POLYMERIC NANOPARTICLES FOR ORAL DELIVERY OF BIOPHARMACEUTICALS: AN OVERVIEW
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Abstract
Biopharmaceuticals, cutting-edge medications sourced from living organisms, embody the zenith of therapeutic progress driven by biotechnological breakthroughs. While oral drug delivery is convenient, it proves challenging for biopharmaceuticals due to the complex barriers in the gastrointestinal tract. Their delicate structure and susceptibility to degradation in the gut pose formidable obstacles. This scientific conundrum necessitates innovative solutions to ensure their effectiveness. Pseudomonas aeruginosa's Exotoxin A demonstrates the difficulty in traversing the intestinal epithelium, necessitating innovative strategies. Researchers utilize mucoadhesive, biodegradable polymers like alginate and chitosan to create nanoparticles. These nanoparticles form a protective gel in the stomach's acidic environment, enhancing drug stability and absorption. Chitosan and alginate collaborate in nanoparticle formulations, improving mucosal adhesion and prolonging drug retention. Introducing non-toxic Exotoxin A enhances trans-epithelial transport, validated by in vitro studies on Caco-2 cell monolayers and accumulation in the rat small intestine's lamina propria. Utilizing green fluorescent protein as a model within alginate-chitosan nanoparticles showcases their potential for oral drug delivery. Bacterial toxins play a crucial role in enhancing trans-epithelial transport, endorsing these nanoparticles. This fusion of biotechnology and polymer science offers a promising solution for biopharmaceutical oral delivery challenges, highlighting alginate-chitosan nanoparticles as versatile carriers for transformative drug delivery advancements.

Introduction
The dynamic field of biopharmaceuticals burgeons alongside advancing biotechnology, furnishing a scientific foundation for the meticulous design and performance evaluation of drug products. While injections remain a staple, their invasiveness and low patient compliance spur exploration into alternative delivery routes. Crucially, each manufacturing step influences drug release and availability at the site of action. Oral dosage forms hold promise, yet face hurdles such as low permeability across the gastrointestinal epithelium and susceptibility to gastric acidity and enzymes. To surmount these challenges and devise successful oral delivery strategies, innovative approaches are imperative. This chapter delves into the intricacies of the gastrointestinal tract anatomy and underscores the pivotal considerations for administering biopharmaceuticals. A particular focus lies on ligand-mediated nanoparticle uptake, with nPE [Non-toxic Pseudomonas exotoxin A] serving as a key ligand. Furthermore, the chapter expounds upon the essential physicochemical properties of nanocarriers, underscoring their significance in navigating the complexities of drug delivery.

Advantages and challenges of oral biopharmaceutical delivery
Biopharmaceuticals, comprising medicinal products derived from biological sources and manufactured via biotechnology, represent a significant milestone in modern medicine. The pivotal breakthroughs of 1970 propelled the advancement and commercialization of these groundbreaking therapies. One key innovation was the development of hybridoma technology, enabling the mass production of identical antibodies. This revolutionized the field by offering a reliable method for generating large quantities of specific antibodies crucial for targeted therapies and diagnostics. Additionally, genetic engineering emerged as a game-changer, facilitating the scalable production of proteins. This breakthrough allowed for the efficient manufacturing of complex therapeutic proteins, paving the way for the production of biopharmaceuticals on an industrial scale. Together, these landmark discoveries laid the foundation for the flourishing field of biopharmaceuticals, offering new avenues for treating diseases and improving patient outcomes. [1]. Since the approval of the first recombinant protein drug, human insulin, by the US Food and Drug Administration in 1982, the prevalence of biopharmaceuticals in the market has steadily risen [2].

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By 2016, approximately 650 protein drugs had received approval worldwide, with over 1300 recombinant pharmaceuticals in various stages of development. Biopharmaceuticals have demonstrated efficacy in treating a wide array of conditions, including cancer, autoimmune diseases, genetic disorders, and metabolic ailments [3,4]. With their increasing adoption, the global market for biopharmaceuticals is projected to reach a staggering $386.7 billion by the conclusion of 2019 [5].

