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Bio-Adhesive Nanomedicine for Targeted Drug Delivery

Abstract *The bio/muco-adhesive auxiliary agents are considered as a promising strategy for the administration of various challenging drugs including peptides, proteins and oligosaccharides therapeutics. The delivery of such therapeutics is hampered due to rapid degradation, restricted uptake, short residence time, poor solubility and limited half-life. The recent emergence of nanomedicine based on bio/mucoadhesive auxiliary agents is offering new avenues to overcome these drawbacks. Hence, it is important to understand the mechanism of nanoscale bio/mucoadhesion, protocols for investigating bio/mucoadhesive potential at nanoscale. This chapter is an endeavor to focus on the mechanism of bio adhesion and the bio/mucoadhesive auxiliary agents that are used in the design of the targeted oral, nasal, ocular, vaginal, and buccal nanomedicine with their properties that affect the bio/mucoadhesion*

Key Words: Bio Adhesion, Mucoadhesion, Nano Cargoes, Polymers, Targeted Drug Delivery System.

Introduction

Several biopharmaceutical shortcomings (e.g., physicochemical instability, poor aqueous solubility, low bioavailability, rapid clearance, non-specific distribution) barricade the dosage form development, delivery and targeting approved therapeutic moieties and the development of new therapeutic moieties. For instance, short residence time and physicochemical instability, characteristics of wider therapeutic moieties including peptides, proteins and oligosaccharides, represents the utmost remarkable impediment to assure the bioavailability of therapeutics administered by extravascular routes. Therapeutics residence time on biological and mucosal surfaces can be extended by bio/muco-adhesive auxiliary agents designed to attach to tissues and mucosal membranes. The adhesive phenomenon has been extensively explored in pharmaceutical developments and has been demonstrated as extended associations between two materials when in contact with each other. In biological sciences, it is frequently stated as bio adhesion when at least one of the materials participated in adhesive process is biologic in nature or, even more precisely, as mucoadhesion if biologic material is a mucosal surface.

Over the past several years, this bio/muco-adhesive property has emerged valuable for pharmaceutical potential to optimize systemic delivery through retaining a dosage form in intimate contact with the absorption site (e.g., the nasal, ocular, vaginal, and buccal mucosal tissues) or localized drug delivery via retaining a formulation at the site of action (e.g., within GIT). Bio/muco-adhesive auxiliary agents could also be used as therapeutic moieties in their specific right, to shield and protect damaged tissues (gastric ulcers or lesions of oral mucosa) or behave as lubricating agents (in the

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oral cavity, eye and vagina). Nanomedicine based on bio/muco-adhesive auxiliary agents has even greater potential. Not only can they adhere to mucosal tissues, but also offer controlled/ prolonged drug release, specific distribution, increased surface area and improved bioavailability by shielding drug from degradation. However, the amount of knowledge regarding nature and strength of the interfacial forces involved in establishment and duration of bio/muco-adhesive nanomedicine is limited. Mainly, traditional approaches towards characterization of mucoadhesive nanomedicine are insufficient. Hence, the present paper describes bio/muco-adhesive auxiliary agents, employed in latest development of oral, nasal, ocular, vaginal, and buccal nanomedicine with their capabilities that affect the bio/mucoadhesion and its mechanism. Moreover, techniques for it characterization and challenges confronted in future for a fruitful bench-to-bedside translation.

Nanomedicine Significance of Mucoadhesion for Challenging Drugs

Conventional drug delivery system has hampered the drug delivery to the potent level. It has shortcomings of being undergoing the GIT's enzymes degradations, less emptying time of intestine, rapid clearance, pH related biodegradation, entrapment in the mucosal mesh, leading to the lesser availability to the till the epithelium and targeted site. These factors are affecting the drug absorption and overall bioavailability. Such issues are optimized using polymers encapsulating the drug along with muco-enhancers, reaching targeted site, thus mucoadhesion optimized the drug delivery(Pridgen, Alexis, & Farokhzad, 2014). Muco-enhancers are used because out of two types of mucus, slippery and firm mucus, firm forms the unstirred water layer. So after mucoadhesion mucopenetrating auxiliaries and water absorption and movement toward epithelium, bioavailability and targeting optimization is enhanced i.e. BCS class 2 and 4 are improved(Netsomboon & Bernkop-Schnürch, 2016).

Naturally, a viscoelastic and mucoadhesive layer of mucus, in luminal surface of mucosal tissues, present in body i.e. in gastrointestinal tract, airways, lungs, vagina and in some other parts (Dünnhaupt, Kammona, Waldner, Kiparissides, & Bernkop-Schnürch, 2015). It functions to be selective membrane for the gases, hormones, microbes, pathogens, water and nutrients. The trapped substances move to GIT and get processed by the Hcl and get emitted(Cone, 2009). Mucus is mainly composed of glycoprotein, lipids, salt and 95% of water, making it hydrated and adhesive in nature. It is secreted by the goblet cells or the salivary glands, present as, adhered to mucosal layer in gel form or in soluble or suspended form(Smart, 2005).

Healthy mucus being less viscoelastic, easily transported and cleared, as compared to pathologically altered mucus. In the pathological state there is more salt and water secretions with decreased functionality and being impermeable to microbes, resulting, microbes infiltration causing the inflammation, through various pathways(Fahy & Dickey, 2010). There may be physical and chemical irritations to the mucus that causes their pathology i.e. within 20hours of single exposure to cigrete smoke there is hyperplasia of mucus secretory cells(Gevers, 1987). So in all the diseases it is not possible to adhere to the drug to mucus, when mucus has undergone pathological changes. As mucus is hydrophilic so it acts as a barrier to hydrophobic drugs in several ways, as being, dynamic barrier, steric barrier and interactive barrier. So our dosage forms need modification as per requirement, bio adhesion is one of these modification of dosage form(Boegh & Nielsen, 2015).

