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Oral therapeutic proteins: a review on current strategies, challenges, and development

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ABSTRACT
Oral therapeutic proteins represent an extinguish class of macromolecules that could be used as pharmaceutical agents due to their proven pharmacological properties. These exist as an array of compounds with variable structures and functions. Oral therapeutic proteins could be used as an efficient replacements of chemotherapeutics as they originate from biological sources, hence project the minimal risk of adverse and toxic effects. Due to the complex structure of proteins their functional modification proves to be an obscure process, where the risk of losing the pharmacological potential is present. In addition, delivery systems also play a significant role in the treatment and subsequent pharmacological effect. In the vista, oral delivery proves to be feasible due to the protein structure related issues being faced in other delivery systems like parenteral, nasal, ocular, and trans-mucosal etc. However, oral delivery method too is facing the hurdles of its own kind. Proteins ingested are susceptible to the physicochemical parameters of gastrointestinal tract, where they get denatured. Also, they sometimes get acquainted with immune response if the host body treats them as abnormal and foreign. Oral proteins mainly face three kinds of barriers namely biochemical, mucus, and epithelial barrier. However, the research has been managed to enhance the oral protein delivery, despite the presence of these barriers. Apart from structural modifications to proteins, absorption enhancers, enzyme inhibitors, mucoadhesive polymeric systems, and particulate carrier systems are also used to overcome these barriers. Furthermore, few systems like microparticles, microspheres, nanoparticles, lipid-based delivery systems, polymeric nanoparticles, and inorganic nanoparticles have also been used. This review focuses the current perspectives and challenges faced in the field of oral therapeutic proteins. It also emphasizes on the methods being followed in the current scenario to enhance the pharmacological efficiency of oral therapeutic proteins.
Key words: *Oral therapeutic proteins, drug delivery systems, oral delivery, barriers, nanoparticles*

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INTRODUCTION

Proteins and peptides are important building blocks of all living organisms and have emerged as a very promising class of therapeutic entities in recent times [1]. Protein therapeutics can also be assembled based on their molecular types that comprise antibody-based drugs, anticoagulants, blood factors, bone morphogenetic proteins, engineered protein scaffolds, enzymes, Fc fusion proteins, growth factors, hormones, interferons, interleukins, and thrombolytics. Sometimes they are also categorized on molecular mechanism of their activity for example mAbs which bind non-covalently to target, enzymes which interact covalently, serum albumin which exerts its activity without specific interactions and so on [2, 3].

Blohm *et al.* identified therapeutic proteins as proteins or polypeptides that are potentially appropriate as a therapeutic drug that can be used in treatment because of their native role in the human body. These may be mutagenesis or proteolysis-prepared derivatives, hybrid proteins, protein aggregates or other molecular conjugates [4, 5]. Although the rationale behind functional properties of therapeutic proteins has been defined, novel insights could always be found as a part of incessant research on pathogenic diseases like diabetes and cancer [6, 7]. The use of therapeutic proteins in the treatment of diseases has many benefits over traditional drugs because they perform a highly complex and diverse range of body

functions that cannot be imitated by simple chemical compounds [8]. As proteins action is very specific, therapeutic proteins are less likely to interact with normal biological processes and to cause adverse effects. Because many of the proteins that are used for therapeutic purposes are naturally generated by our body, these agents are often well-tolerated and less likely to cause immune responses, and protein therapeutics offer an efficient substitute for treatment of genetic disorders without the need for gene therapy [6].

Since the past 3 decades, the production of lot of protein therapeutics has seen a huge rush and offers the benefits of greater focus, greater activity, and less toxicity [5]. However, concerns have been raised about their structural complexity and difficulties in modification [9]. As a result, potential protein treatments that have failed to date [10]. Thus the protein structure and route of administration are the crucial factors that directs the pharmacokinetics and efficacy of the medication in any therapeutic intervention; prompted researchers to create new delivery mechanisms that are capable of more effectively delivering such a class of drugs[11, 12]. The oral route is one of the most appropriate and accepted route to treat therapeutic complexes. The oral delivery of drugs and proteins would outweigh the injection-related pain and discomfort, and would also reduce the chances of infections induced by needle reuse and comes handy in case of repeated administration. In addition, oral utterance is less exorbitant to produce, as it is not necessary to produce them under sterile conditions. In order to overcome challenges such as low permeability, bioavailability, lack of lipophilicity, and inactivation of rapid enzymatic embarrassment in the GI tract, improvement in oral protein utterance needs to be overcome [13]. These aggressive protein physicochemical properties provide pharmaceutical utterance researchers with a monumental challenge [14-16].

The protein drug supplied by oral administration can transmit via the GI tract and the mucus layer of the intestinal epithelium and enter the portal [17, 18]. The ideal delivery system should therefore maintain the highest degree of protein activity and should have certain features such as: high capacity to encapsulate and load drugs, continuous and regulated release, reliability and ease of processing, etc [19]. Therefore, drug delivery systems (DDSs) should be engineered to minimize adverse reactions while ensuring site-specific delivery, proper administration, enhanced patient compliance, and enhanced shelf-life of the product[20,21]. Over the past several decades, engrossment in developing successful DDSs for biological entities has developed dramatically as the number of recombinant proteins being explored for therapeutic applications has increased [22]. The success of these new therapeutics depends on successful DDSs that enable drug access at the right time, length and drenching to their target site(s) [23, 24].