Therapeutic proteins present distinct advantages over conventional small drug molecules. Firstly, their unique structure and function afford highly specific pharmacological effects, minimizing interference with biological processes [6]. Additionally, since many therapeutic proteins are naturally produced by the body, they are generally well-tolerated and exhibit lower immunogenicity. Furthermore, the specific structures and engineering efforts involved in protein therapeutics offer pharmaceutical companies extensive patent protection, thereby providing crucial financial incentives and benefits for their development and commercialization [7].

Despite over 35 years of clinical use, recombinant proteins are predominantly administered via parenteral routes such as intravenous, intramuscular, or subcutaneous injection. However, frequent injections can lead to adverse reactions at the administration site and may decrease patient compliance [8]. Moreover, the risk of infection from needle misuse or reuse and the associated social stigma further limit their widespread acceptance [9]. Oral delivery of biopharmaceuticals presents an attractive alternative to parenteral administration, yet it is hindered by the physicochemical properties of these molecules and barriers within the gastrointestinal tract.

The oral route of drug delivery offers numerous advantages over other non-invasive methods such as pulmonary, intranasal, and transdermal routes. It is not only the most convenient for patients but also boasts high compliance rates [10]. Additionally, oral formulations eliminate the need for direct healthcare professional involvement, making them more cost-effective compared to parenteral administration. Moreover, oral dosage forms require aseptic conditions but not the same level of sterility as parenteral formulations, resulting in cost savings during manufacturing [11].

Orally delivered insulin holds particular promise due to its ability to target the liver directly via hepatic portal circulation, mimicking physiological insulin release from pancreatic beta cells. In contrast, subcutaneous insulin injection delivers the dose to the systemic circulation first, with only a fraction reaching the liver. By replicating this physiological route, oral insulin is expected to offer additional clinical benefits, including reduced risk of hypoglycemia, less weight gain, and decreased hyperinsulinemia associated with systemic insulin therapy [12].

**Acidic and enzymatic environment in the GI tract**

The acidic environment (pH ~1-2) in the stomach alters the ionisation state of amino acids, which acts to unfold proteins through the disruption of interactions involved in secondary or tertiary structures [13]. Additionally, such acidity can also break some peptide bonds [14]. Generally, four categories of digestive enzymes are present in the GI tract: lipases, proteases, amines, and nucleases. These enzymes are responsible for the breaking down of fats, peptides/proteins, carbohydrates and nucleic acids respectively. Exposure of therapeutic proteins to proteases, such as trypsin and chymotrypsin released from the pancreas into the duodenum, may lead to the hydrolysis of peptide bonds or the chemical modification of the functional groups, for example, oxidation and de-phosphorylation. All these biochemical reactions will denature or degrade proteins, which may change their pharmacological effects [15].

**The Mucus layer**

Mucus lines the moist surfaces of the body like lungs, sinuses, mouth, stomach and intestine. It is a viscous material that is adherent to the surface of the intestinal epithelium. It is composed of secretions released from specialised goblet cells. Mucus is a complex hydrogel composed of proteins, carbohydrates, lipids, salts, antibodies, bacteria and cellular debris; but the main component of mucus is the glycoprotein mucin that is viscous and has elastic gel like properties [16]. Mucus is responsible for lubricating the GI tract to assist the passage of substances as well as providing a barrier to protect the epithelium from pathogens and noxious substances [17]. At the apical surface is the glyocalyx; membrane bound mucins (MUC) entangle and cross link with other molecules, such as glycolipids and glycoproteins, to form an adherent ~500 nm thick coating layer between the cells and loosely held mucus [18]. Specifically, MUC 3 is expressed in the apical membrane of enterocytes and goblet cells as part of this process to produce extended rod like structures, that form a component [200 – 1500 nm] of the glyocalyx [19]. Frey et al. [1996] showed the glyocalyx is a size selective barrier with a functional pore size of 7.4 – 28.8 nm [20]. There is constant turnover of the adherent layer and the glyocalyx to remove the potentially damaging compounds on the lumen surface [21].