Most appealing feature of nano-DDS is particle size range, 1 – 100 nm, making dosage form to cross the barriers, controlling the pharmacokinetics and pharmacodynamics of drug, and optimizing the pharmacokinetic parameters to enhance the drug delivery and patient compliance. The elucidated side effects, interaction, dosing frequency, biotransformation and toxic effects are also optimized(Jiang, Kim, Rutka, & Chan, 2007). Mucoadhesive nanocargo have peculiar physical and chemical properties at atomic level irrespective of them in bulk form i.e. high surface are to volume ration, three dimensional structural modification flexibility, cargo to hydrophobic drugs and poor water soluble molecules made them saving lives efficiently(Desai, 2012).

Bio adhesion a state in which out of two substances that will hold together, one is of biological nature, for extended residence period. Its plethora, leading the actual adhesion via polymeric chain hydration, intimate contact to mucus, entanglement with mucus mesh, creation and lysing of bonds i.e. disulfide bridges, hydrogen bonds, Vander Waals bonds, hydrophobic and hydrophilic interactions etc.(Sonvico et al., 2018). Several swell able and non-swellable polymers are used for mucoadhesion, enhanced contact tend to achieve optimized response of delivery. Prolong residence time,

immobilization of drug carrier at surface site, drug localization, increase in the concentration gradient, direct contact with epithelium prior to absorption are some challenges achieved by nano drug delivery system(Sachan Nikhil & Bhattacharya, 2009).

Various other techniques were used to target the drug but in the early 80's professor joseph R.robinon pioneered the concept of mucoadhesive drug delivery(Bernkop-Schnürch, 2005a). As compared to other dosage forms, Nana Cargoes (NCs) have advantage of being encapsulated, protected from activation and more probability to deliver the drug at the target site by overcoming the biological barrier, unlike other dosage forms that gets distributed throughout the body. NCs also enhance the patient compatibility as it reduces frequency of dosing and toxicity of drugs(Ladavière & Gref, 2015). NCs are also able to overcome various drug resistance mechanisms i.e. efflux pump, multi drug resistance protein family (MDRP) and deliver thousands of drug molecules to the target site(Fahmy, Fong, Goyal, & Saltzman, 2005). Various polymers with ability to adhere to the mucus membrane are formed and used as carriers and excipients in the dosage forms, for targeted drug delivery. This enables to bypass first pass metabolism, increased residence time of drug at target side maintaining high concentration gradient at the target site, acting as driving force for passive uptake of drugs at targeted site(Bernkop-Schnürch, 2005a).

Bio adhesion is a phenomenon involving the interfacial forces to hold the two materials, in the case when adhesiveness is with the mucus, it is referred as mucoadhession(Smart, 2005). Mucoadhesion is optimizing the drug delivery by retaining the drug for subjected time period, at the site of action. The nature of mucus is enabling scientists to make such dosage forms that get adhere to mucus by several different bonding i.e. ionic bond, covalent bond, van-der-Val forces etc.(Smart, 2005)

In the Nano targeted drug delivery various cargos are used mostly hydrophilic macromolecules having hydrogen bonding ability, also called as the first generation mucoadhesives(Smart, 2005). Usually the ligands are used to target the NCs that mostly bind to overexpressed receptors at the site of action, two techniques are mostly used to target active targeting and passive targeting(Fahmy et al., 2005). Various routes i.e. parenteral, oral, intraocular, transdermal, pulmonary inhalation, are available to deliver the nano cargo to the body(Azarmi, Roa, & Löbenberg, 2008). Various bio adhesive dosage forms are available such as tablets, patches, micro particles, ointments, pastes, gels, vaginal rods, pessaries and suppositories(Smart, 2005). Various drugs have the issue to reach the cell compartment, do targeting the endothelial cells or making drug mucoadhesive helps to overcome this issue(Ding, Dziubla, Shuvaev, Muro, & Muzykantov, 2006). Nano drug delivery had made the control possible to prevent the drug release in extracellular matrix before reaching the targeted cell site(Ladavière & Gref, 2015). There are various theories that explain the mechanism of these bio adhesive dosage form i.e. electron theory, diffusion theory, adhesion theory etc. and they will be explained later in this chapter(Smart, 2005). In the latest world of bio adhesive drug delivery the carries and cargo are modifying i.e. thiol groups are used in carbomers and ethyl hexyl acrylate with acrylic acid, many others will be explained latter(Smart, 2005). Now days instead of mucus, epithelial layer is selectively targeted to enhance the drug retention time at the targeted site.(Ensign, Schneider, Suk, Cone, & Hanes, 2012)

Mucoadhesive Polymers

Characteristics of Mucoadhesive Polymers

The polymers used in the Nano cargo for mucoadhesion must have some specific characters there glimpse are:(Bagan et al., 2012)

- Have a modifiable flexibility so get entangle with mucus in a better fashion.
- Molecular length and weight must be optimum to get significant chain inter-penetration.
- Must have charge, cationic or anionic for greater degree of adhesion.
- Bearing various functional groups to undergo the various bonding and strengthen the adhesion.
- With surface energy property for optimum spreading phenomenon.
- Not be toxic or irritating agent to the mucus layer.
- Must not decompose during the retention time of dosage form.
- Not too costly so that prepared dosage form must be in appropriate range of cost.
- Drug loading and drug release must be optimum and controlled.
- Must have mucus enhancement and local enzyme agonistic property.

Classification of Mucoadhesive Polymers

Polymers for mucoadhesion are classified on various

basis natural, synthetics, charge based cationic and anionic, biodegradable, biocompatible etc. Mostly used classification is natural and synthetic ones(Punitha & Girish, 2010).