The present article intended to encompass the possibilities being explored in oral delivery of therapeutic proteins and the challenges associated with their development focussing on the strategies employed in oral delivery systems; future prospect and consequences of different approaches of oral therapeutics.

STRUCTURAL PERSPECTIVES IN THERAPEUTIC PROTEIN DEVELOPMENT

Proteins are basically macromolecules with molecular weights that range from approximately 5,000 kDa to several million[25]. Proteins are investigated as oligomeric proteins with two or more polypeptide chains; subunits or protomers are called their constituent chains. A protein's polypeptide chain is formed into a particular three-dimensional structure, which is referred to as protein conformation [26].Fibrous proteins consist of chains of polypeptides that are ordered along a single alliance in collateral position to capitulate long fibers or sheets. The globular proteins that form the peptide/protein pharmaceuticals are the second class. The polypeptide chains in globular proteins are hardly folded into compact spherical or globular shapes [27, 28].

The development and production of therapeutic protein drugs is expanding each year in terms of the amount of product required worldwide [29, 30]. A low molecular weight compound is subjected to sequential preclinical and clinical review in the classical drug discovery and development processes to assess the efficacy, utility and protection of the compound as a potential pharmaceutical agent for human use. Therapeutic protein development and processing are highly complex processes. A typical protein drug, for example, can contain more than 5,000 critical process steps, several times more than the number needed to produce a small-molecule drug. Protein therapeutics also have several secondary and tertiary structures that need to be preserved. Moreover, chemical processes cannot be fully synthesized and must be generated in living cells or organisms; as a result, cell line selection, species origin and culture environment all affect the characteristics of the final product[31]. Furthermore, nearly all biologically active proteins necessitate post-translational alterations that can be affected by using heterologous systems of speech. Furthermore, when they are synthesized by cells or animals, complex purification processes for the products are involved. An evaluation of the physicochemical and biological properties must be carried out for any given protein compound, including an assessment of purity, stability, biological activity in vitro and in vivo, molecular size in solution, pharmacokinetics, biological

half-life, pharmacodynamics, route of administration including distribution aspects and consideration of the physiological barrier itself [4, 11]. It is highly desirable that therapeutic proteins be complex in terms of their large molecular size, post-translational modifications and biological materials involved in their development process, the ability to increase specific functional characteristics for maintaining product safety and the effectiveness achieved through protein engineering strategies. In addition to increasing production yield and product purity, a number of protein-engineering platform technologies are currently used to increase the circulating half-life, targeting, and functionality of new therapeutic protein drugs [30].

PROTEIN ADMINISTRATION AND NEED FOR ORAL DELIVERY

To achieve either systemic or local delivery of proteins, several administration routes may be used. For small therapeutic molecules, various routes of drug administration, such as parenteral (intravenous, intramuscular, and subcutaneous), oral, nasal, ocular, transmucosal (buccal, vaginal, and rectal), and transdermal, can be used [23, 32]. On the other hand, due to their large size and structure, the routes of administration for proteins are limited. The administration of active molecules through a delivery system is only successful if they are delivered directly to the disease site in a timely manner, with patient convenience and assurance of a quality product delivered [33].

The oral bioavailability of most peptides and proteins is very low because of their vulnerability to the strong acidic environment and proteolytic enzymes in the gastrointestinal tract. Peptides and proteins are also substances of high molecular weight and thus do not crucify the intestinal mucosa easily. Administration is the only presently obtainable measure of conveying in situations where the drug is excessively metabolized and/or bound at the site of injection, protein and peptide drugs [34]. In addition to the usual barriers,supplemental barriers associated with the systemic delivery of peptide/protein drugs are due to immunogenicity, such as thrombophlebitis, tissue necrosis, etc. While amino acids and small peptides are not immunogenic in themselves, macromolecular proteins are often recognized by the body as "non-self" which then responds to the production of a particular antibody [28, 35]. Sometimes abnormal developments in health improvement and sub-standard level of disease management also provoke the hunt for substitute delivery routes; patients tend not to like injections, so a non-parenteral articulation is sometimes considered to be preferable to patients and clinicians alike. Therefore, it becomes essential to acknowledge the alternative ways of administration [4, 36].

In the context, oral delivery provides specific advantages, including steady and controlled delivery, ease of administration, likelihood of solid formulations, patient satisfaction and enhanced immune response in the case of vaccines[17]. A wide surface area coated with a viscous mucosal coating often paves the way for the attachment of drugs and eventual absorption. In addition, within mucus, rapt drug molecules are shielded against the shear stress induced by flowing gastric juice. The abundance of enterocytes, in particular microfold cells covering the Peyer patches, in many sections of the intestine, the lymphoid section of the small intestine makes the human intestinal epithelium extremely absorbent [32, 37].

