International Journal of New Findings in Health and Educational Sciences (IJHES)
ISSN 2980-0641
DOI : 10.63053/ijhes.24

Investigation of Drug Release Systems for Application Probiotic Delivery

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ARTICLE INFO

Keywords:
Probiotics,
Release System,
Hydrogels,
Microcapsules

ABSTRACT

Probiotics are live microorganisms that have a positive effect on our health and bring many benefits when consumed in sufficient doses. Maintaining the viability of probiotic bacteria during oral administration can be challenging due to the harsh conditions they face, such as the acidic conditions of the stomach. However, delivery systems for probiotics are very different and important in terms of effectiveness for patient health. These release systems can be categorized into conventional formulations, pharmaceuticals, and non-conventional products, mainly food-based commercial products. In this review, we focus on polymeric carriers and methods applied to encapsulate probiotics in them. Microcapsule technology has been proposed as a successful strategy with key factors including the ability of microcapsules to transport viable functional bacteria in sufficient numbers, protect against harsh physiological conditions, and survive formulation processes to improve their efficacy after oral administration. Also, biodegradable polymers or hydrogels as carriers of probiotics can protect bacteria from the acidic environment of the stomach and increase their survival during storage and consumption. Overall, by combining advances in carrier materials and microcapsules, there is promising potential to improve probiotic delivery and increase health benefits for patients.

Introduction

Currently, people's awareness of the importance of health has increased significantly. Consumers are interested in consuming food products with positive effects on their health. These types of functional foods include components such as vitamins, antioxidants, proteins, and probiotics and have other benefits besides providing nutrition. (Tamjidi et al, 2013) But unfortunately, many bioactive components used in the food industry are sensitive to the production process and storage conditions, and their exposure to oxygen, high temperature, specific pH or light may be harmful. (Aditya et al, 2017) Probiotics are defined as live microorganisms by the World Health Organization that confer various health benefits to the host when administered in sufficient amounts in the gastrointestinal tract. Probiotics can have health benefits such as inhibiting the growth of pathogenic or harmful bacteria, maintaining the balance of intestinal microbiota, reducing cholesterol levels, producing vitamins and antimicrobial agents, stimulating the immune system, relieving constipation, and improving calcium absorption. (Hill et al, 2014) Lactobacillus and Bifidobacterium are two genera of lactic acid bacteria that are used in probiotic products. The famous species of these two genera include Bifidobacterium breve, Bifidobacterium longum, Lactobacillus fermentum, Lactobacillus plantarum and Lactobacillus rhamnosus, which play a role in maintaining human health. These bacteria are able to convert lactose and other carbohydrate sources into lactic acid, which is used by other gut bacteria to make short-chain fatty acids. This action creates an acidic environment in the intestine, which is unfavourable for many pathogenic bacteria (Deshpande et al, 2018). Probiotics are currently used as food supplements with possible effects against gastrointestinal and non-gastrointestinal diseases. These include pathogenic bacterial and viral infections, constipation, acute diarrhoea, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), atopic dermatitis, stomach ulcers, colon cancer, coronary heart disease, and urinary tract infections. et al., 2020)

While the potential for the use of probiotics in the treatment or even prevention of gastrointestinal diseases is high, their clinical efficacy is low due to conflicting clinical trial results for many diseases. Probiotics must be able to survive the harsh environment of the stomach, remain metabolically active, and be released (in a controlled manner) in sufficient amounts at the site of action in the lower gastrointestinal (GI) tract to produce beneficial health effects. Technology to trap small amounts of bioactive compounds or microorganisms in small polymer capsules has been proposed as an effective solution to improve the survival, resistance and targeted release of sensitive microorganisms. In principle, the goal of microencapsulation is to create an environment that protects the bacteria from exposure to external factors (such as low gastric pH) during digestion and subsequently reduces cell damage or cell death before their release at the target site. (Chua et al, 2017) In the study of nanomaterials for encapsulating probiotics, it has been shown that these nanostructures can preserve and protect probiotics against the acidic conditions of the stomach and are released in the intestinal tract. As live supplements, probiotics can have beneficial health effects on the digestive tract. The human intestinal system contains microbes, fungi, yeasts, viruses, and protozoa, and some bacteria may be recognized as pathogens in humans. (Bosch et al, 2012) Probiotic bacteria have many health benefits, including boosting the immune system, they help digestion, protect against pathogenic bacteria, improve lactose tolerance, reduce cholesterol levels and provide treatment for various diseases including Crohn's disease, ulcerative colitis and IBS. Probiotics should be able to co-exist as part of the natural gut flora. (Reiff et al, 2010) Preserving the life of probiotics during production, storage and passing through the digestive system is very important. Drug release systems are designed to preserve probiotics in adverse conditions. Hydrogels based on polysaccharides are used as an effective method to construct release systems. These systems should be able to store probiotics under mild conditions and decompose in the digestive tract under appropriate conditions. The use of polysaccharides including alginate, carrageenan and chitosan can meet these requirements. Protection systems ensure the survival and delivery of sufficient amounts of probiotics to the destination. (Kwiecien, 2018) There are many formulations for the delivery of probiotics, but the viability and effectiveness of these systems are varied. Using tablets and capsules without or with chemical and structural changes is a common and