Natural Polymers

Natural polymers are further of two types' polysaccharides and protein based. They are of algae, plant, and microbes' origin. They are of interest to pharmaceutical scientists because of variety of ranges in molecular weight, functional and reactive groups, diverse chemical composition and other properties that makes them modifiable as per demand. As being of natural sourced they are highly stable in human body environment, safe, biodegradable and less toxic. Hydrophilic nature makes them to interact with biological tissues non-covalently, making them significant and optimum for bio/mucoadhesion(Hamid Akash, Rehman, & Chen, 2015).

Table 1. Mucoadhesive Property of Various Polymers

Name	Mucoadhesive property	Reference
Agarose Chitosan Gelatin Hyaluronic acid Carrageenan Pectin Sodium alginate. **Cellulose derivatives** Carboxy methyl cellulose. Thiolated Carboxy methyl cellulose. Sodium Carboxy methyl cellulose. Hydroxyethyl cellulose, Hydroxypropyl cellulose, Hydroxypropylmethylcellulose, Methylcellulose, Methyl hydroxyethyl cellulose.	1. Cheap and often extracted from renewable resources. 2. Biodegradable, nontoxic. 3. Purity may vary. 4. Induces a strong immunogenic response. 5. Hydrophilic. 6. Properties cannot be controlled. 7. Only surface modification possible. 8. Bio adhesive.	(A. Alexander, Ajazuddin, Swarna, Sharma, & Tripathi, 2011; Khan, Mahamana, & Pal, 2014; Sonia & Sharma, 2012)

Synthetic Polymers

Synthetic polymers are further classified chemically as polyether, polyesters, poloxamers and RP polymers. They have been used by scientists because of being able to increase pharmacokinetics, retention time, circulation time of therapeutic substance thus attaining optimized control on disease by such modification. They are passively used as drug carriers(Punitha & Girish, 2010; Tangri & Madhav, 2011).

First Generation Mucoadhesive Polymers

First generation polymers are mostly classified as anionic, cationic and non-ionic polymers and out of these three anionic and cationic are of most interest because of their enhanced mucoadhesive strength(Punitha & Girish, 2010).

- Anionic polymers being negatively charged, carboxyl and sulphate functional groups show more mucoadhesion and less toxicity, example includes Poly Acrylic Acid (PAA).
- Cationic polymers are positively charged and mostly used and studied chemical under this heading is chitosan. Chitosan is least toxic, optimized biodegradation, biocompatibility, and optimum cross linking with mucin thus favoring mucoadhesion and enhanced therapy via various Para-cellular routes mostly by neutralizing the fixation of anionic sites within tight junctions among mucosal cells.

Novel Polymers/Second Generation Polymers

The need for second generation came because of non-specificity, more susceptibility to mucus turnover rate and less adhesion time of first generation mucoadhesive. To overcome these certain polymers were made and known as second generation novel polymers for mucoadhesion i.e., thiolated polymers, lectins. So, they are cytoadhesive and site specific.

Thiolated Polymers

Thiolated polymers are special multifunctional second generation polymers also known as thiomers. They have free thiol groups on the polymeric backbone, makes it an optimized excipient. It also helped to form the disulfide bonds with mucus glycoprotein on the mucosa membrane thus favoring the mucoadhesion, with cysteine rich domains, interchain disulfide bond helps polymeric network to strong cohesive forces that gives more stability to the mucoadhesive Nano cargo by strong covalent bonding to mucin. By mobilizing the thiol groups the mucoadhesion of chitosan and acrylic acid were optimized. So far, glycoprotein thiomers have shown the best mucoadhesion via thiosulphide exchange reaction and by an oxidation reaction. They also have ability to enhance permeation ability via Para cellular uptake mediated by glutathione aided opening of tight junctions. Mostly used ones are chitosan iminothiolane, PAA homocysteine, PAA cysteine, alginate cysteine(Tangri & Madhav, 2011).

Classification Based on Physical Properties

They are also classified on basis physical properties of different factors that are summarized below in the table

Table 2. Classifications Based On Physical Properties

Hydrophilic	Hydrophobic	Anionic/cationic	Uncharged	References	Biocompatible	biodegradable	Possible mechanism of formation of bioadhesive bonds
Cellulose derivatives CMC, Thiolated CMC, Na CMC, Hydroxyethylcellulose, HPC, HPMC, Methylcellulose, Methylhydroxyethylcel-lulose. Others Poly-N-2-hydroxypropylmethacrylamide, Polyhydroxyethylene	Polymers based on poly(meth)acrylic acid Carbopol, Polycarbophil, Polyacrylic acid, Polyacrylates, Copolymer of acrylic acid and PEG, Copolymer of methylvinyl ether and Methacrylic acid, Poly-2-hydroxyethylmethacrylate, Copolymer of acrylic acid and Ethylhexylacrylate, Polymethacrylate, Polyalkylcyanoacrylates:- Polyisobutylcyanoacrylate, Polyisohexylcyanoacrylate.	Aminodextran, dimethylaminoethyldex-tran, chitosan, quaternized chitosan Chitosan-EDTA, PAC, carbopol, polycar-bophil, pectin, sodium alginate, Na CMC, CMC	Hydroxyethylated starch, HPC, PEG, PVA, PVP	(Kharenko, Larionova, & Demina, 2009; Patil, Tiwari, & Repka, 2016; Punitha & Girish, 2010)	Esters of hyaluronic Acid Polyvinyl acetate Ethylene glycol	Poly(lactides) Poly(glycolides) Polycarprolactones Polyalkyl-cyanocrylates Polyorthoesters Polyphosphoesters Polyanhydrides Polyphosphazenes Chitosan Polyethylene oxide	Covalent Cyanoacrylate Hydrogen bonds Acrylates, carbopol, polycarbophil, PVA **Electrostatic interactions** Chitosan

Nature of Bio/Mucoadhesive Interactions and Auxiliary Agents

Morphology of mucus made scientists to adherence of drugs to it and overcome various factors. There are certain factors that play vital role in modulation of adherence to mucus i.e. temperature, pH., shear stress etc. in the optimized environment of these conditions significant mucoadhesion is possible and so do desired effect(das Neves, Bahia, Amiji, & Sarmento, 2011).