**Low permeability across the epithelium**

The low permeability of therapeutic proteins has direct impact on their bioavailability and thus limits their pharmacological effects [22]. A greater challenge than these two hurdles of harsh environment and mucus layer is the low permeability of crossing the intestinal epithelium. The size of most therapeutic proteins ranges from ~3.5 kDa [calcitonin] to ~150 kDa [monoclonal antibodies], all being too large and too hydrophilic to readily diffuse across enterocytes driven by the concentration gradient in a manner similar to many small molecule drugs; this intracellular pathway is size dependent (> 700 Da) [23, 24] and a minimum level of lipophilicity is needed for molecules to partition into the cell membrane, and to be transported across the cell [25]. Molecules with a lower degree of lipophilicity can travel across the epithelium via the paracellular pathway, meaning via the intercellular spaces between two adjacent cells. Unfortunately, this pathway is restricted to relatively small compounds (< 3.5 kDa), which is not applicable for most therapeutic proteins [26]. Even when...
the biopharmaceutical is extensively protected from the acidic environment in the GI tract, less than 1% of therapeutic protein can reach the systemic circulation.

**Overcoming these barriers**

The anatomy of the GI tract and challenges of transporting therapeutic proteins via the oral route, various experimental techniques have been made to improve the bioavailability of therapeutic proteins over the years.

**Absorption enhancers**

Absorption enhancers are substances that can promote the absorption of a poorly absorbed drug across the intestinal epithelium. Various mechanisms of absorption enhancers have been proposed: 1) disrupting the structural integrity of the cell membrane temporarily to make it more permeable for drug transport [27]; 2) increasing the retention time on the mucus layer; 3) opening tight junctions to utilise the paracellular pathway [28].

Some absorption enhancers result in enhanced permeability not only by one mechanism. For example, the cationic polymer chitosan has the combined effects of mucoadhesion and reversibly opening tight junction structures; and Carbopol polycarboxylate derivatives can inhibit enzymatic activities as well as opening tight junctions through the removal of extracellular calcium ions [29,30]. Recently, bile acid/salt derivatives have attracted considerable interest as absorption enhancers. Mournir et al., [2002] demonstrated that co-administration of insulin with bile acid/salt derivatives [such as deoxycholate and cholate] led to a hypoglycaemic effects in rabbits. Perhaps, bile salts were masking the hydrophilic protein surface via micelle formation [43,44]. It has been suggested that the apical sodium dependent bile salt transporter in the ileum can be targeted for enhancing oral absorption of macromolecules physically complexed with bile acid/salt derivatives [32]. It is significant to observe that this mechanism is used for the absorption of bile acid from the proximal and distal ileum back to the liver.

Since absorption enhancers have the potential to reduce the barrier function of the intestinal epithelium, toxicologic effects clearly need to be evaluated. Taking into consideration the physiologic environment in the small intestine, it appears difficult to control and maintain the enhancer concentration sufficiently well use to use this strategy; these enhancers would be dispersed and diluted in varied volumes of intestinal fluid [33].

**Enzyme inhibitors**

Co-administration of protein drugs with protease inhibitors, such as aprotinin an inhibitor of trypsin and chymotrypsin, soybean trypsin inhibitor, and FK-448 [a chymotrypsin inhibitor] has shown increased permeability of proteins [34]. The choice of protease inhibitor depends on the structure of delivered protein and its specificity towards the enzyme. For example, Yamamoto et al. [1994] showed significant hypoglycaemic effect following large intestinal co-administration of insulin with Na-glycocholate, camostat mesilate and bacitracin, while marginally enhanced insulin absorption was followed following co-administration with soybean trypsin inhibitor and to a moderate degree of aprotinin [35]. In addition, the cocktail of enzymes present in different segments of GI tract is different, which would make the choice of inhibitors more difficult. Furthermore, due to the potential denaturation of enzyme inhibitor itself in the gut, excessive amount of inhibitor is required. If a long term treatment is considered, normal protein digestion might be affected [36].

The enzyme inhibitor act as a promising candidate such as mucoadhesive polymers. Hutton et al. [1990] firstly reported the inhibitor properties of polycrylicate on intestinal proteases [37]. Lueßen et al. [1995] proposed that the inhibitor effect of polycryliclate derivatives is due to the formation of complexation with divalent cation ions (calcium ions), so that the ion dependent enzymatic effects are prevented [50]. Although these polymers are generally regarded as safe, protective effects on the polymer embedded proteins might not be sufficient [34].