For example, insulin-natural physiology influences the oral route; in normal human physiology, the food intake is recognized by the pancreas by the revolt in the level of blood glucose, insulin is released according to the requirement that further releases into the vein of the hepatic portal and reaches the liver that is its main place of action. On the contrary, pulmonary, nasal or buccal insulin is transferred to systemic circulation both in injections and in other routes. Insulin, directed by routes other than oral, travels through the systemic circulation to the liver so that only about 20% of the dose is available to the liver [38]. Oral insulin has benefits over other forms of delivery of insulin as it sticks to the normal physiological pathway, i.e. it first reaches the liver and subsequently the peripheral tissues through hepatic portal circulation. As a result, the natural physiological route of insulin can be imitated to a significant extent by oral delivery. However, the absorption mechanism of oral drugs is more complicated compared to other pathways [39, 32].

CHALLENGES ASSOCIATED WITH ORAL PROTEIN DELIVERY

Despite its remarkable beneficial effects, oral protein delivery has been linked with considerable trouble because the drug has to remain intact and should pass through the mucosa in the tract before being accessible[21]. Therefore, the intestinal epithelium is considered to be the main physical barrier that must be managed when biological elements are delivered by systemic action through an oral road [40, 18]. Problems regarding the therapy of oral drugs may be broadly classified as biological barriers and technical challenges. Biological barriers include every organic element that deny the medication used orally, or blocks the target from being consumed successfully. Technological challenges, on the other hand, arise that oral delivery devices are difficult to create orally via movement [32].

The high mole, proteins and patient biopolymers are functional in a number of functions such as enzymes, structural aspects, hormones and immunoglobulins within the body and involved in various biological activities. Nevertheless, in his faction, great molecular size as well as size of hydrophilic and hydrophobic systems in nature make proteins difficult to escape into the cells and other containment areas and therefore retains small permeability properties via various mucosal processes and organ membranes [18].The effectiveness of the most physiologically active proteins and peptides due mainly to their tertiary structure, and are declined under different physical or chemical environments, and results in denatured or degraded proteins and peptides that result in a successive loss of biological effect, which makes these molecules substantially instable [23].

In addition, the degradation of protein and peptides in the stomach and duodenum is very high, and in the ileum and colon it is reduced significantly. Several delivery systems were established in order to reduce drug exposure to proteolytic enzymes that target absorption from the colon and ileum. There is also another issue, however, such as a potential change of the colon microflora, delaying the substance intake, risk of the drug and other potentially harmful compounds along with medications [41].The body may also develop an images (peptides) and a skinspasmos can sometimes result in somewhat healthy nutrients, causing protein to nil responses [6, 42]. Therefore, due to pricey intermediate technologies used in the designing of sch healing proteins and peptides, production costs are generally high [43].

APPROCHES USED FOR ORAL PROTEIN DELIVERY

In view of the aforementioned multiple challenges correlated with the efficient oral release of therapeutic proteins, several techniques for the manufacture of therapeutic proteins of enhanced bioavailability were researched as [23, 34, 44]. The following methods were used for the production of oral protein delivery programs.

Absorption enhancers to augment permeation through the intestinal epithelium

As listed below, in many instances the body permits were extraordinarily poor for large hydrophilic molecules such as peptides and protein, which seriously and adversely affected their bioavailability[35]. The utilization of permeation factors has been shown to enhance the implementation of therapeutic protein medications with the goal of increase the permeation rate through the intestinal hindrance by modifying epithelium properties on a short or by stimulating paracellular pathways in order to allow epithelial junctions available or by transcellular pathways dismaying the mucosal atmosphere with a small amount[45]. Detergents, surfactants, bile salts, and chelating agents are all absorption enhancers used as speech elements. Even with temporary opening of close junctions, long alkyl chain enhancing products, including fatty acid, sodium caprate and acyl carnitine, have shown similar increased absorption [47]. Zonula ocludens is considered to be a healthy and successful enhancer for moving macromolecules, such as insulin, through mucosal barriers across varied intestinal epithelium straight junctions transiently [47].

While the advantage of activators is an effective technique to improve immunity through the intestinal epithelium, some associated risks have been identified. That is the use of permeation strengtheners. This may modify the breathing condition of the intestines by enhancing the systemic sensitivity to dietary antigens and probable risk for hypothesis diseases, although there are zero clinical information available so far supporting this risk [48, 49]. In order to change the properties of the mucus layer, to disrupt the microbiome and to diminish the defensive role of intestinal epithelium against luminal bacteria, more frequently used excipients with widely accepted safe status including surfactant form permeation enhancers were seen to [50]. The best advantage for the long-term increase is that when an installation improves penetration the biomembrane is disordered or even dissolves the biomembrane, meaning local inflammatory effects of penetration can occur [18].

