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Bioadhesion: an Approach Towards Mucoadhesive Drug Delivery System

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ABSTRACT

Bioadhesion can be defined as the process by which a natural or a synthetic polymer can adhere to a biological substrate. When the biological substrate is a mucosal layer then the phenomena is known as mucoadhesion. The substrate possessing bioadhesive property can help in devising a delivery system capable of delivering a bioactive agent for a prolonged period of time at a specific delivery site. The current review provides a good insight on mucoadhesive polymers, the phenomenon of mucoadhesion and the factors which have the ability to affect the mucoadhesive properties of a polymer. This review also considers the basic mechanisms by which mucoadhesive can adhere to a mucous membrane in terms of the nature of the adhering surfaces and the forces that may be generated to secure them together. Mucosal adhesion is backed by several theories which include electronic, adsorption, wetting, diffusion, fracture and mechanical. Stages of mucoadhesion include contact stage and consolidation stage.

Keywords: Mucosa, mucoadhesion, mucoadhesive polymers, drug delivery.

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INTRODUCTION

Bioadhesion can be defined as a phenomenon of interfacial molecular attractive forces amongst the surfaces of the biological substrate and the natural or synthetic polymers, which allows the polymer to adhere to the biological surface for an extended period of time[1-4]. Bioadhesive polymeric systems have been used since long time in the development of products for various biomedical applications which include denture adhesives and surgical glue[5-8]. The adhesion of bacteria to the human gut may be attributed to the interaction of lectin-like structure (present on the cell surface of bacteria) and mucin (present in the biological tissues)[9-12]. In general, various biopolymers show the bioadhesive properties and have been utilized for various therapeutic purposes in medicine[2,13]. The bioadhesive polymers can be broadly classified into two groups, namely specific and nonspecific[14]. The specific bioadhesive polymers (e.g. lectins, fimbrin) have the ability to adhere to specific chemical structures within the biological molecules while the nonspecific bioadhesive polymers (e.g. polyacrylic acid, cyanoacrylates) have the ability to bind with both the cell surfaces and the mucosal layer. The use of mucoadhesive polymers for the development of pharmaceutical formulations dates back to 1947, when attempts were made to formulate a penicillin drug delivery system for delivering the bioactive agent to the oral mucosa using gum tragacanth and dental adhesive powders[15]. Improved results were reported when carboxymethylcellulose and petrolatum were used for the development of the formulation. Subsequent research resulted in the development of a mucoadhesive delivery vehicle which consisted of finely ground sodium carboxymethylcellulose (SCMC), pectin, and gelatin. The formulation was later marketed as Orahesive®. Another formulation which entered into the clinical trials is Orabase®, which is a blend of polymethylene/ mineral oil base. This was followed by the development of a system where polyethylene sheet was laminated with a blend of sodium carboxymethylcellulose and poly (isobutylene) which provided an added advantage of protecting the mucoadhesive layer by the polyethylene backing from the physical interference of the external environment[16-18]. Over the years, various other polymers (e.g. sodium alginate, sodium carboxymethylcellulose, guar gum, hydroxyethylcellulose, karya gum, methylcellulose, polyethylene glycol (PEG), retene and tragacanth) have been found to exhibit mucoadhesive properties. During the period of 1980s poly (acrylic acid), hydroxypropylcellulose, and sodium carboxymethylcellulose were widely explored for the development of formulations having mucoadhesive properties. Since then the use of acrylate polymers for the development of mucoadhesive formulations have increased

many-fold, various authors have investigated the mucoadhesive properties of different polymers with varying molecular architecture[19-21]. After a lot of research, the researchers are of the view that a polymer will exhibit sufficient mucoadhesive property if it can form strong intermolecular hydrogen bonding with the mucosal layer, penetration of the polymer into the mucus network or tissue crevices, easy wetting of mucosal layer and high molecular weight of the polymer chain. The ideal characteristics of a mucoadhesive polymer matrix include the rapid adherence to the mucosal layer without any change in the physical property of the delivery matrix, minimum interference to the release of the active agent, biodegradable without producing any toxic byproducts, inhibit the enzymes present at the delivery site and enhance the penetration of the active agent (if the active agent is meant to be absorbed from the delivery site)[22].

The need to deliver 'challenging' molecules such as biopharmaceuticals (proteins and oligonucleotides) has increased interest in this area. Mucoadhesive materials could also be used as therapeutic agents in their own right, to coat and protect damaged tissues (gastric ulcers or lesions of the oral mucosa) or to act as lubricating agents (in the oral cavity, eye and vagina).