Mucoadhesive Interactions

Mechanism of Bio Adhesion

The mechanism of mucoadhesion involves various interactions and theories and factors. The mucoadhesive interactions may be physical or

chemical with mucus layer but before that their landing and adhering to mucus is usually classified into two series(Velmurugan & Ashraf Ali, 2013):

1) Contact stage
2) Consolidation stage

Contact Stage

Initial physical contact among nano cargo and mucus layer involving wetting, spreading, swelling and surface tension phenomenon , developing initial contact with mucus layer after landing to it(Khan et al., 2014; Velmurugan & Ashraf Ali, 2013).

Consolidation Stage

Chemical bonding occurs between Nano polymer and mucus mesh. This chemical bonding strength is important, depends on penetration among polymeric chains and mucin interaction. To signify this stage there must solvation among polymer groups and their similarity among chemical structure. Chemical bonds formed here will be described later i.e. covalent, hydrogen etc.(Khan et al., 2014;Velmurugan & Ashraf Ali, 2013)

Chemical Bonding Involved

As described earlier consolidation stage consistency depends upon chemical bond causing interaction among polymer and mucin causing mucoadhesion. Several bonding involved are as follows(Khan et al., 2014; Smart, 2005).

- Ionic bond- caused by interaction of ions, oppositely charged among each other involving electrostatic interaction.
- Hydrogen bond-hydrogen atom carrying slightly positively charged being attracted to electronegative species i.e. oxygen or nitrogen etc. this involves covalent bonding. This bond is weaker then ionic and covalent bond. Hydrogen is said to be shared and bond formed is generally weaker.
- Covalent bond-here there is sharing of electrons to fill vacant orbitals, in pairs. They are strong ones as there is complete sharing of electrons.
- Van-der-Waals bonds- formed by dipole interactions and dipole induced dipole interactions in charged molecules. They are the weakest form of interactions.
- Hydrophobic bonds- they are made because of hydrophobic effect involving indirect bonds, involving non-polar groups in aqueous solution.

Theories of Bio Adhesion

Mucoadhesion and bio adhesion are not yet clearly defined about their mechanism of adhesion and interaction but several theories are used to explain this as follows(Lalge, Sharma, Bhandari, Garud, & Garud, 2014; Smart, 2005)

Electronic Theory

As the nano cargo polymer and mucus have opposite charge and electronic chemistry is different. This theory suggests that transfer of electrons occurs between Nano cargo and adhering (mucus) layer that results in formation of electrical double bond at interface due to difference in electronic structure of contacting materials that gives adhesion due to attractive forces.

Wetting Theory

This is the oldest theory and best fits to liquids and less viscosity bio adhesives. It says that bio adhesion is embedding procedure, firstly spreading of polymer onto adhering surface as prerequisite for adhesion, followed by penetration of adhesive agent into surface of substrate then harden and anchors, overcoming all surface tension effects present there. This calculates the contact angle and thermodynamic adhesion by the duper's equation:

$\omega A = \gamma b + \gamma \tau - \gamma bt$

ωA is the thermodynamic factor and so γb, $\gamma \tau$, and γbt shows surface tension of bio adhesive polymer, substrate, and interfacial tension.

Contact angle must be less for best adhesion and this rule is evaluated by spreading coefficient (sab) using the equation:

SAB: ¼ cB _ cA

Here cA is the surface tension of liquid A and cB energy (surface tension) of liquid B.

Adsorption Theory

This theory says that after initial contact, adhesion is on the basis of chemical interaction between the polymer and mucin, primary and secondary chemical bonds i.e. hydrogen bond and van der Waal's forces are mainly involved in adhesion. Chemisorption theory, subsection of it, says that interaction across interface occurs because of strong covalent bonding. The intensity of bonding is related to polymer's property, so do adhesion.

Diffusion Theory

This theory explains mucoadhesion as entanglement

of both polymer and mucin chains involved. The bond after initial contact, strength is based directly on degree of penetration. The polymer's chemical similarity to the mucin, enhance the process of adhesion. Penetration of 0.2-0.5 micrometer is needed for best bonding among polymer and mucin and adhesion increase as the penetration does. Penetration is contact time dependent. Penetration depth is given by:

l = (t Db) 1/2

In this t is contact time and Db is coefficient of diffusion of polymer in mucin. When the depth of penetration is coinciding with the polymer chain length equally then adhesion strength for a polymer is almost at optimum. So the concentration gradient is driving factor in this and it depends on the molecular chain length of polymer used in nano cargo, causing the formation of semi adhesive bond after penetration.

Mechanical Theory

This theory explains that interlocking of the liquid adhesive into the irregularities on a rough surface is implemented to increase the surface area available with an enhanced viscoelasticity and plasticity. This helped to overcome the energy loss during join failure. So this explains the filling of irregularities on a rough surface by mucoadhesive liquid, interfacial area for interaction, this adds to energy dissipation, is increased by the roughness. It is believed that mucoadhesion is not defendable with single theory; it is always explained by mingling the different theories, Lee et al. (2000)

Fracture Theory

This is different from all other theories as it describes about the detachment after the adhesion process has occurred. Forces required for the detachment is described by this theory as failure of the adhesive bond at the interface occurs that results the detachment process. This occurs at the weakest point, among cohesive force failure between the polymer and the mucin. The tensile strength produced during this can be found as

Sm = Fm/Ao

Fm is the maximum force of detachment and Ao is total surface area involved in the interaction process. So this theory only explains the force required for separation from the adherent layer for polymer. It is also used for calculating the rigid and semi rigid bio adhesive material in which polymer chain do not penetrate the adherent layer.