Enzyme inhibitors improve proteins and peptides stability in the GI tract

One of the major barriers to oral protein delivery is proteins that a range of proteolytic enzymes such as endopeptidases (for example, pepsin, trypsin, cis drugs, exopeptidases may quickly degrade, including Carb and B, can cleave these units into smaller fragments and monomer components in a rather short period of time, and can be switched against to proteins[35]. However, researchers have used a wide variety of kinds of enzyme inhibitors such as the aprotinin and soya trypsin inhibitor, the mesilat and chromostatin, to reduce the breaches in protein by different protein degrading inhibitors but the long-time delivery of such protease inhibitor resulted in a scarcity of these enzymes for human beings [18]. An updated class of enzyme, duck, and chicken enzymes have recently been found and formulations have been developed where insulin ovomucoide and duck enzymes have provided 100 percent protection against the activity of the botanical disruptors [51, 18]. Serine protease (serpotenic) inhibitors may form covalent protease compounds which are also known to protect peptides against peptide attacks. Additional CMC- Ela displayed an increased elastase inhibitor action by almost 33percent of the

therapeutic agent remaining stable on enzyme attack after four hours of incubation of nerve [52, 15]. Based on structural studies, the problem arises in an inhibitory member of the arising band in conformation change, known as stressed transition and a corresponding modification, which is the most critical step of a targeted protease inhibitions mechanism. As an alternative, although an enzyme inhibitor might improve the stability of peptides in the GI system considerably, feedback control has been demonstrated to promote increased secretion, and, hypertrophy, and pancreas hyperplasia have been caused and are leading to major toxic effects in chronic chemotherapy period of treatment [35].

Mucoadhesive polymeric systems increase residence time of therapeutic proteins

Delayed absorption is a bigger issue with the bioavailability of drug proteins that make Peptides and proteins simpler for enzyme breakup and have a short half-life in vivo [41, 35, 18]. Throughout the entire growth process and release of the drug to its desired locations, the protein degradation occurs at different stages. To avoid the degradation of therapeutic proteins, numerous strategies are being used. Proper enclosures with biocompatible and resilient Polymers could provide a healthy framework to avoid the preservation, protection and safe drifting of these particularly volatile macromolecules in the long lasting. Wide research was done into cellular systems like thiols like chitosan, lactic co-glycolic acid (PLGA), thiolated polymer, and alginate, thus prolonging your time spent at the pharmacological disposal site and alginate chitosan [53, 54]. To boost the drug concentrations to the gradient and allow an immediate absorption without degradation of the luminal fluid without disintegration or decompression, they maintain intimate contact with the mucus; [15]. Microenvironment-responsive hydrogel allowed the stomach to protect a protein, but release the small intestine. As a result, it may be a promising oral peptide and protein carrier for[22]. Orally adhesive polymeric chemical alone may also not have an effective protection against proteolytic enzymes in an oral formula. Thus, conjugates of mucoadhesive polymer protease inhibitor were generated to achieve effective protection [18, 49, 52].

Particulate carrier systems for protein delivery

In order to protect protein in the GI tract from acid and enzymatic degradation in the oral protein supply, various particles, such as emulsions, liposomes, microspheres, and nanoparticles, should be used [34, 18]. Such particulate systems provide medicine is superior across the epithelial mucosa with improved deliveries, frequency modulation, and aim the distribution of medications to very specific locations within the intestine [17]. A cumulative result of an acidic pH of the belly, bile and pancreatic later is ideal to inhibit oxidation of oral thickening by the administration of liposomes. Nevertheless, microspheres constructed by spray spraying, double emulsion, and phase diagrams from natural or artificial paint technology may also be used as an alternate solution for mouth distribution safety approach to treat [55]. In relation to their origin, proteins encapsulated in polymeric nanoparticulate carriers are less susceptible to enzymatic degradation through their link with polymer, on the other side. In specific, as orally distributed, nanoparticles for the delivery are ingested into the gut epithelium, in particular via the Peyer patches [12, 56, 57]. The method for the absorption of protein encapsulated nanoparticles is an efficient procedure, is driven by particle size, surface load, ligands, and the changing nature of particle behavior in the gut [58, 15].

Modifying the physicochemical nature of macromolecules

Peptides and proteins specifically minimize entry through the GI epithelial cell membranes. The lowest lipophilibility level is required for the molecules to get into an epithelial cell membrane to be absorbed. For example, the medications and biological analogy approach may prevent protection from the breakdown by protein or other enzymes present in the GI tract [18, 43]. Improvements may be used to maximize macromolecules' pharmacokinetic characteristics, but caution must still be taken not to diminish their medicinal effectiveness [49]. In order to resolve the problem of low biological activity, a new lipidization technique was recently introduced to ensure active polypeptides are regenerated from their lipid conjugates after oral absorption in order to ensure the recovery of their virus on oral antibodies[35]. Many medications have been lipidized using this machinery, and modified macromolecules display an improvement in epithelial absorption and GI property with increasing sensitivity to other temperature and in various situations [49, 59].

Modification of proteins to reduce immunogenicity

Variation of the structure of the protein will inhibit immunogenicity of proteins by reorganisation by mending a sequential transformation or maximizing the development of a sequential therapeutic protein by various forms of polymers. Numerous systems were developed to deal with the issue of immunogenicity in which therapeutic proteins can be structurally changed without lessening their immunogenicity capacity. This can be accomplished by PEGylation, site-specific mutagenesis, doping, and morality. This includes shaming and humanization of monoclonal antibodies in all large societies. The most popular way of altering protein structures is the insertion of protein polyethylene glycol (PEG) molecules in a procedure called PEGylation into protein. During the loop, proteins in functions together

are joined at one end to the PEGs enabling them to react to the amines, the cystein and other protein acids it is assumed to enhance the stability of the structures and the physical stability and thereby extend the half-life *in vivo*. Proteins have a fixating immune coefficient, by using ingredients. Furthermore, PEGylation implicitly weakens the protein immunological evidence by reducing protein congestion, as well as shielding epitopes of immunogenic protein from the immune system.