Mucous Membranes

Mucous membranes (mucosae) are the moist surfaces, lining the walls of various body cavities such as the gastrointestinal and respiratory tracts. They consist of a connective tissue layer (the lamina propria) above which is an epithelial layer, the surface of which is made moist usually by the presence of a mucus layer. The epithelia may be either single layered (e.g. the stomach, small and large intestine and bronchi) or multilayered/stratified (e.g. in the oesophagus, vagina and cornea). The former contain goblet cells which secrete mucus directly onto the epithelial surfaces, the latter contain, or are adjacent to tissues containing, specialized glands such as salivary glands that secrete mucus onto the epithelial surface. Mucus is present as either a gel layer adherent to the mucosal surface or as a luminal soluble or suspended form. The major components of all mucus gels are mucin glycoproteins, lipids, inorganic salts and water, the latter accounting for more than 95% of its weight, making it a highly hydrated system. The mucin glycoproteins are the most important structure-forming component of the mucus gel, resulting in its characteristic gel-like, cohesive and adhesive properties. The thickness of this mucus layer varies on different mucosal surfaces, from 50 to 450 µ m in the stomach, to less than 1 µ m in the oral cavity. The major functions of mucus are that of protection and lubrication (they could be said to act as anti- adherents)[23-25].

Other than the low surface area available for drug absorption in the buccal cavity, the retention of the dosage format the site of absorption is another factor which determines the

success or failure of buccal drug delivery system. The utilization of mucoadhesive systems is essential to maintain an intimate and prolonged contact of the formulation with the oral mucosa allowing a longer duration for absorption. Some adhesive systems deliver the drug towards the mucosa only with an impermeable product surface exposed to the oral cavity which prevents the drug release into oral cavity. For example, Lopez and co-workers designed bilaminated films to provide unidirectional release of drug and avoid buccal leakage. They contained a bioadhesive layer made up of chitosan, polycarbophil, sodium alginate and gellan gum while backing layer made up of ethyl cellulose.

Sites for Mucoadhesive Drug Delivery Systems

The common sites of application where mucoadhesive drug delivery systems have the ability to delivery pharmacologically active agents include oral cavity, eye conjunctiva, vagina, nasal cavity and gastrointestinal tract. The current section of the review will give an overview of the above-mentioned delivery sites.

The buccal cavity has a very limited surface area of around 50 cm^2 but the easy access to the site makes it a preferred location for delivering active agents. The site provides an opportunity to deliver pharmacologically active agents systemically by avoiding hepatic first-pass metabolism in addition to the local treatment of the oral lesions. The sublingual mucosa is relatively more permeable than the buccal mucosa (due to the presence of large number of smooth muscle and immobile mucosa), hence formulations for sublingual delivery are designed to release the active agent quickly while mucoadhesive formulation is of importance for the delivery of active agents to the buccal mucosa where the active agent has to be released in a controlled manner. This makes the buccal cavity more suitable for mucoadhesive drug delivery[27].

Like buccal cavity, nasal cavity also provides a potential site for the development of formulations where mucoadhesive polymers can play an important role. The nasal mucosal layer has a surface area of aroundb150-200 cm^2. The residence time of a particulate matter in the nasal mucosa varies between 15 and 30 min, which have been attributed to the increased activity of the mucociliary layer in the presence of foreign particulate matter[28].

Ophthalmic mucoadhesives also is another area of interest. Due to the continuous formation of tears and blinking of eye lids there is a rapid removal of the active medicament from the ocular cavity, which results in the poor bioavailability of the active agents. This can be minimized by delivering the drugs using ocular insert or patches[29-31].

The vaginal and the rectal lumen have also been explored for the delivery of the active agents both systemically and locally. The active agents meant for the systemic delivery by this

route of administration bypasses the hepatic first-pass metabolism. Quite often the delivery systems suffer from migration within the vaginal/rectal lumen which might affect the delivery of the active agent to the specific location[32-34].

Gastrointestinal tract is also a potential site which has been explored since long for the development of mucoadhesive based formulations. The modulation of the transit time of the delivery systems in a particular location of the gastrointestinal system by using mucoadhesive polymers has generated much interest among researchers around the world

MUCOADHESION THEORIES

There are following mucoadhesion theories:

Electronic Theory

Electronic theory is based on the premise that both mucoadhesive and biological materials possess opposing electrical charges. Thus, when both materials come into contact, they transfer electrons leading to the building of a double electronic layer at the interface, where the attractive forces within this electronic double layer determines the mucoadhesive strength.

Adsorption Theory

According to the adsorption theory, the mucoadhesive device adheres to the mucus by secondary chemical interactions, such as in Van der Waals and hydrogen bonds, electrostatic attraction or hydrophobic interactions. For example, hydrogen bonds are the prevalent interfacial forces in polymers containing carboxyl groups. Such forces have been considered the most important in the adhesive interaction phenomenon because, although they are individually weak, a great number of interactions can result in an intense global adhesion.

