Biotechnology Applications in Gastrointestinal Drug Delivery Systems: A Comprehensive Review

Hiba Abdulameer Mohammed[1], Yusur Falah Faraj[2], Roaa Mokram Hamed[3]

Alkarkh University of Science[1]
National center of hematology/ Mustansiriya university, Iraq[2,3]
[1]hiba.abdulameer@kus.edu.iq
[2]yusur_falah@uomustansiriyah.edu.iq
[3]roaamokram89@uomustansiriyah.edu.iq

Abstract— Biotechnology has become a game-changing technology for creating sophisticated pharmaceutical products targeting the gastrointestinal (GI) tract. Physiological challenges and opportunities for drug delivery design include the unique physiological properties of the GI system, such as the pH gradient, enzymatic environment, mucous barrier and resident microbiome. This review offers a detailed discussion on various biotechnological systems that can be used to deliver drugs in the GI system, such as polymeric and lipid-based nanoparticles, systems that are responsive to the pH or enzymes of the GI tract, liposomal or vesicular delivery systems, stimuli-responsive hydrogels, in situ delivery of therapeutic proteins using genetically engineered probiotic bacteria to target the GI tract, recombinant biopharmaceuticals (monoclonal antibodies and biosimilars) for inflammatory bowel disease (IBD), and a review of biotechnological platforms to deliver oral vaccines targeting the gut-associated lymphoid tissue (GALT). The physiological rationale, design principles, mechanisms of action, and current clinical evidence of each technology are discussed. This review was designed to give the researchers and clinicians an overview of the therapeutic landscape of gastrointestinal diseases changed by biotechnological innovations.

Keywords—Biotechnology, drug delivery, nanoparticles, recombinant probiotics, CRISPR, monoclonal antibodies, gastrointestinal tract, inflammatory bowel disease, oral vaccines

I. INTRODUCTION

The development of therapeutic agents for gastrointestinal (GI) disorders has been transformed by the use of Biotechnology, which supports the design of new drug delivery systems, recombinant protein therapeutics, and genetically engineered probiotics. Combined with molecular biology, genetic engineering, nanotechnology and pharmaceutical sciences, there are unprecedented opportunities for site-specific, sustained and targeted delivery of drugs to the GI tract. Oral delivery of drugs has many drawbacks such as degradation in the stomach due to gastric acid and digestive enzymes, irregular intestinal absorption, first-pass hepatic metabolism, and low bioavailability for many drugs. These limitations have been addressed by biotechnological solutions, by rationally designing delivery platforms which take advantage of the specific physiology of each GI segment [3].

This review discusses the current developments of biotechnological applications in gastrointestinal drug delivery systems such as nanoparticles, liposomes, hydrogel matrices, genetically engineered microorganisms, recombinant biopharmaceuticals, and steps towards a

specific therapeutic strategy using CRISPR. A physiological basis for each approach is presented, as well as the pertinent preclinical and clinical studies that substantiate their use in the management of GI diseases.

II. NANOPARTICLE-BASED DRUG DELIVERY SYSTEMS

A. Polymeric Nanoparticles

Polymeric nanoparticles (NPs) is one of the most widely studied biotechnological platforms for the delivery of therapeutics in the GI tract. Usually, these particles have a diameter of between 10 and 1000 nm and can be made from biodegradable polymers like poly(lactic-co-glycolic acid) (PLGA), chitosan, alginate and poly(epsilon-caprolactone) (PCL) [4]. The mucoadhesive nature of the chitosan nanoparticles is especially beneficial for GI drug delivery, since the mucin glycoproteins in the intestinal mucous layer have a negative charge, causing electrostatic attraction between the chitosan and the negatively charged mucous layer, which increases the residence time of the encapsulated drug at the absorptive surface and, consequently, its bioavailability [5].

A pH-responsive nanoparticle has been developed to take advantage of the pH difference in the GI tract. The advantage of using eudragit coated nanoparticles is that they are resistant to the acidic environment of the stomach (pH 1-3) and released at the higher PH of the small intestine (pH 6-7.4) or colon (pH 7-8). Such strategy has been demonstrated to increase the local levels of anti-inflammatory drugs (e.g. budesonide and 5-aminosalicylic acid (5-ASA)) while decreasing systemic drug-related effects in the treatment of inflammatory bowel disease (IBD) [6, 7].

B. Lipid-Based Nanocarriers

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are suitable systems for delivering lipophilic drugs and bioactive compounds via the GI tract. They are comprised of physiological lipids that are identified by the lipid absorption pathway in the intestine and taken up by enterocytes along with the formation of mixed micelles and packing into chylomicrons for transport through the lymphatic system [8]. The oral bioavailability of many drugs, including curcumin (from <1% to ~30-40% after delivery with NLC) and cyclosporine A (for treating IBD), can be greatly enhanced using this lymphatic route since it avoids first-pass hepatic metabolism.