Characterization Of Bio/Mucoadhesive Profile For Nano Cargoes System

a. Direct Methods

b. Indirect Methods

Introduction:

The potency of the mucoadhesive dosage is measured, extent of force and time for detachment, by various methods and these methods show close relation to the in vivo properties as of invitro. There is always limitation that whether the bond has broken on mucoadhesive surface or detachment at the interface has happened. The most of data regarding the characterization of mucoadhesive polymers/dosage cargo is provided via either direct or indirect method.(das Neves et al., 2011)

Direct Methods

This is the technique in which the tissue explants are used to access what will be happening in vivo, checking the in vivo response. This technique includes cytoadhering method, ex vivo methods, in vivo administration/ex vivo analysis, in vivo imaging. Cytoadhering is mostly used method. In this method the mucoadhesive tissue of interest is evaluated usually by incubating the fluorescent labeled nanoparticles with cultured cells i.e. caco-2cells with mucus producing HT-29 cells. Another approach of nano cargo uptake is also experimented in this method and cross section or multiangle visualization is done at lower incubation temperature of cells. In the ex vivo method the fluorescent nano cargo are used, and their retention is measured that mimics it's in vivo response and washing with aqueous is also done to co relate the in vivo physiological washing process and dye is removed via organic solvent. Confocal laser microscopy is also used in this process to quantify the polymer used. The preparation of mucosa, sampling and animals used limits this test as its disadvantage. In the in vivo administration/ex vivo analysis, there is direct or indirect administration of radioisotope or fluorescent labeled nano cargo to the targeted tissue. Then the animals are sacrificed and observed quantitatively and qualitatively using various techniques i.e. fluorometric assay, auto radiographic detection. In the in vivo imaging method, the real imaging of Nano cargo traffic is taken, involves the use of x ray photograph of nano cargo using some contrast agent i.e., barium sulfate. This method is more realistic in the world of Nano drug technology dosage form and helps to evaluate the mucoadhesion with the pharmacological response of nano cargo.

Indirect Methods

The most common method to study the mucoadeshion via in vitro techniques, provide the basic mechanisms of Nano cargo mucoadhesion using the invitro developed mucin solution films as per requirement. It includes the methods of mucin particle, micro gravimetric, atomic force microscopy, optical techniques and diffusion particle tracking. Mucin particle method involves the measurement of amount of mucin directly involved to adhere to the Nano cargos on the aqueous media, thus determine mucoadhesive potential. It involves the use of size variation, z potential or electrophoretic movement of complexes formed. The micro gravimetric method involves the evaluation done on the basis of adsorbed mucin with quarts microbalance. Quartz crystal resonator gets covered by the mucin and incubated Nano cargos. Change in resonance frequency is measured, converted to adsorbed mass of Nano cargo and relative change is measured and pharmacokinetics is evaluated with various parameters. Atomic force microscopy, topographical studies, probe is used to scan the sample at molecular level then shows the attraction or repulsion, measured. Atomic force microscopy is for imaged study of samples using the close scan via probe accessing the close view of attraction or repulsion at molecular level producing the 3d image. In this method the contrast of the topo graphed mucosal cells, incubated with polymers, shows the mucoadhesive potential by accessing the roughness. Optical technique is used to access the adhesion potential among the Nano cargo and respective mucin, provides the quantitative data by using the incident light on immobilized substrate bounded to an analyte. Plasmon resonance technique is also used and the refractive index based calculations are done to get the results. Diffusion/ particle tracking methods are used to measure the diffusion in reference to mobility of different types of the particles and detect their interaction with components of aqueous media (biological), nature of interaction between the body fluids and Nano cargo is accessed in a way that they are made fluorescent and made to interact with body fluids and 2D videos are taken using video microscopy. This makes to access the diffusion, quantitatively.

Approaches for Improving/Subsiding Mucoadhesion of Nano Cargoes System

a. Targeted oral Nano cargoes system.
b. Targeted nasal Nano cargoes system.
c. Targeted ocular Nano cargoes system.
d. Targeted buccal Nano cargoes system.
e. Targeted vaginal Nano cargoes system.

Targeted Drug Delivery

All the dosage forms being synthesized at enhanced level of scientific development is intended to produce the desired effect in the body against the undergoing pathology, reaching the specific site of ailment is always the desired aspect of scientists, so from past few decade they are trying to make the Nano drug delivery to be targeted using various technologies. Major aim of targeted delivery is to transport the therapeutic agent to intra cellular site without harming the normal cells. Nano cargo being small in size bypasses the disadvantages of conventional drug delivery, making them targeted to desired site, adds a lot to it. Mostly the process includes to emphasize the loading the drug into a suitable carrier, load it with targeted ligands, intended for site specific delivery, ligands are mostly blended with formulation or may be coated to it. Mostly biodegradable polymeric Nano cargos are developed. Cell internalization is mostly used to check biological activity of the loaded drug cargo; mostly clathirin and caveolae are mostly used for this. Some good points of targeted drug delivery are(Fahmy et al., 2005; Lambkin & Pinilla, 2002)

1. By passing multi drug resistance
2. Enhanced interactions with target through multivalency
3. Synergistic effect between target ligand and encapsulated drug

The mostly used transport mechanisms in this targeted drug delivery of Nano cargo are as follow(Lambkin & Pinilla, 2002)

1. Para cellular.
2. Trans cellular.
3. Trans-cytotic.
4. Carrier mediated.

Targeted Oral Nano Cargoes System

Mucoadhesive Buccal Nano Cargo System

Oral mucosal pathology is quite common with advance oral diseases treatment is also recently developed as per the pharmaceutical dose technology development. Too much saliva is a big issue in buccal cavity is a major issue in the bioavailability of this drug route as it washes away the most of the drug by scavenging effect of saliva, and no control release rate can be obtained at specific site of action. The drug in buccal cavity do not gets equally bioavailable, less retention time, in certain

areas as there is not uniform saliva in the oral/buccal cavity. The poor patient compliance like bitter taste and size of some drugs also alter the therapeutic effect. To cope all this barriers recently mucoadhesive nano cargo are developed including buccal tablets, buccal films, buccal patches, buccal gels that are used to treat various diseases including infections and cancer of oral mucosa(Paderni, Compilato, Giannola, & Campisi, 2012). Some general character of good mucoadhesive buccal nano cargo dosage form is as follows(Punitha & Girish, 2010).