effective way to deliver probiotics to the intestine. By changing their excipients, the tablets improve the amount of live probiotic cells in the intestine. By adding enteric polymers, adhesion enhancers, and controlled release enhancers, they improve delivery problems of probiotics. Tablets and capsules are used as the preferred method for delivering probiotics to the human body due to their greater stability in storage. However, during tablet compression, the temperature may reach up to 60°C, which can kill heat-sensitive bacteria. Other formulations such as microcapsules and seeds can keep bacteria alive during manufacturing, storage and delivery to the desired site (Solanki et al, 2013).

Encapsulation of probiotics

Encapsulation generally refers to a process that traps one material in another material and produces particles on various scales from nanometers (nanoencapsulation), to micrometres (microencapsulation) or millimetres. In this process, the enclosed part is known as the core material, internal phase, active agent or cargo phase. The material used to enclose the core material is called the covering membrane, carrier, shell, external phase, or matrix. For probiotics encapsulation, freeze-drying methods are used, which is also referred to as cryopreservation. Encapsulation systems can be produced in different shapes such as spherical, cylindrical, oval or irregular shapes, and methods such as spray drying, freeze drying, emulsion and melt extrusion are used to produce the matrix type. (Shishir et al, 2018) A microcapsule is a the capsule type is micrometre in size and consists of a solid, liquid or gas core surrounded by a semi-permeable membrane or shell. In the probiotic formulation, the membrane acts as a protector for the probiotic cells against external conditions, and nutrients and metabolites can be transported through the membrane (Chen et al, 2012).

Microencapsulation is an important technique to preserve the viability of probiotics during processing, storage and gastric acid conditions. This method provides the possibility of working with probiotics and access to their controlled doses. In food applications, microencapsulated probiotics are added to products such as yoghurt, cheese, ice cream, nutrition bars, biscuits, vegetables and frozen water. In this method, biopolymers (such as proteins or carbohydrates) are used as carrier materials to encapsulate probiotics, which minimizes molecular mobility during storage time and prevents their degradation. (Homayouni et al, 2008) The microencapsulation method is widely used in the food and pharmaceutical industries and protects probiotics by creating small capsules. These capsules create a functional barrier between the cells and the environment and prevent damage to the probiotic bacteria, thereby improving the viability of the probiotics. Encapsulated cells are able to reach the site without adverse effects of environmental factors such as oxygen and pH. (Manojlovic et al, 2010) Microencapsulation, by protecting probiotics from the acidic environment of the gastrointestinal tract, can improve the survival of probiotic cells. The low pH stomach environment damages many probiotics, such as Lactobacillus rhamnosus and Bifidobacterium longum, and the bile salt conditions in the gut also cause severe damage to probiotics. One of the key requirements in encapsulation is the ability of the encapsulating material to release the loaded probiotic cells from the microcapsules at the site of delivery. Encapsulated cells can be released by various mechanisms such as biodegradation, pH change, mechanical rupture and diffusion. (Yadav, 2019) In recent years, encapsulating materials with mucoadhesive properties have been improved, which helps in the targeted delivery of probiotics in the gastrointestinal tract. Mucosal adhesion is an important factor in the adhesion of encapsulating materials to mucous membranes and creating temporary persistence in the digestive system, which leads to increasing the shelf life of probiotics, reducing the number of times of administration, improving bioavailability and improving spatial targeting. Improving the adhesion of microcapsules by using adhesive encapsulating materials is an excellent strategy for delivering probiotics to the intestine (Nagpal, 2012).

Nanostructured encapsulating materials

Nanostructured materials for encapsulating probiotics are composed of natural (such as carbohydrates, gums and proteins) and synthetic polymers and have their own mechanical, chemical, physical and biological properties. The use of new nanomaterials with different shapes, textures and compositions for the microencapsulation of probiotics has been investigated recently.

Due to their unique properties, nanostructured microcapsules improve the protection of probiotics against harsh environments. The nanomaterials used are non-toxic and compatible with probiotics and the body, and some of them are able to release the loaded probiotics under certain conditions such as acidic pH (Byun et al, 2010).