**Chemical modification of proteins**

Some absorption enhancers described above can also be conjugated to proteins, for example, DOCA [deoxycholic acid] was conjugated with heparin to target ASBT [39]. Attempts have been made to chemically conjugate proteins with functional groups in order to improve their lipophilicity, and thereby to enhance their uptake. Hashizume et al. [1992] demonstrated that conjugation of insulin with fatty acids [palmitic acid] not only increased the lipophilicity of insulin but also reduced its degradation [40]. The oral insulin product hexyl insulin monoconjugate -2 [HIM2] has demonstrated efficacy in both type I and type II diabetics, HIM2 contains recombinant human insulin covalently modified with a single amphiphilic oligomer [41,42]. However, this strategy is not reported in market till date.

Ideally, functional groups [conjugation groups] would detach or cleave from the delivered protein before its arrival in the systemic circulation, so that the structure and efficacy of protein would remain the same. Otherwise the conjugated protein would be considered to be new drug, requiring through safety and toxicology data for a regulatory filing. Thus, the conjugate effects on pharmacokinetics and other pharmacological activities of such delivered proteins must be considered carefully [43].

**Novel carrier systems**

Numerous carriers have been exploited for oral protein delivery, such as microspheres, liposomes, emulsions and nanoparticles [NPs]. All of these systems have the potential to increase the bioavailability of a protein loaded into them, but each also has unique challenges to overcome before being developed into an efficient delivery system. For example, the physicochemical stability of emulsions is one critical drawback for this strategy [11].

**Biodegradable polymeric nanoparticles**

Biodegradable polymers are materials that can break down naturally either by inherent instability or through the action of enzymes [43]. The first record of biodegradable polymer in medicine is the catgut suture [made from sheep intestine], dating back to at least 100 AD [44]. Although the first industrialization of a synthetic polymer was started in the 1910s by Baekeland, the concept of synthetic biodegradable polymer was not introduced until the 1980s [45]. Since then, biodegradable polymers have innumerable uses in the biomedical field, particularly in tissue engineering and drug delivery [46].
Various biodegradable polymers have been emerging for the preparation of NPs, such as PLA [polyactic acid], PLG [polyglycolic acid], PLGA [poly [lactic co-glycolic acid]], PEG [polyethylene glycol] and PCL [polyacrylactone]. Four methods are commonly used for the preparation of NPs from these polymers [47] they are Solvent evaporation [46], Nanoprecipitation [47], Salting out [48] and Emulsion diffusion [49].

Advantages of nanoparticles

The formulation strategy discussed herein involves utilizing nano-carriers, a concept pioneered by Peter Speiser in the 1960s [50]. Mohanraj and Chen [2006] highlighted several advantages of nano-carriers as drug delivery systems across various administrations, including oral, nasal, and parenteral routes. Firstly, nano-carriers offer high drug loading capacity without the need for chemical reactions, preserving the bioactivity of fragile drugs. Secondly, they enable site-specific targeting, enhancing therapeutic effects while minimizing unintended side effects. Thirdly, the release properties of encapsulated drugs can be controlled, enhancing drug delivery precision. Additionally, nano-carriers’ size, charge, and other characteristics can be manipulated to achieve active or passive drug targeting after administration [51].

Specifically concerning the oral route, nano-carriers offer a high surface area per mass, facilitating increased contact with epithelial cells and thereby enhancing uptake. Studies have demonstrated the heightened bioavailability of nano-carrier-delivered drugs [52]. Moreover, nano-carrier architecture provides protection for loaded proteins against enzymatic or acidic environments, ensuring drug stability during transit through the gastrointestinal tract [53].

Importance of specific nanoparticle characteristics

Size

Particle size and size distribution are critical characteristics influencing the pharmacokinetics, biodistribution, toxicity, and targeting capabilities of nanoparticles [NPs]. Moreover, size directly affects drug loading, release, and NP stability. The size of NPs significantly influences cellular uptake and transcytosis, with the impact varying depending on the cell type [54]. Desi et al. [1997] demonstrated that NP uptake by polarized Caco-2 monolayers is dependent on size, while Win et al. [2005] identified the optimal size range for NPs as 100-200 nm. These findings underscore the importance of carefully controlling NP size to optimize their biological behavior and therapeutic efficacy [54, 55].

Surface properties

The surface properties of nanoparticles [NPs] profoundly influence their interactions with the surrounding environment, ultimately dictating their fate in vivo. NP hydrophobicity determines the degree of adsorption of blood components, particularly proteins, which can lead to opsonization and subsequent phagocytosis by immune cells [51]. Coating NP surfaces with hydrophilic polymers or surfactants, such as polyethylene glycol, polysorbate 80, or polyoxamer, mitigates protein adsorption, enhancing NP circulation time in the bloodstream [56].