Wetting Theory

The wetting theory applies to liquid systems which present affinity to the surface in order to spread over it. This affinity can be found by using measuring techniques such as the contact angle. The general rule states that the lower the contact angle then the greater the affinity. The contact angle should be equal or close to zero to provide adequate spreadability.

Diffusion Theory

Diffusion theory describes the interpenetration of both polymer and mucin chains to a sufficient depth to create a semi-permanent adhesive bond. It is believed that the adhesion force increases with the degree of penetration of the polymer chains. This penetration rate depends on the diffusion coefficient, flexibility and nature of the mucoadhesive chains, mobility and contact time. The adhesion strength for a polymer is reached when the depth of penetration is approximately equivalent to the polymer chain size. In order for diffusion to occur, it is

important that the components involved have good mutual solubility, that is, both the bioadhesive and the mucus have similar chemical structures. The greater the structural similarity, the better the mucoadhesive bond.

Fracture Theory

This is perhaps the most-used theory in studies on the mechanical measurement of mucoadhesion. It analyses the force required to separate two surfaces after adhesion is established . This force, s_m, is frequently calculated in tests of resistance to rupture by the ratio of the maximal detachment force, F_m, and the total surface area, A_0, involved in the adhesive interaction (equation 1):

$$Sm = Fm/A0 \quad (1)$$

Since the fracture theory is concerned only with the force required to separate the parts, it does not take into account the interpenetration or diffusion of polymer chains. Consequently, it is appropriate for use in the calculations for rigid or semi-rigid bioadhesive materials, in which the polymer chains do not penetrate into the mucus layer.

Mechanical Theory

Mechanical theory considers adhesion to be due to the filling of the irregularities on a rough surface by a mucoadhesive liquid. Moreover, such roughness increases the interfacial area available to interactions thereby aiding dissipating energy and can be considered the most important phenomenon of the process. Lee, Park, Robinson, 2000 had described that it is unlikely that the mucoadhesion process is the same for all cases and therefore it cannot be described by a single theory. In fact, all theories are relevant to identify the important process variables.

The mechanisms governing mucoadhesion are also determined by the intrinsic properties of the formulation and by the environment in which it is applied. Intrinsic factors of the polymer are related to its molecular weight, concentration and chain flexibility. For linear polymers, mucoadhesion increases with molecular weight, but the same relationship does not hold for non-linear polymers. It has been shown that more concentrated mucoadhesive dispersions are retained on the mucous membrane for longer periods, as in the case of systems formed by in situ gelification. After application, such systems spread easily, since they present rheological properties of a liquid, but gelify as they come into contact the absorption site, thus preventing their rapid removal. Chain flexibility is critical to consolidate the interpenetration between formulation and mucus. Environment-related factors include pH, initial contact time, swelling

and physiological variations. The pH can influence the formation of ionizable groups in polymers as well as the formation of charges on the mucus surface. Contact time between mucoadhesive and mucus layer determines the extent of chain interpenetration. Super-hydration of the system can lead to build up of mucilage without adhesion. The thickness of the mucus layer can vary from 50 to 450 µm in the stomach to less than 1µm in the oral cavity. Other physiological variations can also occur with diseases.

MECHANISMS OF MUCOADHESION

The mucoadhesive must spread over the substrate to initiate close contact and increase surface contact, promoting the diffusion of its chains within the mucus. Attraction and repulsion forces arise and, for a mucoadhesive to be successful, the attraction forces must dominate.

Each step can be facilitated by the nature of the dosage form and how it is administered. For example, a partially hydrated polymer can be adsorbed by the substrate because of the attraction by the surface water[35].

Due to its relative complexity, it is likely that the process of mucoadhesion cannot be described by just one of these theories. Lee, Park, Robinson, 2000 had described the mechanism of mucoadhesion in four different approaches. These include:

Dry or partially hydrated dosage forms contacting surfaces with substantial mucus layers (typically particulates administered into the nasal cavity).

- Fully hydrated dosage forms contacting surfaces with substantial mucus layers (typically particulates of many mucoadhesive that have hydrated in the luminal contents on delivery to the lower gastrointestinal tract).
- Dry or partially hydrated dosage forms contacting surfaces with thin/discontinuous mucus layers (typically tablets or patches in the oral cavity or vagina).
- Fully hydrated dosage forms contacting surfaces with thin/discontinuous mucus layers (typically aqueous semisolids or liquids administered into the esophagus or eye). It is unlikely that the mucoadhesive process will be the same in each case. In the study of adhesion generally, two stages in the adhesive process supports the mechanism of interaction between mucoadhesive materials and a mucous membrane Thus, the mechanism of mucoadhesion is generally divided in two stages, the contact stage and the consolidation stage.