Adding Novel functionality to macromolecules

Several common methods such as endogenous transport systems and mediated transport of peptides can be used to facilitate bio-availability for oral protein. Possible use of endogenous mobile cell transport and additional transportation molecules that are known into the GI field by endogenous cell transporters could help create a simpler and more convenient alternative of increasing intestinal ablution, the application of endogenous transmission systems of peptide and protein. These endocytosis mediated by receptors as well as by membrane vectors, such as vitamin B12, transferrin, haemoaggulitin of anaesthetic, toxic and lectin and are engineered to bind protein molecules in order to increase the intercellular deliveries to the target cells. Described as protein transduction domains (PTD), CPPs consists of protein residues of from 3 to 30 protein residues [60]. There has been a significant volume of tiny peptides, such as TAT and oligoarginine and penetratin. These have been used to install individual protein and peptide formulations into cells, since such particular ones have been found. It was understood that the permeation happened to occur by the cell membrane producing a certain sign of membrane, leading to greater absorption of oral methods of both proteins and peptides. The Peptide enables macromolecules, microparticles, Li and a nanoparticle to be introduced by the hybridization into cells or tissues with the target molecules to be made possible by this method. The peptide policy focuses on not-a particular deliveries and is tailored to the improving bioavailability and targeted purpose across the oral direction of oral proteins and peptides. In fact, these approaches have been used to achieve enhanced bioavailability by many companies and companies.

BARRIERS FOR THEIR ORAL DELIVERY OF THERAPEUTIC PROTEINS

While the oral route of administration has several benefits over invasive and other non-invasive delivery paths, until administered via the oral route, therapeutic proteins lose their bioavailability due to their ease of enzyme degradation, instability at differing pH through GIT, low solubility, physiological barriers in GIT and hepatic metabolism, and many other properties of absorption [18, 61]. There are still some enormous challenges facing oral delivery of therapeutic protein, the main barriers of which can be split into the biochemical barrier, mucus barrier, and intestinal epithelial barrier [62, 35].

THE BIOCHEMICAL BARRIER

The biochemical barrier for oral provision of protein is the first barrier whereby stomach and intestine have the most active and biochemical bioavailability barriers to the ingestion of orally ingested proteins in the gastronic acidic environment (pH 1,2-3,0) and the intestinal alkaline environment (pH 6,5-8,0) [62]. Various enzymes such as pepsin, trypsin, chymotrypsin and elastase are presented. Moreover, the brush border membrane contains sucrase and dozens of peptitdases, which have a high level of protein and peptide degradation. The pH gradient thus influences pharmacokinetics of orally administered drugs greatly, together with changing gastric emptying rates and gastrointestinal Motility. The successful oral supplementation of protein based drugs requires the disactivation and/or protection from enzymes and pH changes [18].

THE MUCUS BARRIER

Mucus is a hydrogellikeviscoelast that coat the gastrointestinal tract secreted by cellulose, acting as a semi-permeable dynamic barrier for exchanging nutritions, water, gasses, and hormones, etc. It also acts as a physical obstacle that restricts the transfer of medicines and other molecules from the lumen to the epithel [63]. Before epithelial surfaces can be contacted, nanoparticles containing drugs can be trapped and immobilized. Protein medicine must enter the mucus and the glycocalyx layer, then it will be successful in the systemic circulation, but both these layers have a limiting effect on protein drug diffusion [19, 64]. The interaction of protein and mucine fibers through electrostatic ion interactions, interactions between van der waals, the hydrophobic forces, and hydrogen links thus leads to structural changes and poor protein intake [65]. Thus, substances used in the provision of medicinal products need to be able to move easily through mucus or else to improve the residence time in the intestines through mucoadhesion, in order to overcome this barrier [49].

The epithelial barrier

Another physical barrier between intestinal lumen and systems circulation is the epithelial lining present below the mucus barriers consisting of the bi-layered phospholipid membrane and cholesterol. The macromollecules can be passive diffusion into the epithelial cell [32]. The piercing of the cell membrane of the lipid bilayer is difficult for the proteins as it is hydrophilic and has a high molecular mass. As a result, the

intestinal cell is a major barrier in the absorption of therapeutic proteins[66].In the lining of the small intestine, enterocytes are a popular cell group and they promote the flow of nutrients and water from intestinal lumen into the blood stream. Highly lipophilia molecules are passively diffused easily through the cellular barrier, while large molecules are actively internalized through enterocytes or M cells to the opposite membrane. However, the insertion of the substance through the cellular barrier allows a drug to penetrate the underlying vasculature directly [63, 67].