Furthermore, NP surface charge plays a crucial role in their behavior and fate within specific bodily sites or tissues. The uptake behavior of NPs is also influenced by the type of cells encountered. Based on current knowledge, an optimal NP size for intestinal transcytosis falls within the range of 100-200 nm in diameter. However, uncertainties remain regarding the impact of surface charge, underscoring the importance of employing NPs with modifiable surface charge characteristics to tailor their interactions with biological systems.

Nanoparticles targeted to the stomach

Formulations targeting the stomach often employ mucoadhesive materials to enhance gastric retention, with chitosan being a predominant choice. This naturally occurring polysaccharide contains primary amines that readily interact with mucin glycoproteins, thus serving as an effective mucoadhesive in delivery systems. However, chitosan’s rapid dissolution and ionization in the acidic stomach pH pose challenges. To address this, chitosan is complexed with polyanions to form nanoparticles resistant to rapid dissolution in acidic conditions [57].

Chang et al. developed amoxicillin-loaded nanoparticles comprising chitosan and poly-glutamic acid complexes, demonstrating reduced dissolution at acidic pH. In a study assessing therapeutic potential, fluorescently labeled nanoparticles were incubated with gastric adenocarcinoma [AGS] cell monolayers infected with H. pylori. Confocal microscopy revealed nanoparticle interaction with AGS cells at the site of H. pylori infection, showcasing their potential in targeted drug delivery for gastric ailments [58].

Lin and colleagues devised polyelectrolyte nanoparticles utilizing fucose-substituted chitosan and heparin for targeted treatment against H. pylori infection. Fucose was strategically incorporated to target fucose receptors on H. pylori. These nanoparticles were loaded with amoxicillin, with in vitro release studies revealing 40% release of encapsulated amoxicillin within the first 2 hours at pH 1.2. To mitigate rapid release, genipin-induced crosslinking of chitosan-heparin nanoparticles was employed, resulting in slower amoxicillin release in vitro [59].

Electron microscopy studies showcased the attachment of amoxicillin-loaded, genipin-crosslinked heparin-chitosan nanoparticles to H. pylori, leading to distortion of bacterial shape. Subsequent in vitro antimicrobial studies demonstrated superior efficacy of these nanoparticles against H. pylori compared to non-fucosylated counterparts and amoxicillin solution. In vivo experiments in mice revealed nearly 4-fold enhanced efficacy of orally administered amoxicillin-loaded, genipin-crosslinkedfucose-chitosan-heparin nanoparticles in clearing H. pylori infection compared to amoxicillin solution [59].

Further investigations by Lin et al. underscored the potential of berberine-loaded genipin-crosslinkedfucose-chitosan-heparin nanoparticles in H. pylori infection treatment, yielding similar promising results. The visualization of the in vivo gastric retention duration of fucosylated chitosan-heparin complex nanoparticles and chitosan-heparin complex nanoparticles presents an intriguing avenue for future exploration [60].

In a recent study, Lin and colleagues introduced stomach-targeted nanoparticles aiming to enhance oral treatment efficacy against gastric cancer. Given that various cancers, including gastric cancer, exhibit carbohydrate antigens and heightened fusosyl transferase expression, the researchers devised nanoparticles comprising fucosylated chitosan, PEGylated chitosan, gelatin, and epigallocatechin gallate.
in curcumin bioavailability and maximum plasma concentration compared to curcumin solution. Remarkably, both nanoparticles and curcumin solution showed similar Cmax values within 100 minutes, suggesting the stomach as the primary site for systemic absorption [63].

In vivo biodistribution studies further revealed a four-fold increase in curcumin levels in the stomach of mice treated with ethyl cellulose-curcumin nanoparticles compared to curcumin solution in 2 hours post-administration. To validate enhanced gastric retention of ethyl cellulose nanoparticles, Suwannateep et al. excised the stomachs of treated mice after 2 hours and subjected them to scanning electron microscopy imaging. These findings highlight the potential of ethyl cellulose nanoparticles for improving gastric retention and systemic absorption of curcumin, offering promising implications for enhanced oral drug delivery [63].