Stage 1 Contact stage:

An intimate contact (wetting) occurs between the mucoadhesive and mucous membrane.

Stage 2 Consolidation stage:

Various physicochemical interactions occur to consolidate and strengthen the adhesive joint, leading to prolonged adhesion. In some cases, such as for ocular or vaginal formulations, the delivery system is mechanically attached over the membrane. In other cases, the deposition is promoted by the aerodynamics of the organ to which the system is administered, such as for the nasal route. On the other hand, in the gastrointestinal tract direct formulation attachment over the mucous membrane is not feasible. Peristaltic motions can contribute to this contact, but there is little evidence in the literature showing appropriate adhesion. Additionally, an undesirable adhesion in the esophagus can occur. In these cases, mucoadhesion can be explained by peristalsis, the motion of organic fluids in the organ cavity, or by Brownian motion. If the particle approaches the mucous surface, it will come into contact with repulsive forces (osmotic pressure, electrostatic repulsion, etc.) and attractive forces (van der Waals forces and electrostatic attraction). Therefore, the particle must overcome this repulsive barrier[36].

In the consolidation step, the mucoadhesive materials are activated by the presence of moisture. Moisture plasticizes the system, allowing the mucoadhesive molecules to break free and to link up by weak van der Waals and hydrogen bonds. Essentially, there are two theories explaining the consolidation step: the diffusion theory and the dehydration theory.

According to diffusion theory, the mucoadhesive molecules and the glycoproteins of the mucus mutually interact by means of interpenetration of their chains and the building of secondary bonds (Smart, 2005). For this to take place the mucoadhesive device has features favoring both chemical and mechanical interactions.

For example, molecules with hydrogen bonds building groups (–OH, –COOH), with an anionic surface charge, high molecular weight, flexible chains and surface-active properties, which induct its spread throughout the mucus layer, can present mucoadhesive properties[26].

According to dehydration theory, materials that are able to readily gelify in an aqueous environment, when placed in contact with the mucus can cause its dehydration due to the difference of osmotic pressure. The difference in concentration gradient draws the water into the formulation until the osmotic balance is reached. This process leads to the mixture of formulation and mucus and can thus increase contact time with the mucous membrane. Therefore, it is the water motion that leads to the consolidation of the adhesive bond, and not the interpenetration of macromolecular chains. However, the dehydration theory is not applicable for solid formulations or highly hydrated forms.

Factors Affecting Mucoadhesion

Several factors have been identified as affecting the strength of the solid mucoadhesive

joint. Many studies have indicated an optimum molecular weight for mucoadhesion, ranging from circa 10^4 Da to circa 4×10^6 Da, although accurately characterizing the molecular weight of large hydrophilic polymers is very difficult. Larger molecular weight polymers will not hydrate readily to free the binding groups to interact with a substrate, while lower molecular weight polymers will form weak gels and readily dissolve. The flexibility of polymer chains is believed to be important for interpenetration and entanglement, allowing binding groups to come together. As the cross-linking of water-soluble polymers increases, the mobility of the polymer chains decrease, although this could also have a positive effect in restricting over hydration. Studies have shown that the mucoadhesive properties of polymers containing ionisable groups are affected by the pH of the surrounding media. For example, mucoadhesion of poly(acrylicacid)s is favoured when the majority of the carboxylate groups are in the unionised form, which occurs at pHs below the pKa. However, in systems with a high density of ionisable groups (e.g. carbomers or chitosans), the local pH within or at the surface of a formulation will differ significantly from that of the surrounding environment.

The strength of adhesion has been found to change with the initial 'consolidation' force applied to the joint, or the length of contact time prior to testing. The presence of metal ions, which can interact with charged polymers, may also affect the adhesion process[37-38].

Before discussing about the commonly used mucoadhesive polymers, the different theories which have been proposed to explain the phenomenon of mucoadhesion will be discussed. Furthermore, different factors affecting mucoadhesion, methods of evaluation of mucoadhesive properties of polymers and the potential biological sites where mucoadhesion can play an important role will be taken up for discussion.

Polymers in Mucosal Drug Delivery

Mucoadhesive delivery systems are being explored for the localization of the active agents to a particular location/ site. Polymers have played an important role in designing such systems so as to increase the residence time of the active agent at the desired location. Polymers used in mucosal delivery system may be of natural or synthetic origin. In this section we will briefly discuss some of the common classes of mucoadhesive polymers.