Nanocellulose

Cellulose is the most widespread and abundant natural biological macromolecule, which consists of disordered (amorphous) and ordered (crystal) regions. Nano-sized cellulose, known as nitrocellulose, has recently attracted much attention due to its properties including low risk of toxicity, biocompatibility, and tunable surface properties. Nanocellulose exists in two forms: cellulose nanocrystals (CNC) and cellulose nanofibers (CNF). Nanocelluloses can be modified with various additives, and the physical and chemical properties of these materials have made them attractive for biomedical applications, including drug delivery and probiotic delivery systems. (Razavi et al, 2021) The use of nanocellulose improves the properties of probiotic delivery systems. makes it possible Cellulose nanocrystals (CNC) are also used as a long-lasting material in the formulation of drugs and improve the mechanical properties of the carrier. The use of CNC in formulations improves the compressive strength of dried microbeads. CNF is known as a filler for various biomaterials that have strong intermolecular bonds. Aqueous hydrogels are formed for cell encapsulation by CNFs, and the carboxyl groups in CNFs can be used to change the pore size of cellulose macrogels in order to improve their loading capacity. (Zhang et al, 2018) Cellulose nanostructures can also be To be effective for the development of controlled release systems. Nanocellulose can be obtained from various sources including plants and microorganisms. BNC is biosynthesized as a primary extracellular metabolite by several bacterial species such as Komagataeibacter xylinus (K. xylinus) during a fermentation process that results in the secretion of high-quality cellulose ribbons from microfibrillar bundles. BNC is non-toxic and comes in various shapes, sizes and surface structures depending on the manufacturing process. (Huq et al., 2017)

Chitosan nanoparticles

Chitosan is a natural multi-cationic polysaccharide derived from chitin, a natural macromolecule that is the main constituent of arthropod exoskeleton and fungal cell walls. The cationic properties of chitosan, due to its primary amino groups, have high potential as well as its biocompatibility, non-toxicity and low cost for a wide range of biomedical applications including tissue engineering, drug delivery and wound dressing, as well as in the food industry and It is used medicinally. (Krasaekoopt et al, 2006) With its positive charge, chitosan can create an electrostatic attraction with the mucous adhesive layer, and the physical entanglement between chitosan and mucous components can increase the adhesion of chitosan-based capsules to the gastric mucosa. Recently, chitosan nanoparticles (CSNPs) have been proposed for micro- and nano-encapsulation of cells and molecules, including probiotic bacteria, respectively, and delivery systems based on CS nanoparticles (CSNPs) have high adhesion strength compared to CS due to their high surface-to-volume ratio. have shown themselves. (Mavad et al, 2018)

Eudragit S100 nanoparticles

Eudragit S100 is a non-toxic anionic polymer synthesized from methacrylic acid and methacrylic acid methyl ester. The solubility of Eudragit S100 is pH dependent and is insoluble in strongly acidic solutions. Multiwalled microcapsules based on Eudragit S100 nanoparticles, alginate and chitosan were developed to improve the viability of probiotic bacteria Lactobacillus acidophilus and Lactobacillus rhamnosus in simulated digestive conditions. Since the chitosan layer is porous, it's coating with Eudragit S100 nanoparticles covers the porous beads and forms a protective bubble that protects the enclosed core from adverse environments. Also, the strength of the enclosed beads and improving the viability of bacteria Probiotic is better compared to single-coated beads. (Soares and Crema, 2020)

Magnesium oxide nanoparticles

Magnesium oxide (MgO) is a practical semiconductor that has many applications including pharmaceuticals and optoelectronics. MgO is medically prescribed to relieve cardiovascular disease and stomach problems. It also has high mechanical strength, thermal stability and low cost. Magnesium oxide nanoparticles (MgO NPs) have been used in the industry for pharmaceuticals,

toxic waste cleanup, and toxic gas removal. Probiotics enclosed in microcapsules containing MgO have shown better survival than free bacterial cells. There are two potential reasons for this improved cell survival: MgO nanoparticles fill the pores inside the alginate-gelatin microgels, which may inhibit the oxygen exposure of probiotics, and MgO nanoparticles neutralize hydrogen ions and, in an acidic environment, from Probiotics protect in gastric fluids. (Yao et al, 2018)