In a follow-up investigation, the aforementioned research team assessed the efficacy of clarithromycin-loaded ethyl cellulose nanoparticles for treating H. pylori infection. As anticipated, these nanoparticles demonstrated superior efficacy in clearing H. pylori infection in mice compared to clarithromycin suspension [64]. Furthermore, similar positive outcomes were observed when evaluating Garcinia mangostana extract loaded into ethyl cellulose nanoparticles. These findings underscore the potential of ethyl cellulose nanoparticles as a versatile platform for enhancing the therapeutic efficacy of various drugs and natural extracts, presenting promising implications for the treatment of H. pylori infection and other ailments [65].

In a recent breakthrough, researchers synthesized polyethylene oxide acrylate [PEO-Acr] substituted ethyl cellulose to craft nanoparticles with potential mucus microstructure-modulating properties. By utilizing acrylate-terminated ethyl cellulose, the nanoparticles were designed to engage in thiol-ene click reactions with mucin-derived thiols, a process facilitated by biocompatible reducing agents such as tris[2-carboxyethyl] phosphine hydrochloride [TCEP] or vitamin C [66].

Ex vivo experiments conducted on porcine stomachs demonstrated that fluorescent PEO-Acr-terminated ethyl cellulose nanoparticles exhibited enhanced binding to the mucosal surface in the presence of TCEP compared to their non-modified counterparts. Moreover, in vivo assessments in H. pylori-infected mice revealed intriguing results. When co-administered with vitamin C, clarithromycin-loaded PEO-Acr-modified ethyl cellulose nanoparticles showcased superior efficacy in eradicating H. pylori compared to both non-modified clarithromycin-ethyl cellulose nanoparticles and clarithromycin suspension [66].

This innovative approach not only highlights the potential of PEO-Acr-modified ethyl cellulose nanoparticles in targeting mucosal surfaces but also underscores the synergistic benefits of combining nanoparticles with vitamin C for enhanced therapeutic outcomes in H. pylori infection treatment [66].

As outlined, the focus of many studies utilizing mucosalhesive nanoparticles has centered on addressing H. pylori infections. A recent investigation by Navabi and colleagues unveiled that both acute and chronic H. pylori infection led to a slowdown in mucin production and turnover within the murine gastric mucosa. While the normal mucus turnover rate in mice typically occurred within approximately 6 hours, this process...
extended to over 12 hours in H. pylori-infected mice. Consequently, Navabi et al. suggested that nanoparticles adhering to gastric mucus might not remain retained for more than 6 hours in healthy mice or 12 hours in H. pylori-infected mice [67].

In human subjects, H. pylori infection has been associated with heightened mucosal permeability and disruption of the mucosal barrier. Notably, clinical trials investigating antibiotic-loaded mucoadhesive hydrogels have failed to demonstrate any superiority in efficacy compared to conventional treatment regimens [67]. These findings underscore the complex interplay between H. pylori infection, mucin dynamics, and nanoparticle retention, highlighting the need for further research to optimize nanoparticle-based approaches for the treatment of H. pylori infections and related gastric disorders [67].

Currently, research in the field has not explored the integration of muco-inert nanoparticle formulations for targeting the stomach. The effectiveness of mucoadhesive materials and their nanoparticle formulations in stomach targeting remains unconfirmed. There is a notable gap in our understanding regarding the utility of muco-adhesive nanoparticles for this purpose. Consequently, further investigations are warranted to explore the potential of muco-adhesive nanoparticles and elucidate their role in targeted drug delivery to the stomach.

Conclusion
The efficient oral delivery of biopharmaceuticals via nanoparticle formulations is highly coveted due to its simplicity and patient acceptability, alongside the challenge of achieving targeted delivery with minimal side effects. Nanoparticles offer numerous advantages such as targeted drug delivery, enhanced surface-to-volume ratio, and sustained release, thus improving oral bioavailability. However, the design challenge lies in balancing complexity with simplicity to ensure clinical translation. Leveraging natural biological processes within the gastrointestinal tract can optimize formulation outcomes. Moreover, careful selection of experimental methods is crucial for preclinical validation, with evolving techniques offering insights into distinguishing pathways for clinical trials. Recent research has focused on nanoparticle modifications to enhance gastrointestinal uptake, underscoring the vast potential of nanomedicine in revolutionizing oral drug delivery efficacy.

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Conflict of Interest
No Conflict of Interest

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