- Must adhere abruptly to buccal mucosa and possess particular and potent mechanical strength.
- pH must be bio variable and must be viscoelastic
- Must possess the proper spread ability, wetting, swelling and solubility and biodegradation.
- Must peel, tensile and shear potency in bio adhesive range.
- Must be free of leaching impurities and must be nonirritant and most compliant to patient.
- Must be cost effective.
- Should be able to bio adhesive in both dry and liquids state.
- Must able to be bear potency of local enzyme inhibition and permeation enhancement.
- Molecular weight should be optimum to make drug in size.
- Sufficiently optimum shelf life.
- Should not develop secondary infections related to dental care.
- Required optimum spatial chemistry and optimum no of adhesive groups to serve as bio adhesive.

There are two types of drug deliveries used in buccal mucosa one is keratinized and other is non-keratinized and they are selected on the ability of permeability as keratinized i.e. gingival and hard palate, are not good in drug permeation so they are used for local drug delivery instead of systemic and have less ADR and drug interactions. While non-keratinized are further divided as sublingual and buccal, good for systemic drug delivery. Out of these, sublingual is more potent for abrupt systemic delivery as compared to buccal because of it thin nature. So to treat acute diseases sublingual route is used when abrupt onset of action is required and for chronic diseases when controlled release is require the buccal route is used. So overcoming all these short comings mucoadhesive drug carriers were developed in form of various dosage forms to increase the retention time and bioavailability and this do not alter the normal physiology and patient can do all eating, drinking, talking activity(Paderni et al., 2012).

In formulation of buccal mucoadhesive nano drug delivery system two types of approaches are developed monolithic (matrix) types and reservoir (controlled). In the former the drug release is by diffusion mechanism and in later drug in between the backing layer and polymeric membrane controlling the drug release, with the property to be being nonirritant, good mucoadhesive, good compliance for patient, sustained release. In the bio adhesive system firstly there comes the bio adhesive tablets for oral disease, mostly oval or flat with 5 to 8mm and thickness of 2mm, adhere to mucus in presence of saliva and release the drug, without any discomfort to patient. Chlorhexidine is used in these to swell and gel formation thus controlling the release profile of dosage form, forming gel. Double layer or 2 layers are developed to prevent any drug loss and leakage thus promoting unidirectional absorption in oral cavity. Both fast release and control release dosage forms are available. Nystatin a bi-layered bio adhesive tablet is available to treat the oral candidiasis; long term used is non-compliant for patient as it lacks the physical flexibility. These tablets should not disturb once they are adhere to mucosa because this might cause the drug leakage(Paderni et al., 2012).

Bio adhesive patches and films are also available as mucoadhesive therapeutic agents. They are mostly for buccal drug delivery and potent in systemic bioavailability in good time. They are consisting of appropriate polymer with loaded drug, with ability to adhere to mucus and backing layer for unidirectional drug release, with penetration and permeation enhancers and enzyme inhibiters. They are complex in formation with high cost of production but still they are better in enhancing retention time of drug, better bioavailability and good flexibility. Good flexibility makes them to have good patient compliance with reduced interactions and better level of therapeutics in treatment goal. So they are preferred over adhesive tablets(Paderni et al., 2012).

Mucoadhesive semi solid gels and ointment are also used as they are easy to disperse throughout oral mucosa, intimate contact with mucosa and abrupt drug release but no accurate, no drug control release. Their retention time is not valid even using the bio adhesive polymer. Mostly used for narrow therapeutic index drug, as they give less retention time. Mostly used in the diseases like traumatic ulcers, oral mucositis, oral lesions, hyposalivation etc.

Bio adhesive liquids like oral rinse and sprays are also developed; being fine mist they coat the entire mucosa. So they actually increase surface area from where drug could permeate and get bioavailable. They are capable of film forming property so they are of high mucoadhesive and viscoelastic composition. They must form the spray with suitable ovality pattern and particle size, oval shape particle size, as it will be more oval there will be more permeability across mucosa and thus bioavailability and desired therapeutics. They are used against the lichen planus, aphthous stomatitis, mucositis, hyposalivation, malignant disorder i.e. leukoplakia and several immunological diseases(Paderni et al., 2012). The lipophilic buccal spray and phospholipid vesicles are developed to deliver the peptide through this route, cubic and lamellar liquid crystalline phases of glyceryl monooleate drug carriers are also in use. A formulation named as Orlin, generax biotechnology, is developed for precise delivery of insulin using metered dose inhaler as fine droplets directly into mouth, in level 3 of clinical phase trials, this novel pain free and controlled drug release insulin delivery has more patient compliance(Punitha & Girish, 2010). Changing morphology based phospholipid vesicles are also used for insulin delivery, more close to liposome, but peculiar in ability to change the structure in response to mechanical stress using less energy, allowing better drug permeation and better bioavailability, mostly sodium collate and sodium deoxycholate are sued in this regard.(Punitha & Girish, 2010)

The polymer selection here is of great importance as the Nano cargo made is much prone to biodegradation in buccal cavity, mostly protein and polypeptide drugs. One of strategy use in this is auto degradation of buccal enzymes by the use of polymers i.e. acrylic acid, by Ca+2 ions depletion that serves to change the morphology of trypsin and regulate the auto degradation. Mostly the polyacrylates, cellulose derivatives and chitosan are used that shows permeation enhancing and enzyme inhibition properties. Lectin is also used because of its virtual property of being mucoadhesive. Thiolated polymers are one of the advanced ones, forming disulphide bonds with cysteine part of mucus glycoprotein, lining mucus membrane and by immobilizing the thiol groups the properties of polymers i.e. chitosan and acrylic acid were increased up to the 100-250 times(Punitha & Girish, 2010).