In addition to the barriers mentioned above, some physicochemical properties of proteins medicine also influence therapeutic protein permeability [34, 35]. First, molecular dimension controls the spread of medicines through the epithelial layer and known large molecules with less diffusivity. The molecular size, however, significantly decreases the permeability [68].In comparison, several distinct molecular-size conformations can be assumed by the proteins in solution. Therefore the conservation of pHA throughout the formulation and sterilization is essential, as any changes in the conformation can affect the permeability of the membrane. As permeational systems are known to be stereo-selective, the stereospecificity for protein medicines should also be retained. The distribution of charges and division coefficient of the peptide are also important for the prediction of peptide permeability through oral mucosa [68, 69]. The load density impact can be altered by changing the pH of the medium and thus by promoting the absorption of protein peptides ionization[62, 35]. Proteins usually have a complex solubility compared to their pH profile because of their amphoteric nature, their aqueous solubility depends on the pH, metal ion, ion strength and temperature. At the isoelectric point, even where a drug is neutral or no net charge, water solubility is minimal. Proteins are therefore very hydrophilic unless the N and C terminals are blocked. The lipophilicity of proteins should thus be increased by better absorption by passive diffusion [70]. In addition, protein self-aggregation tends to alter its intrinsic properties. Additives such as ionic surfactants can, however, stabilize protein formulation against self-aggregation[68].

SYSTEMS FOR DELIVERY OF PROTEINS

The applicability and effectiveness of therapeutic proteins depend on the conception of adequate supply systems [5]. Improper protein formulation will result in degradation, denaturation and/or aggregation resulting in immunogenic effects and loss of pharmacological activity [9]. The efficiency of therapeutic proteins can be increased through approaches such as emblazoning in microparticulate, microsphere, nanoparticulate, chemical modification using hydrophilic polymers and recombinant protein engineering [69, 5].

MICROPARTICLES AND MICROSPHERES

Microparticles are tiny, rigid particles or small liquid droplets bound to a natural/synthetic polymer and scaled by up to 0.1–200 mm in diameter [71]. Microparticles are very useful ways of carrying proteins which, due to reduced water solubility, are otherwise difficult to supply. The delivery mechanism for microparticulate offers long duration controlled release of drugs. A variety of methods such as double-emulsion, single emulsion, phase separation (coacervation), ultrasonic atomisation, spray-drying, and microfluidics will lead to encapsulation of proteins into polymer microparticles[55]. Micro-particular scale, polymer molecular mass, degradation intensity, polymer charging, hydrophilicity-hydrophobicity ratio, micro-particle size polydissipation, protein loading quantity as well as microenvironment surrounding effects emanate kinestics of encapsulated microparticles. Currently, many microparticle formulations are on the market to distribute protein and various microparticles for preclinical application for therapeutic proteins such as morphogenetic bone protein-2, insulin, recombinant human epidermal development, and recombinant human erythropoietin (EPO), etc. are being created. Proteins are clearly an important group of therapeutic agents, but most of them require many therapies for limited half life in vivo [5].They are also extremely unstable in biological fluids and due to their high molecular weight are not completely absorbed. Microsophere is useful as a long-term dosage method to resolve these disadvantages associated with protein medications [72]. Spherical particles scaling from 1 to 50 microns are separate microspheres. The membrane emulsification using calcium ion and polymer solidification has recently been used to develop an alginate-chitosan microsphere, in which influence of particle dimensions, surface morphology and the microspheric zeta potential were investigated. Since then, they were used profitably as vectors for insulin peptide orally, where packing efficiency was 56,7%, whereas immunological activity was notable 99,4%. Only 32 percent were released in the stomach and intestine during the transit time and lasted longer in the blood (14 days). In addition, during absorption the structure remained unchanged. Therefore, diabetic rats have reduced their blood glucose level strongly and remained firm for about 60 hours [73].

Nanoparticles

Nanotechnologies established as an incredible promise for medicines have emerged as forward-thinking drug delivery systems and are used to target treatment proteins to selective organs/tissues, particularly nanoparticles such as liposomes nanoparticles, polymer nanoparticles, inorganic nanomaterials and

nanoparticles containing cell-penetrating peptides, etc [11]. The ingestion of particles inside the intestine increases the efficiency and hydrophobicity of particles that have been administered orally as part of the nanoparticles [74]. In addition, in the various parts of the intestine, the extent and pathway of nanoparticles is diverse [75]. The above-mentioned technology offers several advantages, for example; protecting proteins against premature degradation or dematuration in the biological environment and improving the systemic circulation half-life of proteins with poor pharmacokinetic characteristics. Protein nanoparticles also control continued or tunable release to diseased tissues, cells and intracells, thus enhancing drug safety and efficacy, as well as helping to maintain therapeutic concentrations in the field of medicines [35, 75].