Hydrophilic Polymers

The polymers within this category are soluble in water. Matrices developed with these polymers swell when put into an aqueous media with subsequent dissolution of the matrix. The polyelectrolytes extend greater mucoadhesive property when compared with neutral polymers [42]. Anionic polyelectrolytes, e.g. poly (acrylic acid) and carboxymethyl cellulose, have been

extensively used for designing mucoadhesive delivery systems due to their ability to exhibit strong hydrogen bonding with the mucin present in the mucosal layer [43]. Chitosan provides an excellent example of cationic polyelectrolyte, which has been extensively used for developing mucoadhesive polymer due to its good biocompatibility and biodegradable properties[44]. Chitosan undergoes electrostatic interactions with the negatively charged mucin chains thereby exhibiting mucoadhesive property[42]. The ionic polymers may be used to develop ionic complex with the counter-ionic drug molecules so as to have a drug delivery matrix exhibiting mucoadhesive property. In a recent study, partially neutralized poly (acrylic acid) complex was developed in the presence of levobetaxolol hydrochloride, a potent cardiac β-blocker. The delivery system was prone to dissolution as the time progressed due to the release of the incorporated drug[45]. Mucoadhesive microcapsules can be designed with same principle by using orifice-ionic gelation method. This technique has been used to design a delivery system of gliclazide, an anti-diabetic drug, using sodium alginate, sodium carboxymethyl cellulose, carbopol 934P and hydroxy propylmethyl cellulose. The delivery system showed the release of gliclazide for an extended period of time due to its mucoadhesive properties[46]. Non-ionic polymers, e.g. poloxamer, hydroxypropyl methyl cellulose, methyl cellulose, poly (vinyl alcohol) and poly (vinyl pyrrolidone), have also been used for mucoadhesive properties[47]. The hydrophilic polymers form viscous solutions when dissolved in water and hence may also be used as viscosity modifying/enhancing agents in the development of liquid ocular delivery systems so as to increase the bioavailability of the active agents by reducing the drainage of the administered formulations[48]. These polymers may be directly compressed in the presence of drugs so as to have a mucoadhesive delivery system[49].

Numerous polysaccharides and its derivatives like chitosan, methyl cellulose, hyaluronic acid, hydroxypropyl methylcellulose, hydroxypropyl cellulose, xanthan gum, gellan gum, guar gum, and carrageenan have found applications in ocular mucoadhesive delivery systems[42]. Cellulose and its derivates have been reported to have surface active property in addition to its film forming capability[50]. Cellulose derivatives with lower surface acting property are generally preferred in ocular delivery systems as they cause reduced eye irritation. Of the various cellulose derivates, sodium carboxymethyl cellulose has been found to have excellent ocular mucoadhesive property. Cationic cellulose derivatives (e.g. cationic hydroxyethyl celluloses) have been used in conjunction with various anionic polymers for the development of sustained delivery systems[51].

Hydrogels

Hydrogels can be defined as three-dimensionally crosslinked polymer chains which have the ability to hold water within its porous structure. The water holding capacity of the hydrogels is mainly due to the presence of hydrophilic functional groups like hydroxyl, amino and carboxyl groups. In general, with the increase in the crosslinking density there is an associated decrease in the mucoadhesion[52]. Thielmann et al. reported the thermal crosslinking of poly (acrylic acid) and methyl cellulose. They reported that with the increase in the crosslinking density, there was a reduction in the solubility parameters and swelling which resulted in a reduction of mucoadhesion[52]. Hydrogels prepared by the condensation reaction of poly (acrylic acid) and sucrose indicated an increase in the mucoadhesive property with the increase in the crosslinking density and was attributed to increase in the poly (acrylic acid) chain density per unit area[53]. Acrylates have been used to develop mucoadhesive delivery systems which have the ability to deliver peptide bioactive agents to the upper small intestine region without any change in the bioactivity of the peptides. In a typical experimentation, Wood and Peppas developed a system in which ethylene glycol chains were grafted on methacrylic acid hydrogels and were subsequently functionalized with wheat germ agglutinin. Wheat germ agglutinin helped in improving the intestinal residence time of the delivery system by binding with the specific carbohydrate moieties present in the intestinal mucosa[54]. In addition to the drug targeting, mucoadhesive hydrogel based formulations for improving the bioavailability of the poorly water soluble drug. Muller and Jacobs prepared a nanosuspension of buparvaquone, a poorly water soluble drug, by incorporating it within carbopol and chitosan based hydrogels. The mucoadhesive delivery systems showed improved bioavailability of the drug when compared over the nanosuspension. This was attributed to the increased retention time of the delivery system within the gastrointestinal tract[55].

Thiolated polymers:

The presence of free thiol groups in the polymeric skeleton helps in the formation of disulphide bonds with that of the cysteine-rich sub-domains present in mucin which can substantially improve the mucoadhesive properties of the polymers (e.g. poly (acrylic acid) and chitosan) in addition to the paracellular uptake of the bioactive agents[56]. 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[24].