Mucoadhesive Oral Drug Delivery System

Numerous routes for drug delivery has been used since a long ago, time to time modified and developed as per research. Oral route is mostly accepted conventional drug delivery route; ease of administration, noninvasive and economical. This system needs several modifications as per disadvantages of it i.e. swallowing issues, irritability, high first pas effect, lower absorption, less retention time in stomach causing less absorption, poor control of release, biodegradation etc. minimizing all these factors new oral dosage forms are developed recently i.e. mucoadhesive drug delivery system, fast dissolving tablet, orally disintegrating tablets, water independent swallow tablets(Gupta, Bhandari, & Sharma, 2009). The monolithic and reservoir type of dosage are modified to be mucoadhesive dosage forms, improving conventional methods, with the quality to increase retention time, bioavailability and therapeutic effect adhering more drug loading capacity and non-irritancy and flexibility in acceptability. Oral mucoadhesive system has various dosage forms i.e. tablets, patches, film, adhesive semi solids and adhesive liquid(Chen & Langer, 1998).

Our interest is with mucoadhesive Nano cargo for oral drug delivery. Particles made with biodegradable or other mucoadhesive polymers are of great importance in recent advancement in pharmaceutical drug development, they slow down the transit time increasing the retention time thus increasing the bioavailability. It was found that poly-fumaric anhydride-co-sebacic anhydride proved strong bio adhesion in intestine, delayed the intestinal transit as compared to simple alginate preparation. Insulin is poorly oral bioavailable and being degrade by stomach environment, less control on dosage studies, showed that loading insulin in polyalkylcyanoacrylate nano cargo presented to lower postpartum hyperglycemia by 50% being administered intragastric another study showed that packing of insulin in mucoadhesive nano cargo poly fumaric anhydride and poly lactide-co-glycol-ide 50:50(p(FA:PLGA)), showed better control on hyperglycemia. Some other bio adhesive nano polymer i.e., amine modified graft polyester, thiolated trimethyl chitosan nano cargo are also used. Mucoadhesive tiolated oral tablets were also developed for insulin, consisting chitosan and thiol group, showing the increased retention time and proper drug absorption with dose control(Andrews, Laverty, & Jones, 2009). Similarly the plasmid DNA having B-galactosidase reported gene was enclosed into mucoadhesive Nano cargo, microsphere (p (FA: SA) then administered orally, showed b-galactosidase activity in intestine, stomach, liver(Chen & Langer,

1998;Plapied, Duhem, des Rieux, & Préat, 2011). M cells have also been in consideration of mucoadhesive Nano cargo delivery, used to deliver the vaccine, being able to transfer from lumen to cell. Nano cargo that mimic the pathogen chemistry can be used to target the M cells, some ligands of M cells have been found, so graft of these ligands i.e., Pattern Recognition Receptors (PRPs) is used to target the M cells, thus producing the potent immunization. Mucoadhesive polymeric nano cargo for inflamed bowel disease is also made by finding the increase mucus and immune cells at that particular site of pathology. Ligand grafted nano cargo, targeted or untargeted, are also developed to enhance the delivery of anti-inflammatory drug in case of colitis(Plapied et al., 2011)

Carbomer are also used in mucoadhesive drug delivery nano cargo system with the property of high molecular weight, strong anionic charge, sufficient chain flexibility, surface energy favoring muco-adhesion, strong hydrogen bond forming hydroxyl and carboxyl group, they form bond with mucus thus potentiate the muco-adhesion. Several tablets are formed using carbomer muco-adhesive technology i.e., diclofenac sodium, indomethacin, sulpiride, tenoxicam, hydrochlorothiazide, sodium fluoride, metronidazole, aminophylline, verapamil HCL, mesalamine etc. Carbomers mingling with some polymers i.e. Hydroxypropylmethylcellulose and this combination is used to potent the bio adhesive property of oral drugs like propranolol, metronidazole this increase the retention time, mucus turnover and bioavailability. They also increased the bioavailability of insulin and peptide drugs through oral route(Singla, Chawla, & Singh, 2000).

Salmon calcitonin Mucoadhesive thiolated tablets using calcitonin and chitosan with tributylamine conjugation were formed and chronic bone pathologies were treated and proved to be 1.35% better than their i/v dosage form(Bernkop-Schnürch, 2005b).

Enterocytes are also targeted as their concentration is higher as compared to M cell in the intestine. Special ligands are used, grafted on the Nano cargo like lectins and folic acid receptors, on the enterocytes that enable the drug orally to get bio adhesive and more permeability and bioavailability(Pridgen, Alexis, & Farokhzad, 2015).