Lipid-based delivery systems

The lipid formulation is another area with a tremendous importance. Liposomes contain bi-layered phospholipides that help to shield proteins or peptide in its lipid center from oxidation and deamidation in the summarized drug/peptide[76]. In addition, liposomes are used to use the lymph system to quickly unload medicines mediated into the flow and thus to bypass metabolism of the first passage [77]. Many different methods have been used for lipid based protein delivery; for example pharmacological effectiveness of calcitonin-loaded wheat germ agglutinin–carbopol modified liposomes upon oral administration was raised by 20-folds and 3-folds when correspond to non-modified and carbopol-modified liposomes. Consequently, both in vitro and in vivo analyses its use as a systematic oral peptide delivery system [71, 78]. Further nanocarbons (SLNs) from solid lipids were developed as well as modified agglutinin (WGA–SLNs). These particles were packed with insulin, and their effectiveness as oral carriers[69]was appreciated. A lot of authors have explained that SLNs as well as SLNs modified by WGA tend to protect insulin with higher efficiency from digestive enzymes degradation in vitro [39]. Liposomes are one of the very important lipid-based formulations, as they provide almost any medicine that is irrespective of their solubility, though their stability cannot be controlled. Kim *et al*. have developed nanoparticles of the lipid micelle-type to actively target lung cancer and deliver therapeutic proteins into tumor cell cytosol. They made lipid nanoparticles using 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), DOPE and apolipoprotein (APOPA-I) and included DSPE-PEG anisamide, which is more specifically expressed in H460 Lung carcinoma to facilitate accurate targeting via signa receptors [79]. In order to increase cytochrome C (cytC) permeability, peptide membrane-permeable (MPS) sequence was conjugated in the cytC covalent and, following high lipophilic MPS-cytC, loaded into the nanoparticles' lipid levels resulting in a significant increase in cellular apoptosis compared with nanoparticles without MPS or MPS-cytC alone[62]. The platformen for hybrid encapsulation of therapeutic agents, designed with biodegradables core-shell liposome polymer gels (nanolipodel; nLG) composed of TGF-β inhibitors (SB505124, SB) complex cyclo-dextrins(CDs) and Ile-2 are subsequently manufactured in Park and co-workers as immunotherapeutic nano-platform for cancer treatment, which integrated advantageous functionality. Wange *et al*. recently published a combinatorial architecture and reversible chemical protein engineering of cationic lipid-like products (termed lipidoids). The Combinatorial Library Strategy was pioneered by Anderson and colleagues for the production of siRNA lipidoids, and the authors of this study expand the class of protein-based materials [39, 32].

Polymeric Nanoparticles

Polymer nanoparticles, such as poly- £-caprolactone, polyacrylamide, and polyacrylate and natural polymers, e.g., albumins, DNAs, or chitosan gelatines are structures of between 10 to 100 NM synthesized from synthetic polymers [12]. A new dimension of biodegradable polymer nanoparticles has been used to increase the oral bioavailability of proteins and peptides and to boost the firmness, release control and therapeutic parameters [80, 75]. In addition, the surface of these polymers can be smoothly modified to avoid opposition. The protein to be transferred can be dissolved, encapsulated or encapsulated to the target region [81, 11]. For decades they have been explored as a promising system for drug distribution, showing improved stability and expanded protection against the degrading effects of the GI climate [11, 82]. In addition, the differing molecular weight and structure of the repeating device and residue on the polymer backbone can influence their molecular characteristics. One of the better ideas as these drugs can be released by desorption, diffusion or nanoarctic degradation into the target tissue is using biodegradable polymer nanosystems to produce nanomedicines where drugs can be immobilized on the PNP surface after a polymerization reaction [83]. The limited toxicity associated with the use of PLGA in the medication supply was replicated by Kumari et al., which is biocompatible with tissue and cells. In addition to the pharmaceutical biodegradable, the nanocarriere conjugates examined for the delivery of protein are stable in blood, non-toxic and NT [84]. They are also non-immune and non-pro inflammatory, and neutrophils are not activated or reticular-endothelial. Its nanometer measurements also stimulate efficient permeation of cell membranes and blood stream stabilization. Polymers are also very convenient sources for the development of several and various molecular structures and can be assembled into separate nanoparticles for several possible medical applications [11, 85, 86]. However, Yao et al demonstrated that the key disadvantage of nanoparticular carrier systems is generally associated with low loading and thermodynamic stability agglomeration [49, 87].

Inorganic nanoparticles

Inorganic nanoparticles with different special inherent physiochemically-induced properties {gold NPs, selenium NPs (SE NP's), silica NPs (Si NPs), alumina, TiO2, zirconium phosphate (ZrP)} have also shown increasing promise for use in medicinal use and have been successfully applied in orally providing therapeutic peptides / proteins, treatment for hyperthermia, disease diagnostics and the sensory system. These materials are born with noticeable acid and enzyme GI stabilities[11]. Many researchers have disclosed the impact on efficient distribution of chondroitin sulphate capped au NPs/Ins; Deng et al. have made the inorganic nanoparticles within Se NPs; Zham and Al. have prepared HAP (Hydroxyapatite) NPs in the form of PEG, along with ins and gallic acid (GA) [9, 75]. In all cases there were effective hypoglycaemic outcomes compared with those without Np. In all cases. However, injected Si NPs covered with HP55 demonstrated an enormous hypoglycemic effect which remained between 40% and 70% in 2–7 hours. So Si NPs with a high specific surface area and high porosity offer excellent biocompatibility and biodegradability [22]. Further Han and colleagues have been using mesoporous silicon nanoparticles, believed to dissolve tau aggregates, a pathological characteristic of Alzheimer's disease, to charge and supply therapeutic proteasomes. In contrast to the free proteasomes, the Ptsm-MSNPN greatly decreased over-expressed tau in cells [88]. Gold nanoparticles are also very significant biomedical uses because their surface can easily be adjusted with thiol group molecules and is biocompatible with special spectroscopic properties [12]. The broad areas on which the nanoparticles are tuned and the golden functionalities make it a magnificent protein scaffold because protein conformation is conserved by customizing nanoparticles to form a monolayer [76]. Tang *et al.* designed gold nanoparticle-stabilized supramolecular nanocapsules (NPSC) to deliver therapeutic caspase-3 proteins which caused ~72% of HeLa cells to undergo apoptosis; one more example of using gold nanoparticles as a carrier of vascular endothelial growth factor (VEGF) for the treatment of ischemic lesions was reported by Kim et al. and results were further established in a hind-limb ischemia mouse model subsequent to the intravenous supervision of nanoparticles wherein VEGF-conjugated gold nanoparticles led to a recovery of blood perfusion over time to 93% of normal tissue, as well as increased blood vessel density, quantified by immunohistochemical staining with CD31 [89, 88]. Jiang *et al.* have reported a graph-based nanostructure as a cancer-specific protease-mediated co-dispensing system that integrates the membrane associated cytokines and chemotherapy agents for the treatment of amalgamation [75, 90].