Lectin-based Polymers:

Lectins are proteins which have the ability to reversibly bind with specific sugar / carbohydrate residues and are found in both animal and plant kingdom in addition to various microorganisms[56]. Many lectins have been found to be toxic and immunogenic which may lead to systemic anaphylaxis in susceptible individuals on subsequent exposure[24]. The specific affinity of lectins towards sugar or carbohydrate residues provides them with specific cyto-adhesive property and is being explored to develop targeted delivery systems. Lectins extracted from legumes have been widely explored for targeted delivery systems. The various lectins which have shown specific binding to the mucosa include lectins extracted from Ulex europaeus I, soybean, peanut and Lens culinarius[57]. The use of wheat germ agglutinin has been on the rise due to its least immunogenic reactions, amongst available lectins, in addition to its capability to bind to the intestinal and alveolar epithelium and hence could be used to design oral and aerosol delivery systems[58].

Mucoadhesive Dosage Forms

The primary objectives of mucoadhesive dosage forms are to provide intimate contact of the dosage form with the absorbing surface and to increase the residence time of the dosage form at the absorbing surface to prolong drug action. Due to mucodhesion, certain water-soluble polymers become adhesive on hydration and hence can be used for targeting a drug to a particular region of the body for extended periods of time. The mucosa lines a number of regions of the body including the gastrointestinal tract, the urogenital tract, the airways, the ear, nose, and eye. These represent potential sites for attachment of any mucoadhesive system and hence, the mucoadhesive drug delivery system may include the following[33] Gastrointestinal delivery system, Nasal delivery system, Ocular delivery system, Buccal delivery system, Vaginal delivery System, Rectal delivery system.

CHARACTERIZATION OF MUCOADHESIVE DOSAGE FORM

No technology has still been developed specifically to analyze mucoadhesion. Most of the tests available were adapted from other pre-existing techniques but are useful and necessary for selecting the promising candidates as mucoadhesives as well as in elucidating their mechanisms of action.

Analysis methods of Mucoadhesion

Since the early 1980s, a vast variety of methods to evaluate the potential mucoadhesive properties of new polymeric materials have been developed. The diversity in physical forms of the mucoadhesive devices invented led to the generation of a wide variety of techniques for

mucoadhesion evaluation.

A large number of methods found in the literature are based on the measurement of the force necessary to separate a mucoadhesive material from a biological membrane. Peel, shear and tensile forces can be determined depending on the direction in which the mucoadhesive material is detached from the biological surface.

Peel forces are measured when evaluating mucoadhesive devices for buccal or transdermal applications. Within the shear strength tests, the Wilhelmy plate method developed by Smart et al. is one of the most remarkable methods. In this method, a glass plate coated with the mucoadhesive material to be tested is submerged in a mucin solution.

A microbalance connected to the plate measures the forces due to surface tension on the plate as the system containing the mucin solution is pulled away from the mucoadhesive material. This force measured is related to the wettability of the mucin on the polymer surface and corresponds to the adhesive force between the mucoadhesive polymer and the mucin glycoprotein.

Tensile tests have been widely used for the evaluation of a large diversity of mucoadhesive devices. For example, Ponchel et al. analyzed the tensile force required to separate a mucoadhesive tablet from animal mucosa. This force is then used to calculate the work of adhesion. This parameter has been extensively used as a good indicator of the mucoadhesive properties of a material and is calculated by the integration of the force vs. displacement curve obtained in the tensile experiments. Other notable mucoadhesion techniques include the method developed by Robinson et al., where human epithelial cells are labeled with fluorescent probes and placed in contact with a mucoadhesive polymer. The interaction between the epithelial cell membrane and the polymer is investigated. More recently, other methods used to examine the molecular interactions at cell surfaces include the force microscopy techniques.

Mikos and Peppas invented the flow channel method in which a mucoadhesive polymer particle is placed on a mucus surface in a Plexiglas-channel. A laminar flow of air is directed over the microparticle, and photographs are taken to analyze the static and dynamic behavior of the polymer particle. Other techniques used for the evaluation of mucoadhesive particles include the electrobalance method and contact angle measurements. The falling film technique developed by Ho and Teng is also a remarkably simple method for the evaluation of mucoadhesive particles. In this method, spherical latex particles are coated with a mucoadhesive material and are suspended in a buffer solution of a known concentration.

The particle solution is then pumped over a rat small intestine cut lengthwise and placed in a cylindrical channel. The eluted solution is collected and the remaining particles in the solution are counted. The portion of particles that remained adhered in the mucosal tissue is an indication of the mucoadhesive properties of the material tested.