Bio Adhesive Vaginal Drug Delivery

Oral and invasive drug delivery routes have their own complications that led scientists to think for the vaginal drug delivery pathway, decade ago. Nature gave vaginal wall a good potency of blood vessels that make a potent drug bioavailability possible to reach the therapeutic impact, bypassing the first pas metabolism. Before 1918 vagina was just an organ, not a drug delivery route after this its absorption and administration for morphine, potassium iodide and atropine were studied. Then various compound were used to deliver to body via this route i.e. estrogen, progesterone, insulin, sodium salicylate, quinine hydrochloride and progesterone. Pessaries and tablets were used as contraceptive and to treat vaginal infections. Transdermal and transmucosal drug deliveries were mostly used initially then there came the concept of control drug delivery system for vaginal route in 1970's. In that decade medroxyprogesterone ring was developed to deliver the drug as contraceptive, controlled delivery. After that various hydrogels, creams and rings are available on mechanism of mucoadhesion, using mucoadhesive polymers i.e., hydroxy propyl methyl cellulose, hydroxyl propyl cellulose, sodium carboxy methyl cellulose, polyethylene oxide, acrylic acid for vaginal drug delivery. Bleomycin was the first mucoadhesive tablet developed for vaginal route to treat the cancer, necrotic tissue, inserted in cervical cleft of patient(N. J. Alexander et al., 2004; Brannon-Peppas, 1993). After that various nano mucoadhesive emulsions were formed to treat infections in vagina i.e. imidazole for fungal infection. The drug distribution in vagina is carried out by the method using vertical thermostatic cellophane tube, formulation are placed, discharge of liquid is detected by relating it to retention time throughout experiment. The impact of gravity on formulation is also accessed by the flow property on incline surface under gravity and various statistical and mathematical models are applied to get results. magnetic Resonance imaging i.e. gadolinium labeled, gamma scintigraphy and cross section imaging, is also done to evaluate the level of achievement of pharmacokinetic and pharmacodynamics by drug in vagina and access the desired therapeutics, latest technology uses the photo bleaching, fluorescence based and coherence interferometry(das Neves, Amaral, & Bahia, 2010). Several advantages of this route includes(N. J. Alexander et al., 2004).

- Provide potent localized and through sustained therapeutic effect as compared to other routes.
- Long dosing regimens and continuous release medicine are used with more patient

compliance via this route, providing longer intervals among dosing frequency.
- First pas effect is bypassed, 95% estrogen is metabolized by oral route
- Biodegradation by GIT is bypassed.
- Missing pills can be avoided.
- Lesser dosing, lesser side effects.
- Lesser activity of amino acids, thus less biodegradation and mire bioavailability.

The pH in vagina is mounted to be acidic (3.5-4.5) by epithelial cells as they convert glycogen to lactic acid by microbial flora of vagina, an important factor in designing of dosage form. This pH changes with age, menstrual cycle. The pH secretions, thickness, estrogen level, microbial flora changes with age that needs to be considered while designing the dosage form. Transvaginal and intravaginal are two most important available routes for drug delivery through vagina, leading from vagina to uterus and systemic circulation either by portal circulation of lymph flow, acid form and cellulose acetate phthalate working on such mechanism. Use of mucoadhesive technology with permeation enhancers are of great interest in modern world of research several mucoadhesive polymers i.e., polycarbophil 934-P, hyaluronic acid, acacia, Carbopol 974P-NF, acrylic acid, sodium alginate, chitosan, tragacanth, carbomer, sodium carboxymethyl cellulose and several copolymers of various compounds and their derivatives are used, in combination with permeation enhancers i.e. Bile salts, benzalkonium chloride, polyethylene glycol, ethoxydiglycol, interesterified stone oil, non-ionic surface-active agents. Hydrated and dehydrated products are available as mucoadhesive nano drug carriers. Dehydrated products become mucoadhesive by dehydration of local mucosa, while hydrated products absorb moisture and become gel and then mucoadhesive, time release additives are also added in such dosage forms i.e., polycarbophils and carbomers. Prochieve is mucoadhesive gel used for hormone replacement therapy working on this pre-described principle (Dobaria, Mashru, & Vadia, 2007). In the administration mucoadhesive or non-mucoadhesive drug delivery one factor is of great importance, uterine first pas' effect. This is of desired nature when mucoadhesive dosage form is meant to produce therapeutic effect in the uterus, then administered in outer one third of vagina but if mucoadhesive dosage form is meant to produce the effect systemically then uterine first pas effect will lower serum drug concentration as it will be more in the uterus than blood. Tablets being, most convenient and cheap are easily administered as wash out period is high and rapid in vagina so with mucoadhesion they tend to be effervescent. The semi solids like gels and ointments are administered at night and patient is said to be in spine position with less irritancy and less leakage of dosage form, gels are mostly of polymer named polycarbophil for dry vagina and in menopause. As the creams has less ability to be bio adhesive and less retentive in the vagina so they are modified using site release technology by KV pharmaceuticals, making cream somehow bio adhesive and less leaky and some product of it are also available i.e., Butoconazole nitrate 2% and Clindamycin phosphate 2%. Whereas the liquid formulation is avoided as there are chances of triggering of some pathological conditions(das Neves et al., 2010).

Conclusion

Bio/muco-adhesive auxiliary agents could also be used as therapeutic moieties in their specific right, to shield and protect damaged tissues (gastric ulcers or lesions of oral mucosa) or behave as lubricating agents (in the oral cavity, eye and vagina). Nanomedicine based on bio/muco-adhesive auxiliary agents has even greater potential. Not only can they adhere to mucosal tissues, but also offer controlled/ prolonged drug release, specific distribution, increased surface area and improved bioavailability by shielding drug from degradation. However, the amount of knowledge regarding nature and strength of the interfacial forces involved in establishment and duration of bio/muco-adhesive nanomedicine is limited. Mainly, traditional approaches towards characterization of mucoadhesive nanomedicine are insufficient. Hence, the present paper describes bio/muco-adhesive auxiliary agents, employed in latest development of oral, nasal, ocular, vaginal, and buccal nanomedicine with their capabilities that affect the bio/mucoadhesion and its mechanism. Moreover, techniques for it characterization and challenges confronted in future for a fruitful bench-to-bedside translation.

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