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A Review on Mucoadhesive Microspheres for Controlled Drug Delivery

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Abstract

Mucoadhesion is a topic of current interest in the design of drug delivery systems. Mucoadhesive micro- spheres exhibit a prolonged residence time at the site of application or absorption and facilitate an intimate con- tact with the underlying absorption surface and thus contribute to improved and/or better therapeutic perfor- mance of drugs. In recent years such mucoadhesive microspheres have been developed for oral, buccal, nasal, oc- ular, rectal and vaginal routes for either systemic or local effects. The objective of this article is review the princi- ples underlying the development and evaluation of mucoadhesive microspheres and the research work carried out on these systems.

Key words mucoadhesion; micosphere; controlled drug delivery

INTRODUCTION-

MUCOADHESIVE MICROSPHERES

Drug delivery systems (DDS) that can precisely control the release rates or target drugs to a specific body site have had an enormous impact on the health care system. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as micro- spheres, nanoparticles, liposomes, *etc.* which modulates the release and absorption characteristics of the drug. Micro- spheres constitute an important part of these particulate DDS by virtue of their small size and efficient carrier characteris- tics. However, the success of these novel DDS is limited due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for provid- ing an intimate contact of the DDS with absorbing mem- branes. It can be achieved by coupling mucoadhesion charac- teristics to microspheres and developing novel delivery sys- tems referred to as "mucoadhesive microspheres."

MUCOADHESION AND MUCOADHESIVE DRUG DE-LIVERY SYSTEMS

Mucoadhesive drug delivery systems are delivery systems which utilize the property of bioadhesion of certain polymers which become adhesive on hydration[1)] and hence can be used for targeting a drug to a particular region of the body for ex- tended periods of time.[2)] Bioadhesion is an interfacial phe- nomenon in

which two materials, at least one of which is bio- logical, are held together by means of interfacial forces.[3] The attachment could be between an artificial material and biological substrate, such as adhesion between a polymer and a biological membrane. In the case of polymer attached to the mucin layer of a mucosal tissue, the term "mucoadhe- sion" is used. The mucosal layer lines a number of regions of the body including the gastrointestinal tract, the urogential tract, the airways, the ear, nose and eye. These represent po- tential sites for attachment of bioadhesive system and hence, the mucoadhesive drug delivery systems could be designed for buccal, oral, vaginal, rectal, nasal and ocular routes of ad-ministration.

ADVANTAGES OF MUCOADHESIVE SYSTEMS

Mucoadhesive systems have three distinct advantages when compared to conventional dosage forms.

1. The mucoadhesive systems are readily localized in the region applied to improve and enhance the bioavailabil- ity of drugs. Greater bioavailability of piribedit,[4] testos- terone and its esters,[5] vasopressin,[6] dopamine,[7] in- sulin[8] and gentamycin[9] was observed from mucoadhe- sive dosage systems.
2. These dosage forms facilitate intimate contact of the for-mulation with underlying absorption surface. This al- lows modification of tissue permeability for absorption of macromolecules, such as peptides and proteins. Inclu- sion of penetration enhancers such as sodium glyco- cholate,[10] sodium taurocholate and L-lysophosphotidyl choline (LPC)[11] and protease inhibitors[12] in the mu- coadhesive dosage forms resulted in the better absorp- tion of peptides and proteins.
3. Mucoadhesive dosage forms also prolong residence time of the dosage form at the site of application and absorp- tion to permit once or twice a day dosing.[13]

MUCOADHESIVE MICROSPHERES

Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1—1000 *m* m in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it, respectively.[14] Mi- crospheres, in general, have the potential to be used for tar- geted and controlled release drug delivery; but coupling of mucoadhesive properties to microspheres has additional ad- vantages, *e.g.* efficient absorption and enhanced bioavailabil-ity of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of drug to the absorption site achieved by anchoring plant lectins,[15] bacterial adhesions[16] and antibodies,[17] *etc.* on the surface of the microspheres.

Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye, nasal cavity, urinary and gastrointestinal tract, thus offering the possibili- ties of localized as well as systemic controlled release of drugs. Application of mucoadhesive microspheres to the mucosal tissues of ocular cavity, gastric and colonic epithelium is used for administration of drugs for localized action. Pro-longed release of drugs and a reduction in frequency of drug administration to the ocular cavity can highly improve the patient compliance.[18] The latter advantage can also be ob- tained for drugs administered intra-nasally due to the reduc- tion in mucociliary clearance of drugs adhering to nasal mu- cosa.[19] Microspheres prepared with mucoadhesive and bio- erodable polymers undergo selective uptake by the M cells of Peyer patches in gastrointestinal (GI) mucosa.[20] This uptake mechanism has been used for the delivery of protein and peptide drugs, antigens for vaccination and plasmid DNA for gene therapy. Moreover, by keeping the drugs in close prox- imity to their absorption window in the GI mucosa. The mu- coadhesive microspheres improve the absorption and oral bioavailability of drugs like furosemide[21] and riboflavin.[22] The concept of a non-invasive single shot vaccine, by means of mucosal immunization, offers controlled release of anti-gens and thus forms another exquisite application of mu- coadhesive microspheres.[23]

POLYMERS USED FOR MUCOADHESIVE MICRO-SPHERES

The properties of the mucoadhesive microspheres, *e.g.* their surface characteristics, force of mucoadhesion, release pattern of the drug, and clearance, are influenced by the type of polymers used to prepare them. Suitable polymers that can be used to form mucoadhesive microspheres include soluble and insoluble, non-biodegradable and biodegradable poly- mers. These can be hydrogels or thermoplastics, homopoly- meres, copolymers or blends, natural or synthetic polymers.

CLASSIFICATION OF POLYMERS

Hydrophilic Polymers These are the water-soluble poly- mers that swell indefinitely in contact with water and eventu- ally undergo complete dissolution, *e.g.* Methylcellulose, hy- droxyethyl cellulose, hydroxy propyl methyl cellulose, sodium carboxy methyl cellulose, carbomers, chitosan and plant gums *etc.*

Hydrogels These are water swellable materials, usually a cross-link polymer with limited swelling capacity, *e.g.* poly(acrylic acid co acrylamide) copolymers, carrageenan, sodium alginate, guar gum and modified guar gum *etc.* **Thermoplastic Polymers** These polymers include the non-erodible neutral polystyrene and semi crystalline bio- erodible polymers, which generate the carboxylic acid groups as they degrade, *e.g.* polyanhydrides and polylactic acid. Var- ious synthetic polymers used in mucoadhesive formulations include polyvinyl alcohol, polyamides, polycarbonates, polyalkylene glycols, polyvinyl ethers, esters and halides, polymethacrylic acid, polymethylmethacrylic acid, methyl- cellulose, hydroxypropyl cellulose, hydroxypropyl methylcel-lulose and sodium carboxymethylcellulose.

Various biocompatible polymers used in mucoadhesive formulations include cellulose-based polymers, ethylene gly- col polymers and its copolymers, oxyethylene polymers, polyvinyl alcohol, polyvinyl acetate and esters of haluronic acid.

Various biodegradable polymers used in mucoadhesive formulations are poly(lactides), poly(glycolides), poly(lac- tide-co-glycolides), polycaprolactones, and polyalkyl cyano- acrylates. Polyorthoesters, polyphosphoesters, polyanhy- drides, polyphosphazenes are the recent additions to the polymers.

SPECIFIC SITE DIRECTEDBIOADHESIVES—THENEXT GENERATION

The specific mucosal surfaces can be targeted using site- specific chemical agents that are anchored onto the poly- meric DDS. The first generation mucoadhesive polymers lack specificity and can bind to any mucosal surface. This limits their use for fabrication of mucoadhesive drug delivery system for a particular tissue. However, the development of polymers and microspheres grafted with mucus or cell-spe- cific ligands have increased therapeutic benefit and made site-specific drug delivery possible (Table 1). Any ligand with a high binding affinity for mucin can be covalently linked to the microspheres and be expected to influence the binding of microspheres. Targeting of the drugs can be achieved by using the following ligands.

Lectins Lectins can be defined as proteins of non-im- mune origin that bind to carbohydrates specifically and non covalently. Lectins can increase the adherence of micropar- ticules to the intestinal epithelium and enhance penetration of drugs.[24] They may be used to target therapeutic agents for different gut components or even for different cells (*e.g.* complex-specific lectins for parietal cells or fucose-specific lectins for M cells).