Staining methods have also been developed for the evaluation of mucoadhesive polymers. A colloidal gold staining technique was developed by Park, where mucin-gold conjugates interacted with a hydrogel surface resulting in a red coloration. More recently, a direct-staining method to evaluate the attachment of a polymer to human buccal cells has been proposed. Hassan and Gallo reported the rheological method for mucoadhesion evaluation. This method is based on the idea that when a mucoadhesive polymer is mixed with mucin, there is a synergistic increase in viscosity. However, the contradictory results obtained in some experiments suggest that this method should not be used as a single technique to evaluate mucoadhesion.

Other techniques used to study the interaction between mucoadhesive polymers and mucin glycoproteins has been done by Huang et al. with the use of the surface force apparatus(SFA). The SFA measures the magnitude and distance dependence of the molecular force acting between two surfaces, with resolutions of the measured force up to 10 nN and distances up to 1Å.

Some in vivo methods to assess mucoadhesion properties of polymers include the gamma scintigraphy and the use of radioisotopes to measure the gastrointestinal transit of the mucoadhesive device.

In Vitro and *Ex Vivo* Tests

In vitro/ex vivo tests are important in the development of a controlled release bioadhesive system because they contribute to studies of permeation, release, compatibility, mechanical and physical stability, superficial interaction between formulation and mucous membrane and strength of the bioadhesive bond. These tests can simulate a number of administration routes including oral, buccal, periodontal, nasal, gastrointestinal, vaginal and rectal. The in vitro and ex vivo tests most prevalent in the literature are reported below.

Techniques Utilizing Gut Sac of Rats

The everted gut sac technique is an example of an ex vivo method. It has been used since 1954 to study in intestinal transport. It is easy to reproduce and can be performed in almost all laboratories. A segment of intestinal tissue is removed from the rat, everted, and one of its ends sutured and filled with saline. The sacs are introduced into tubes containing the system

under analysis at known concentrations, stirred, incubated and then removed. The percent adhesion rate of the release system onto the sac is determined by subtracting the residual mass from the initial mass[34].

Other techniques use non-everted gut sac[35]. The sacs were sealed and incubated in saline. After a stipulated time, the number of liposomes adhered before (N_0) and after (N_S) incubation was assessed with a coulter counter and the percent mucoadhesive was expressed by equation 2.

$$\% \text{ Mucoadhesion} = N_0\text{-}Ns/Ns \quad (2)$$

The mucoadhesive effect of a system can also be evaluated by increases in gastrointestinal transit. Fluorescent tracers are incorporated into a system and quantified them by fluorescence spectroscopy in the stomach and intestinal mucus as a function of time.

Tests Measuring Mucoadhesive Strength

Most in-vitro/ex-vivo methodologies found in the literature are based on the evaluation of mucoadhesive strength, that is, the force required to break the binding between the model membrane and the mucoadhesive.

Depending on the direction in which the mucoadhesive is separated from the substrate, is it possible to obtain the detachment, shear, and rupture tensile strengths36. The force most frequently evaluated in such tests is rupture tensile strength. Generally, the equipment used is a texture analyzer or a universal testing machine. In this test, the force required to remove the formulation from a model membrane is measured, which can be a disc composed of mucin, a piece of animal mucous membrane, generally porcine nasal mucus or intestinal mucus from rats. Based on results, a force- distance curve can be plotted which yields the force required to detach the mucin disc from the surface with the formulation, the tensile work (area under the curve during the detachment process), the peak force and the deformation to failure. This method is more frequently used to analyze solid systems like microspheres, although there are also studies on semi-solid materials (mini-matrices)[36-39]. In addition to rupture tensile strength, the texture analyzer can also, as inferred by its name, evaluate the texture of the formulations and assess other mechanical properties of the system. A mobile arm containing an analytical probe forces down into a sample held in a flask placed on the equipment's platform. Speed rate, time and depth are preset.

From the resulting force-time and force-distance plots, it is possible to calculate the hardness (force required to reach a given deformation), compressibility (work required to deform the product during the compression), and adhesiveness (work required to overcome the attraction forces between the surfaces of sample and probe). Using this technique, it is possible

to perform a previous evaluation of the material's adhesive capacity, evidencing mucoadhesion properties[38-41].

Imaging Methods by AFM, CFSLM, MPEM

Optical microscopes offer insufficient resolution for studying effects at a molecular level. For such inves- tigations, a resolution at micro or nanometric level is needed. Electronic microscopy gives a larger view, but the environmental conditions in which the sample must be submitted are far from the physiological conditions. For instance, the samples are analyzed in a vacuum chamber and generally are covered with a metallic film to avoid changes caused by the electronic rays. SEM in the studies of mucoadhesive gives the topology information in vitro, but the nature of the dosage form in vivo and the nature of transport across the biological barriers are missing.