III. LIPOSOMAL AND VESICULAR DELIVERY SYSTEMS

Liposomes are spherical vesicles containing of one or more phospholipid bilayers which have an aqueous compartment inside, and can be used to encapsulate either hydrophilic drugs, within the aqueous compartment, or lipophilic drugs within the phospholipid bilayer. For GI delivery liposomes ensure the gastric stability of drugs which are sensitive to the acid environment, promote mucosal uptake by fusion with enterocytes and allow a gradual release of the drug [10]. A specialized type of liposomes, bilosomes, are composed of bile salt in the liposome bilayer and are more stable in the GI tract and are more readily transcytosed across the intestinal epithelium, which makes them ideal for oral delivery of peptide and protein based drugs including insulin, calcitonin and vaccines [11].

Vesicles, known as transfersomes and ethosomes, are highly deformable vesicles that have been shown to have the most promising properties for transcellular and paracellular intestinal penetration of the mucus barrier and the epithelial membrane. Oral delivery of poorly absorbed macromolecular drugs such as heparin and interferons has been shown to be promising with these systems [10, 12].

IV. HYDROGEL-BASED DELIVERY PLATFORMS

Hydrogels are polymers in the shape of a network in three dimensions that can absorb huge amounts of water without losing shape. For GI drug delivery, stimuli-responsive hydrogels have been developed that respond to particular physiological stimuli such as pH, enzymatic activity, temperature, and redox potential [13]. The pH-sensitive poly(methacrylic acid-co-ethyl acrylate) hydrogels expand and deliver their payload in the neutral to alkaline environment of the intestine, but are collapsed in the acidic stomach. Azo-bonds or disulfide linkages are included in enzyme-responsive hydrogels to break down them specifically by azoreductases or glutathione produced by the bacteria in the colon, which allows for highly selective delivery to the colon [14].

In situ forming hydrogels (IFH) such as alginate, gellan gum and poloxamer have been developed as localised GI therapy. These systems are administered as a liquid in the form of an ionotropic gel solution which precipitates and creates a depot to release the drug at the target site when exposed to physiological conditions (ions, pH, or temperature). This has been used for delivering proton pump inhibitors (PPIs) to the gastric mucosa and mesalamine to the colonic mucosa in ulcerative colitis [13, 15].

V. GENETICALLY ENGINEERED MICROORGANISMS

B. Recombinant Probiotics

A new and innovative biotechnological strategy for treating GIs is the genetic engineering of probiotic bacteria for the production and delivery of therapeutic molecules within the intestinal lumen. Lactococcus lactis and Lactobacillus spp. have been genetically modified to produce anti-inflammatory cytokines (IL-10, IL-27), trefoil factors (TFF1, TFF3), antimicrobial peptides and single-domain antibodies (nanobodies) against pro-inflammatory targets, like TNF-alpha [16, 17]. The benefits of this are that the therapeutic protein is continuously generated in the mucosa where it is required, thus providing high concentrations at the site of action and low levels in the systemic circulation.

People with cancer who develop oral mucositis have completed Phase II clinical trials with genetically modified L. lactis (AG013) that secretes human trefoil factor 1 (hTFF1) and are being studied for use in GI mucosal healing. Likewise, anti-TNF-alpha nanobodies have been found to be effective in murine models of colitis, with the added benefit of not having the immunosuppressive side effects seen with systemic anti-TNF therapy [17, 18].

C. Engineered Bacteriophages

Another frontier in the field of GI medicine is the use of bacteriophage therapy. Engineered bacteriophages can be engineered to selectively kill a pathogenic bacteria while preserving the commensal microbiota in the gut. Bacteriophages armed with a CRISPR cas3 or cas9 nuclease with guides against the virulence genes or antibiotic resistance determinants of specific pathogens have demonstrated promising results in preclinical models including Clostridioides difficile and adherent-invasive Escherichia coli (AIEC) [19, 20].

VI. RECOMBINANT BIOPHARMACEUTICALS FOR GI DISORDERS

The development of recombinant proteins and monoclonal antibodies has revolutionized the treatment of GI disorders and especially inflammatory bowel disease (IBD) by biotechnology. Anti-TNF-alpha antibodies (infliximab, adalimumab, golimumab), anti-integrin antibodies (vedolizumab that binds to alpha-4-beta-7 integrin), anti-IL-12/23 antibodies (ustekinumab) and

anti-IL-23 antibodies (risankizumab, guselkumab) are all products of recombinant DNA technology [21, 22]. These biologics are genetically engineered in a mammalian cell line (most often CHO, Chinese hamster ovary) to act on specific molecular pathways known to be important in the pathogenesis of IBD.

