelia [46]. The electrostatic interaction between the positively charged amines on chitosan polymer chains (pKa = 6.5) and the negative sialic acid residues on the glycoproteins in mucosal fluid plays a role in the mucoadhesive properties of chitosan [47]. Moreover, chitosan possesses NH_2 groups that can react with mucin forming electrostatic interpenetration [48].

Table 3. Polymers used to optimize mucoadhesive systems.

Polymer	Chemical Structure	Charge	Hydrogel Groups	Structure
Chitosan	Poly[β-(1,4)-2-amino-2-deoxy-D-glucopyranose]	Cationic	NH_2 and OH	
Hyaluronic acid	D-glucuronic acid and N-acetyl–D-glucosamine linked together through alternating β(1,3) and β(1,4) glycosidic bonds	Anionic	COOH and OH	
Alginic acid	β-(1,4)-D-mannuronic acid (M) and α-(1,4)-L-guluronic acid (G) units	Anionic	COOH and OH	
Polygalacturonic acid	Heterogeneous structure bonded via α (1,4) glycosidic linkage.	Anionic	COOH and OH	
Carboxymethyl cellulose	Cellulose derivatives	Anionic	COOH and OH	
Poly(acrylic acid)	Polyacrylates	Anionic	COOH and OH	

Polygalacturonic acid (PgA) is a natural polymer with a heterogeneous structure bonded via α (1,4) glycosidic linkage. Hanafy and his colleagues have demonstrated that PgA has limited dissolution in distilled water, Phosphate buffer saline (pH7.3), and acidic distilled water (pH3), resulting in white turbidity (Figure 3) [49]. This result is consistent with its hydrophobic properties [50]. PgA is not degraded in the upper gastrointestinal tract due to its insolubility in acidic conditions [51]. However, PgA can be dissolved in alkaline distilled water (pH 10), exhibiting a translucent yellow-green color. This means it can be hydrolyzed in the high pH environment of the colon. The cause of this dissolution is related to the pKa of its carboxyl group, i.e., at low pH it is protonated and the polymer is insoluble, while it is deprotonated at higher pH leading to polymer dissolution [52]. It is reported that the combination of PgA with a second polymer into a composite may alter the degree of swelling and can change its mechanical properties [53], improving both the stability of encapsulated drugs and the control of their release.
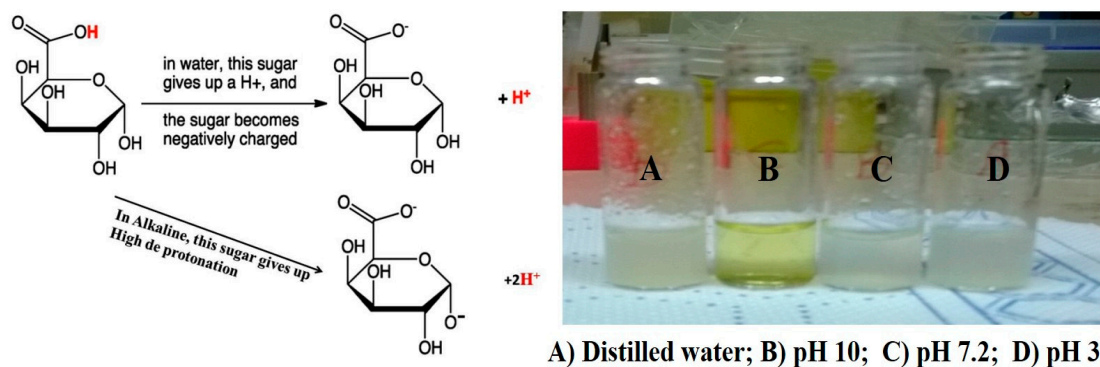
A) Distilled water; B) pH 10; C) pH 7.2; D) pH 3.

Figure 3. Dissolution of PgA by alkaline solution (figure adapted from [49]).

It is reported that the addition of pectin to mucin in deionized water can form an aggregated compound, demonstrating an association between pectin and mucin [54]. Despite both polymers bearing a negative charge, this reaction probably induces a hydrogen–molecular interaction, where electrostatic repulsion may cause the uncoiling of polymer chains, facilitating chain entanglement and bond formation [55].