Table 1. Specific Ligands Corresponding to the Glycosal Groups on Cell Membranes, Which can be Used for Targeting the Mucoadhesive Microspheres to Specific Site

S. No.	Glycosyl groups on cell membranes	Specific ligands	Specific site
	Mannose[56]	*Galanthus nivalis* agglutinin (GNA)	Epithelial cells in stomach, caecum, and colon
	N-Acetyl glucosamine[57]	Wheat germ agglutinin (WGA)	Epithelial cells in stomach, caecum, colon and enterocytes in small intestine
	Lycopersicon esculentum or tomato		Strong binding to M cells lectin (LEA)
	N-Acetyl glucosamine[58]	Lectin ML-1 from Visum album	Endocytosed by villus enterocytes and goblet cells strong binding to epithelial cells in small intestine
	Phytohaemagglutinin[59]	*Phaseolus vulgaris* isoagglutinin	Surface cells of the stomach
	Fucose[60]	Aleuria aurentia agglutinin (AAA)	Specific binding and transcytosis by M cells

A bioinvasive mechanism has been de- scribed for the activity of lectins as targeting moieties. After binding to specific cells, the lectins undergo cellular uptake and subsequently can also exhibit strong binding to nuclear pore membranes.[25] Polystyrene microparticules coated with tomato lectin were shown to be specifically adhesive to ente-rocytes.[26] Tomato lectin is a potential targeting moiety due to its low toxicity and high specificity, but its inactivation due to cross-reactivity with mucus limits its usefulness. The po- tential of tamato lectin can, however, be tapped by exploiting its cellular uptake for drug delivery.[15] The other useful lectin ligands include lectins isolated from: *Abrus precatroius*,[27] *Agaricus bisporus*,[28] *Anguilla anguilla*,[29] *Arachis hypogaea*,[30] *Pandeiraea simplicifolia*,[31] and *Bauhinia pur- purea*.[32] Lectin-mediated drug delivery forms a promising approach for the peroral, specific mucoadhesive formula-tions. The use of lectins for targeting drugs to tumor tissue is currently under intensive investigation as the human carci- noma cell lines exhibit higher lectin binding capacity than the normal human colonocytes.[26]

Bacterial Adhesions Bacteria are able to adhere to ep- ithelial surfaces of the enterocytes with the aid of fimbriae.[33] Fimbriae are long, lectin like proteins found on the surface of many bacterial strains. Their presence has been correlated with pathogencity, *e.g.* adherence of *Escherichia coli* to the brush border of epithelial cells mediated by K99 fimbriae is a prerequisite for subsequent production and cellular uptake of *E. coli* enterotoxin.[34] Thus, the DDS based on bacterial ad- hesion factors could be an efficient mechanism to increase adhesion of mucoadhesive microspheres to epithelial sur- faces.[24] Another study[35] envisaging the importance of bacte-rial adhesion has been carried out using "invasion," which is a membrane protein from *Yersinia pseudotuberculosis*. Cel- lular uptake of polymeric nanospheres functionalized with invasion has been observed using confocal laser scanning mi-croscopy.

Amino Acid Sequences Certain amino acid sequences have complementary parts on the cell and mucosal surfaces and when attached to microparticles can promote binding to specific cell surface glycoproteins.[36] The cell surface glyco- proteins are altered in the presence of disease conditions and these altered protein sequences can be targeted by comple- mentary amino acid sequences attached to the drug delivery device,[37] *e.g.* amino acid sequences such as Arg-Gly-Asp and others, if attached to the matrix, could promote adhesion by binding with specific cell surface glycoprotein.

Antibodies Antibodies can be produced against selected molecules present on mucosal surfaces. Due to their high specificity, antibody can be a rational choice as a polymeric ligand for designing site-specific mucoadhesives.[38] This ap- proach can be useful for targeting drugs to tumor tissues,[39]
e.g. the hyaluronic acid esters (HYAFF) bioadhesive micro- spheres in the presence of a mucosal adjuvant-LTK 63 ad- ministered intranasally are reported to induce a significantly enhanced serum IgG antibody response in comparision to in- tramscular immunization with haemagglutinin obtained from influenza A virus. Polyphosphazene microspheres with ad- sorbed influenza antigen and tetanus toxiod can be adminis- tered intranasally to have increased immune responses.

PREPARATION OF MUCOADHESIVE MICROSPHERES

Mucoadhesive microspheres can be prepared using any of the following techniques.

Solvent Evaporation It is the most extensively used method of microencapsulation, first described by Ogawa *et al.*[40] A buffered or plain aqueous solution of the drug (may contain a viscosity building or stabilizing agent) is added to an organic phase consisting of the polymer solution in sol- vents like dichloromethane (or ethyl acetate or chloroform) with vigorous stirring to form the primary water in oil emul- sion. This emulsion is then added to a large volume of water containing an emulsifier like PVA or PVP to form the multi- ple emulsion (w/o/w). The double emulsion, so formed, is then subjected to stirring until most of the organic solvent evaporates, leaving solid microspheres. The microspheres can then be washed, centrifuged and lyophilize to obtain the free flowing and dried microspheres.