Nanoparticles Containing Cell-Penetrating Peptides (CPP NP's)

CPP are small peptides consisting of positively charged fragments of amino acids which are extremely capable of membrane penetration and help in the intracellular supply of macromolecules or nanoparticles in cells[81]. CPPs are known to provide multiple nanoparticles combined with various protein medicines throughout the intestinal mucoa, thereby increasing the absorption and bioavailability of medication by chemically hybridizing them with the target matter. HIV 1 Tat, penetratin and oligoarginine are among these[91]. This will thus be one of the promising strategies for successful non-invasive protein absorption[71, 92, 61, 18]. However, there is still no clear mechanism for CPP's protein recovery and there are no competent in vivo studies that could show the therapeutic potentials of their use [93].

Chemical modification with hydrophilic polymers

One of the main problems associated with small proteins and peptide therapeutics is the clearance by glomerular filtration from the systemic circulation which may be overcome with chemical modification of proteins and their molecular weight and/or hydrodynamic radius with hydrophilic polymers [5]. PEGylation or hyperglycosylation can be performed, where polyethylene glycol (PEG) chains and sugar molecules are joined to proteins respectively [94]. The addition of sugar moieties is known to increase the protein stability and pharmacokinetic properties, prolong biological half-life and improve stability by increasing protein solubility and immunogenicity [81, 95]. Although it presents some benefits, it requires complex synthesis and causes adverse changes in conformity leading to loss of biological activity and the association of the goal.

Recombinant protein engineering

Protein applications to enhance the use of therapeutic proteins are an important medical area[30]. The development of protein engineering resulted in the second and third generations of therapeutic protein products by mutation, fusion deletion and application characteristics [96, 97]. DNA rejuvenation and recidivist replica could also improve therapeutic proteins [98]. "Antibody engineering" is possible because of advances in recombinant DNA technology. Antibodies are also used in protein engineering applications. Fc-based fusion proteins with a genetically-bonded Fc immunoglobin domain are a promising approach since Fc-fusion provides protein distinguished effectors with the properties of enhancing the protein half life and therapeutic efficacy through FC receptor binding and complementary fixation [96, 99]. Another example of the generic fusion technologies capable of expanding plasma half-life is a recombinant fusion build, consisting of an unstructured polypeptide and a protein drug. Exenatide fusion has been developed by Schellenberger *et al*. and showed ~58 times higher animals half-life and lower immunogenicity [100, 79].

CONCLUSION

Oral therapeutic proteins have brought a remarkable change in the field of biomedicine and they have been a promising area of research. The strategy for development of oral therapeutic proteins has been a daunting task for researchers due to their complex structure, varying and high molecular weight, impermeability through biological membrane, and susceptibility to chemical and enzymatic degradation. Although growing field of biotechnology has allowed cost-effective and pilot-scale production of proteins, there are only few protein formulations in the market. But ongoing research provides new insights into an array of novel techniques that provide successful administration of oral therapeutic proteins without any structural alteration of the protein. As highlighted in the review, nanotechnology offers a great deal of carriers to encapsulate the protein-based drugs. Muco-adhesion based nanoparticles and liposome-mediated nanoparticles play a significant role in the enhancement of absorption by facilitating stronger attachment of the molecule to the mucosal epithelial layers of the gastrointestinal tract. Despite improvisation of the drug permeation by these techniques, they offer only a minimal protection from proteases and acidic pH environment. But polymeric nanoparticles and liposomes have overcome this barrier and offer stable delivery of the proteins even in low pH and protease rich environment. CPPs increase the absorption of the drug across the intestinal mucosal wall, hence enhancing the bioavailability. On the other hand, chitosan-based nanoparticles possess good mucoadhesive properties and allow the controlled release of low molecular weight proteins. Microparticles allow prolong and steady release of proteins and microspheres aid in the maintenance of the protein activity and its sustained release. In the end, one must note that due to the progress achieved in the field of oral therapeutic proteins in recent years, few of the therapeutic proteins have already entered the market and most of the others are in the line to clear the clinical trials. However, in near future, issues related to protein quality, site specific delivery, immune response, and toxicity required to be addressed. Emphasis on delivery systems currently being employed depicts the difficulty in limiting the number of delivery systems. Therefore, introduction of new systems or the modification of the current ones to incorporate all the above mentioned aspects is required.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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