Vedolizumab is an example of gut-selective (anal-specific) immunotherapy as it specifically inhibits binding between alpha-4-beta-7 integrin on circulating lymphocytes and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on endothelial cells of the intestine, thus preventing lymphocyte trafficking to the gut mucosa without systemic immunosuppression [22]. Biosimilars of these agents can be manufactured in much the same way, with a few differences, and have greatly enhanced access to patients as well as mitigated cost of treatment [23].

VII. CRISPR-BASED THERAPEUTIC STRATEGIES

The clustered regularly interspaced short palindromic repeats and CRISPR associated protein (CRISPR) 9 gene editing technology has ushered in new therapeutic opportunities for GI diseases. For gastric and colorectal cancers, the genetics of these cancers have been engineered with CRISPR-Cas9 to produce chimeric antigen receptor T-cells (CAR-T cells) against tumor-associated antigens including claudin 18.2, carcinoembryonic antigen (CEA) and human epidermal growth factor receptor 2 (HER2) [24]. New gene correction strategies based on CRISPR are being developed for monogenic GI diseases, and disruption of oncogenes or reversion of tumour suppressor genes is a promising new approach for hereditary GI cancer syndromes like familial adenomatous polyposis (FAP) and Lynch syndrome [25].

Precision engineering of commensal bacteria is also being applied to modify the gut microbiome, including improvement of the gut microbiome's probiotic activity, such as increased production of short-chain fatty acids, enhance bile salt hydrolase activity, and improve colonization resistance against pathogens [26].

VIII. BIOTECHNOLOGICAL APPROACHES TO ORAL VACCINE DELIVERY

The GI-associated lymphoid tissue (GALT) containing Peyer patches and isolated lymphoid follicles is the largest immunological organ in the body and a promising site for oral vaccination. Recombinant attenuated bacteria (Salmonella, Shigella, Listeria) displaying heterologous antigens, antigen loaded nanoparticles targeted to M-cells overlying Peyer patches and virus like particles (VLPs) also constitute some of the strategies used in biotechnology for oral vaccination. Enhancement of transcytosis across the follicle-associated epithelium and strong induction of both mucosal IgA and systemic IgG immune responses have been observed in the case of surface-modified chitosan and PLGA nanoparticles with M-cell targeting ligands, like Ulex europaeus agglutinin-1 or peptides that specifically bind to claudin-4 [28].

A novel platform of recombinant plant-based oral vaccines, obtained by genetic transformation of edible plant species like rice, potato and lettuce, is emerging as a biotechnological approach that associates antigen production with a natural vector for oral delivery. The plant-produced vaccines are resistant to stomach digestion, and are delivered to the intestine by digestion of the plant cell wall when presented to the bacterial enzyme in the intestine, resulting in efficient antigen delivery to the GALT [29].

IX. CHALLENGES AND FUTURE PERSPECTIVES

Although there have been tremendous advances in developing biotechnological methods for drug delivery to the GI tract, there are still some obstacles. Laboratory-to-industrial scale scaling-up of production of nanoparticles with good batch-to-batch consistency and quality control is technically difficult and expensive. The regulation of genetically engineered microorganisms and the therapeutics based on CRISPR is still evolving, and therefore, there is uncertainty regarding the clinical translation [30]. Biological barriers of the GI tract, such as the gastric acid barrier, the mucus layer, epithelial tight junctions, and enzymatic degradation, still remain as a significant barrier to the oral bioavailability of numerous biotechnological products, especially protein and nucleic acid therapeutics [3].

The future direction of the work involves the development of drug delivery systems that are customized for each individual based on their microbiome profiling and the optimization of nanoparticles through AI, the development of platforms that integrate drug delivery with biosensors for real-time monitoring and feedback-controlled drug delivery, and finally the use of organoid technology and gut-on-a-chip models for more physiologically relevant preclinical testing of novel drug delivery systems for the GI tract [31, 32].

X. CONCLUSION

Biotechnology has had a tremendous impact on the gastrointestinal field, with novel technology products being developed for targeted drug delivery, recombinant protein production, genetic engineering of therapeutic microorganisms, and precision gene editing. Nanoparticle-based systems take advantage of the pH difference and the presence of enzymes in the GI tract for targeted drug delivery. Therapeutic proteins are directly delivered at the mucosal surface via genetically engineered probiotics. Recombinant biopharmaceuticals are precisely directed to specific molecular pathways in inflammatory bowel disease in a way that no other pharmaceutical has ever been. CRISPR technology has the potential to change the treatment of GI cancers and the engineering of the gut microbiome. These technologies are still under development and face a number of translation challenges but have the promise to greatly enhance the therapeutic effects for patients with a variety of gastrointestinal diseases.

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