Hot Melt Microencapsulation This method was first used by Mathiowitz and Langer[41] to prepare microspheres of polyanhydride copolymer of poly[bis(*p*-carboxy phenoxy) propane anhydride] with sebacic acid. In this method, the polymer is first melted and then mixed with solid particles of the drug that have been sieved to less than 50 *m* m. The mix- ture is suspended in a non-miscible solvent (like silicone oil), continuously stirred, and heated to 5° above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting micro- spheres are washed by decantation with petroleum ether. The primary objective for developing this method is to develop a microencapsulation process suitable for the water labile poly-mers, *e.g.* polyanhydrides. Microspheres with

diameter of 1—1000 *m* m can be obtained and the size distribution can be easily controlled by altering the stirring rate. The only disad- vantage of this method is moderate temperature to which the drug is exposed.

Solvent Removal It is a non-aqueous method of mi- croencapsulation, particularly suitable for water labile poly- mers such as the ploy anhydrides. In this method, drug is dis- persed or dissolved in a solution of the selected polymer in a volatile organic solvent like methylene chloride. This mix- ture is then suspended in silicone oil containing span 85 and methylene chloride.[42] After pouring the polymer solution into silicone oil, petroleum ether is added and stirred until solvent is extracted into the oil solution. The resulting mi- crospheres can then be dried in vacuum.

Hydrogel Microspheres Microspheres made of gel-type polymers, such as alginate, are produced by dissolving the polymer in an aqueous solution, suspending the active ingre- dient in the mixture and extruding through a precision de- vice, producing micro droplets which fall into a hardening bath that is slowly stirred. The hardening bath usually con- tains calcium chloride solution, whereby the divalent calcium ions crosslink the polymer forming gelled microspheres. The method involves an "all-aqueous" system and avoids residual solvents in microspheres. Lim and Moss[43] developed this method for encapsulation of live cells, as it does not involve harsh conditions, which could kill the cells. The surface of these microspheres can be further modified by coating them with polycationic polymers, like polylysine after fabrication. The particle size of microspheres can be controlled by using various size extruders or by varying the polymer solution flow rates.

Spray Drying In this process, the drug may be dissolved or dispersed in the polymer solution and spray dried. The quality of spray-dried microspheres can be improved by the addition of plasticizers, *e.g.* citric acid, which promote poly- mer coalescence on the drug particles and hence promote the formation of spherical and smooth surfaced microspheres. The size of microspheres can be controlled by the rate of spraying, the feed rate of polymer drug solution, nozzle size, and the drying temperature.[44] This method of microencapsu-lation is particularly less dependent on the solubility charac- teristics of the drug and polymer and is simple, reproducible, and easy to scale up.[44]

Phase Inversion Microencapsulation The process in- volves addition of drug to a dilute solution of the polymer (usually 1—5%, w/v in methylene chloride). The mixture is poured into an unstirred bath of strong non-solvent (petro- leum ether) in a solvent to non-solvent ratio of 1 : 100, result- ing in the spontaneous production of microspheres in the size range of 0.5—5.0 *m* m can then be filtered, washed with petro- leum ether and dried with air.[45] This simple and fast process of microencapsulation involves relatively little loss of poly- mer and drug.

EVALUATION OF MUCOADHESIVE MICROSPHERES

The best approach to evaluate mucoadhesive microspheres is to evaluate the effectiveness of mucoadhesive polymer to prolong the residence time of drug at the site absorption, thereby increasing absorption and bioavailability of the drug. The methods used to evaluate mucoadhesive microspheres include the following.

MEASUREMENT OF ADHESIVE STRENGTH

The quantification of the mucoadhesive forces between polymeric microspheres and the mucosal tissue is a useful in- dicator for evaluating the mucoadhesive strength of micro- spheres. *In vitro* techniques have been used to test the poly- meric microspheres against a variety of synthetic and natural mucus, frozen and freshly excised tissue *etc.* The different *in vitro* methods include the following.