Poly(acrylic acid) (PAA) is a hydrophilic polymer [56] that adopts a random coil conformation in solution. In their recent work, Hanafy and his colleagues have further demonstrated that these coils have swelling properties under certain ionic strengths and salt concentrations, leading to extended chain conformations in alkaline solutions (Figure 4) [49,57–59]. This is due to the COOH side chain which is pH responsive, i.e., protonated (COOH) at pH ≤ 5 while de-protonated (COO–) at pH > 5 [60]. In aqueous media of appropriate pH and ionic strength, the carboxylic groups ionize and develop fixed charges on the polymer network, generating electrostatic repulsive forces responsible for pH-dependent behavior, causing swelling or shrinking of the hydrogel structure [61]. PAA is expected to form a hydrophilic corona. While in alkaline pH, the interaction of the swelling coils of PAA with water makes it more favorable for protection, as it adsorbs water at a weight many more times than that of its own. [62,63]. Additionally, PAA is poorly soluble in water at low pH, causing structure shrinkage. This mechanical elasticity is predicted to protect the micelles during oral administration and through the gastrointestinal tract.
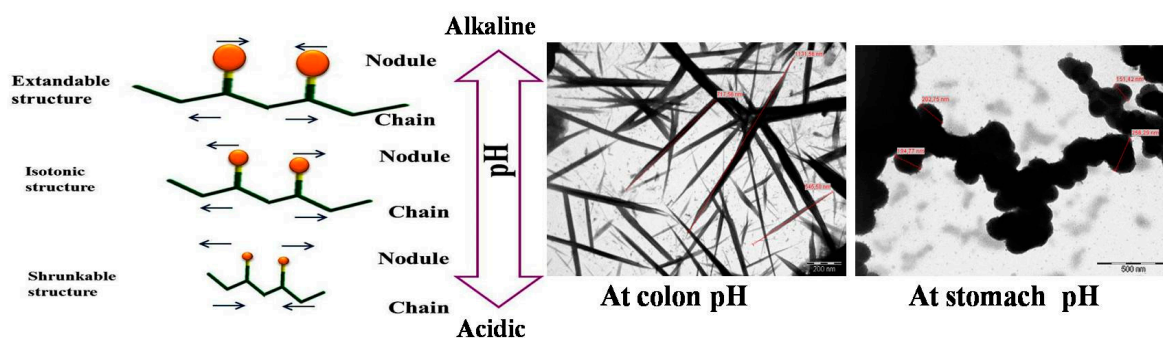

Figure 4. Poly(acrylic acid)extended at alkaline condition and shrunk at acidic condition(figure adapted from [49] and [56]).

Poly(acrylic acid) doped with cysteine can increase mucus glycoprotein adhesion via thiol–disulfide reaction. This reaction can significantly improve the mucoadhesive properties of PAA [64].

Carboxymethyl cellulose (CMC) is a biodegradable polymer and a cellulose derivative. CMC is a good candidate for hydrogel formation because it has a good swelling capacity. Its carboxyl and hydroxyl groups are mainly responsible for hydrogel assembly due to their hydrogen intramolecular reaction [65]. Hanafy and his colleagues fabricated a hydrogel system upon a surface of fluoro-magnetic

nanoparticles by using polyethylene glycol and carboxymethyl cellulose. This hydrogel was constructed using layer-by-layer assembled polymers. Further, their hydrogel properties were used to derive activin-like kinase proteins [66].

The adhesion of pectin [51], polyacrylic acid [60], hyaluronic acid [67], and alginates to mucin makes them stronger electron donors than water. Also, hydroxyl groups can act as electron acceptors. Superior mucoadhesion is further demonstrated for polymers with low molecular weights because they can more easily interpenetrate the mucin network, or display intermolecular interactions with mucin molecules [68–71].

7. Importance of Mucoadhesive Polymers for Optimizing Drug Delivery Systems

Mucoadhesive delivery has received great interest for optimizing drug delivery systems for many reasons. This system can enhance the bioavailability of therapeutic agents by avoiding drug degradation. Hydrophobic and hydrophilic molecules can be integrated inside acore–shell assembly while the surface can be further functionalized by ligands and other moleculesto increase its cellular attachment. This system is suitable for multidrug encapsulation. The physicochemical properties of mucoadhesive systems (e.g., swelling, expanding, and shrinking), are advantageous to reaction with mucosal layers, allowing nanoparticles to be internalized in tissues [49,56,58]. This system can be functionalized with several monomers that could represent other advantages for mucoadhesion either by simple fabrication or in case Layer by Layer assembly for inorganic [72] and metal nanoparticles [66,73,74]. Moreover, this system is suitable for drug delivery in oral, vaginal, nasal, and ocular administration. It may be summarized that mucoadhesive hydrogel nanoparticles represent a new and vital frontier in the optimization of drug delivery systems.

8. Conclusions

Hydrogel systems can be more easily functionalized during fabrication than any other drug delivery system, with the addition of several monomers conferring great physicochemical advantages. These advantages can increase biocompatibility, biodegradability, porosity, and mucoadhesion. Accordingly, any delivery system that is not developed with mucoadhesive moieties should not be recommended for tissues containing mucosal structure (such as ocular, vaginal, nasal, and small intestinal surfaces). Considering this limitation, many efforts have been made to improve mucoadhesion by adding thiol groups to polymer chains.

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