Atomic force microscopy (AFM) is a relatively new technique that overcomes such restrictions, because it can be used under any environmental conditions, in air, liquids or vacuum. It enlarges more than 10^9- fold, which enables visualization of isolated atoms and offers a tridimensional image of the surface. The equipment has a support combined with a probe perpendicularly attached to it. This tip moves toward a plane parallel to the sample, acquiring its topographic characteristics and the tip position is recorded by an optic deflection system: a laser beam is reflected onto the support and its position is then further reflected by a mirror reaching a photodiode sensor. A force-distance curve is plotted to measure the forces between this tip and the surface of interest. This curve is then used in bioadhesion studies. This entails, coating the tip in adhesive material which is generally spherical in shape and then the interaction with the surface, in this case the mucous membrane, can be measured.

Besides AFM, there are other techniques using photographic images, such as fluorescence microscopy and confocal laser scanning microscopy (CSLM)[42-43]. B. R. Masters have described about confocal microspcopy right from its history to the present updates[44]. Hirofumi Takeuchi *et al* has used confocal imaging to see the nature of mucoadhesive microspheres ex-vivo in the gut of rats[45.] Here a tungsten or lazer illuminates and detects the scattered or fluorescent light respectively within the vesicles. A set of conjugated apertures, one for illumination and one for detection of light function as spatial filters.

In confocal microscopy, lateral and axial resolutions are enhanced when compared to standard light microscopy. The axial resolution is responsible for identifying the lodging position of the vesicles ever deep within the tissues. The main advantage of confocal microscope is its ability to optically section thick specimens. Real time video frame can be

captured with a low light video camera which inturn can be connected to a video recorder. Video frames give a live demonstration of the pathway and nature of the transportation of vesicles. Fluorescent dyes for detection used are Calcien AM (for green fluorescent), Rhodamine – 123, Rhodamine – DHPE, fluoroscien – DHPE, nile red[46].

Multi-Photon excitation microscopy (MPEM)is another tool which can be conveniently used to study the living tissues. So the microspheres inside the tissues also can be studied. The important work of Denk, Strickler and Webb which was published in Science in 1990 launched a new revolution in nonlinear optical microscopy. They implemented multi-photon excitation processes into microscopy by integrating a laser scanning microscope and a mode-locked laser which generates pulses of near-infrared light. The pulses of red or near-infrared light (700 nm) were less than100 sec in duration and the laser repetition rate was about 80 MHz.

These pulses have sufficiently high peak power to achieve two-photon excitation at reasonable rates at an average power less than 25 mW that induces minimal photo damage to many types of Biological samples. However, highly pigmented cells and tissues could be subjected to photo-induced thermal damage. The potential benefits of two-photon excitation microscopy include reduced photo bleaching of the fluorophores, improved background discrimination and minimal the photo damage to living cell specimens[47-51].

Falling Liquid Film Method

The chosen mucous membrane is placed in a stainless steel cylindrical tube, which has been longitudinally cut. This support is placed inclined in a cylindrical cell with a temperature controlled at 37ºC. An isotonic solution is pumped through the mucous membrane and collected in a beaker (Figure 6). Subsequently, in the case of particulate systems, the amount remaining on the mucous membrane can be counted with the aid of a coulter counter. For semi-solid systems, the non adhered mucoadhesive can be quantified by high performance liquid chromatography. In this later case, porcine stomach, intestinal and buccal mucus were tested, and also jejunum from rabbits. The validation of this method showed that the type of mucus used does not influence the results. The release systems tested were precursors of liquid crystals constituted by monoglycerides. This methodology allows the visualization of formation of liquid- crystalline mesophase on the mucous membrane after the flowing of the fluids and through analysis by means of polarized light microscopy[52].

CONCLUSION

Mucoadhesive drug delivery system shows promising future in enhancing the bioavailability

and specific needs by utilizing the physiochemical characters of both the dosage form and the mucosal lining. It has to be noted that only a moist surface can bring the mucoadhesive nature of the dosage form. Of late, scientists are trying to improve the bioavailability of active agents by tailoring the properties of the delivery systems instead of designing new active agents. Mucoadhesive polymers may provide an important tool to improve the bioavailability of the active agent by improving the residence time at the delivery site. The various sites where mucoadhesive polymers have played an important role include buccal cavity, nasal cavity, rectal lumen, vaginal lumen and gastrointestinal tract. Development of novel mucoadhesive delivery systems are being undertaken so as to understand the various mechanism of mucoadhesion and improved permeation of active agents. Many potential mucoadhesive systems are being investigated which may find their way into the market in near future.

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