Tensile Stress Measurement. Wilhelmy Plate Technique The wilhelmy plate technique is traditionally used for the measurement of dynamic contact angles and involves the use of a microtensiometer or microbalance. The CAHN dynamic contact angle analyzer (model DCA 322, CAHN instru- ments, Cerritos) has been modified to perform adhesive micro force measurements. The DCA 322 system consists of an IBM compatible computer and microbalance assembly.[46] The microbalance unit consists of stationary sample and tare loops and a motor powered translation stage. The instrument measures the mucoadhesive force between mucosal tissue and a single microsphere mounted on a small diameter metal wire suspended from the sample loop in microtesiometer.[47] The tissue, usually rat jejunum, is mounted within the tissue chamber containing Dulbecco's phosphate buffered saline containing 100 mg/dl glucose and maintained at the physio- logic temperature. The chamber rests on a mobile platform, which is raised until the tissue

comes in contact with the suspended microsphere. The contact is held for 7 min, at which time the mobile stage is lowered and the resulting force of adhesion between the polymer and mucosal tissue recorded as a plot of the load on microsphere versus mobile stage dis- tance or deformation. The plot of output of the instrument is unique in that it displays both the compressive and the tensile portions of the experiment. By using the CAHN soft ware system, three essential mucoadhesive parameters can be ana- lyzed. These include the fracture strength, deformation to failure and work of adhesion.

- Fracture strength: it is the maximum force per unit surface area required to break the adhesive bond.
- Deformation to failure: it is the distance required to move the stage before complete separation occurs. This parame- ter is dependent on the material stiffness and the intensity of strength of adhesion.
- Work of adhesion: It is a function of both the fracture strength and the deformation to failure. It tends to be the strongest indicator of the bioadhesive potential.

This technique allows the measurement of mucoadhesive properties of a candidate material in the exact geometry of the proposed microsphere delivery device and the use of a physiological tissue chamber mimics the *in vivo* conditions. From a single tensile experiment, 11 mucoadhesive parame- ters can be analyzed out of which 3 are direct predictors of the bioadhesive potential.[48)]

The CAHN instrument, although a powerful tool has in- herent limitations in its measurement technique, makes it better suited for large microspheres (with a diameter of more than 300 *m* m) adhered to tissue *in vitro*. Therefore, many new techniques have been developed to provide quantitative infor- mation of mucoadhesive interactions of the smaller micros- pheres.

Novel Electromagnetic Force Transducer (EMFT) The EMFT is a remote sensing instrument that uses a cali- brated electromagnetic to detach a magnetic loaded polymer microsphere from a tissue sample.[49)] It has the unique ability to record remotely and simultaneously the tensile force infor- mation as well as high magnification video images of mu- coadhesive interactions at near physiological conditions. The EMFT measures tissue adhesive forces by monitoring the magnetic force required to exactly oppose the mucoadhesive force. To test a microsphere, it must first be attached to the sample of tissue; magnetic force is then generated by an elec- tromagnet mounted on the microscope vertically above the tissue chamber. After the computer has calculated the posi- tion of microsphere, the tissue chamber is slowly moved down, away from the magnet tip. As the tissue slowly de- scends away from the magnet, the video analysis continu- ously calculates the position of microsphere until the latter is completely pulled free of the tissue. The computer can dis- play the results either as raw data or convert it to a force *ver- sus* displacement graph. The primary advantage of the EMFT is that no physical attachment is required between the force transducer and the microsphere. This makes it possible to perform accurate mucoadhesive measurements on the small microspheres, which have been implanted *in vivo* and then excised (along with the host tissue) for measurement. This technique can also be used to evaluate the mucoadhesion of polymers to specific cell types and hence can be used to de- velop mucoadhesive drug delivery system (MDDS) to target specific tissues.[38)]

Shear Stress Measurement The shear stress measures the force that causes a mucoadhesive to slide with respect to the mucus layer in a direction parallel to their plane of con- tact.[2)] Adhesion tests based on the shear stress measurement involve two glass slides coated with polymer and a film of mucus. Mucus forms a thin film between the two polymer coated slides, and the test measures the force required to sep- arate the two surfaces. Mikos and Peppas[50)] designed the *in vitro* method of flow chamber. The flow chamber made of Plexiglass is surrounded by a water jacket to maintain a con- stant temperature. A polymeric microsphere placed on the surface of a layer of natural mucus is placed in a chamber. A simulated physiologic flow of fluid is introduced in the cham- ber and movement of microsphere is monitored using video equipment attached to a goniometer, which also monitors the static and dynamic behavior of the microparticule.[48)]

CONCLUSION

Mucoadhesive microspheres offer unique carrier system for many pharmaceuticals and can be tailored to adhere to any mucosal tissue, including those found in eyes, oral cavity and throughout the respiratory, urinary and gastrointestinal tract. The mucoadhesive microspheres can be used not only for controlled release but also for enhancing bioavailability, for targeted delivery of the drugs to specific sites in the body. Drug delivery through mucoadhesive microspheres is a promising area for continued research with the aim of achieving controlled release with enhanced bioavailability over longer periods of time, and for drug targeting to various sites in the body.

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