Starch nanoparticles

Starch is one of the most abundant biological polymers in nature, which is produced by many plants and crops such as cereals. Despite its simple chemistry, the starch granule is a complex semicrystalline structure (up to 100 μm long), containing linear amylose and highly branched amylopectin. Amylopectin is a semi-crystalline polysaccharide composed only of D-glucose residues covalently linked together mainly by α-(1 → 4) glucosidic bonds, as well as 4-5% α-(1 → 6) glucosidic bonds. are connected (Odeniyia et al, 2018) Starch nanoparticles (SNPs) and nanocrystals (SNCs) are two nanostructures that have been widely used for biomedical applications, especially drug delivery applications. Both SNPs and SNCs are nanosized. SNCs refer to the crystalline part of starch grains, which remains their amorphous structure after acid or enzymatic hydrolysis. SNCs have a crystalline structure, while SNPs are amorphous. Despite showing promising results for the delivery of bioactive compounds, SNPs may not be excellent candidates for microencapsulation of probiotics. Cells are not protected against higher temperatures by SNP although significantly better viability can be observed at lower temperatures. Unlike SNPs, SNCs protect probiotics by encapsulation in alginate-starch nanocrystals under simulated digestive conditions and during long-term storage at 4°C. This study shows that alginate and starch nanocrystals are suitable materials for probiotic encapsulation. (Thangrongthong et al, 2018)

Nanostructure encapsulation methods

Several methods have been used for the microencapsulation of probiotics, including chemical methods (such as surface polymerization), physical methods (such as spray drying), and physicochemical methods (such as coasorption and ionic gels). Two of the mentioned methods are mentioned here. (Prakash et al, 2016)

electrospinning

Electrospinning has shown great potential to enclose bacterial cells by nanofibers. In this method, probiotic cells are protected from harsh environmental conditions in the intestine and their survival is increased. Electrospun nanofibers based on several polymers (such as polyvinyl alcohol (PVA), fructooligosaccharide (FOS) and alginate) have shown the ability to protect probiotic cells against harsh environments. The simultaneous administration of probiotics and prebiotics (such as oligosaccharides and inulin) is possible with nanostructured encapsulation techniques and has been proposed as a new strategy to improve the survival of probiotics. In cell proliferation, it has also been reported that FOS/PVA nanofibers electrospun can significantly improve the survival of encapsulated cells under wet heat treatment. Electrospun nanofibers may consist of one polymer or a combination of two or more polymers. (Feng et al, 2018)

Layer by layer method

The layer-by-layer (LbL) method is another promising approach to encapsulating and introducing specific probiotic species into the gastrointestinal tract. This strategy is based on coating alternating layers of cationic (such as chitosan) and anionic (such as alginate) polymers on bacteria through electrostatic interaction. The LbL method increases the protection of bacteria against acidic environments and bile salts, thereby multiplying the encapsulated probiotics. The LbL technique can be used to encapsulate different types of probiotic cells and produce homogeneous nanocoatings with precise structure control. It has been shown that encapsulation of probiotic cells using the LbL technique improves bacterial protection in the digestive tract and increases microbial adhesion and growth in the desired locations. (Yilmaz et al, 2020)

Hydrogel-based systems

Hydrogels made of polysaccharides may be used as delivery systems for probiotics when the pore size of the hydrogels is small enough compared to the dimensions of the bacterial cells to allow

entrapment of the probiotic cells in the hydrogel matrix until the network breaks. (Govender et al, 2014)

Polysaccharide hydrogels

Alginate is an anionic polysaccharide extracted from brown algae. This material is used in the form of a hydrogel, and its gelling properties are related to the interaction with polyvalent cations such as calcium. Alginate hydrogel is insoluble in acidic environments and can be used to protect probiotics in the acidic environment of the stomach. The swelling percentage of alginate hydrogel depends on the pH of the surrounding solution, and it decomposes in the intestinal environment. By using alginate to prepare multilayer hydrogels, probiotic cells can be enclosed inside them and protected against acidic conditions. By increasing the number of layers in the alginate hydrogel, the survival of the encapsulated probiotic cells increases. These hydrogels are decomposed in the condition of intestinal fluid and help to release the enclosed probiotic cells. Is. In a one-step method, microcapsules were prepared in a solution containing cross-linking agent (CaCl2) and chitosan. In this method, Ca2+ and protonated amine groups of chitosan interact with negatively charged carboxylic groups of alginate and microcapsules are formed. In the two-step method, first the alginate gelation process is performed and then the microcapsules are coated with chitosan. In this case, the chitosan layer is placed separately on the alginate microcapsules. In a study conducted on Lactobacillus plantarum, probiotic cells in calcium alginate microcapsules coated with chitosan in a two-step method compared to free cells and encapsulated cells. With the single-step method, they showed better survival in simulated digestive conditions. Also, it was found that the excessive proximity of the chitosan layer to the protected cells had a negative effect on the cell viability. This effect has been explained due to the antimicrobial activity of chitosan, especially in acidic conditions. (Sohail et al, 2011)

Probiotic bacteria are mainly consumed with dairy products, but people with lactose intolerance should find alternative sources. Juices can also be considered as an alternative, but due to having a very acidic pH, they are considered a harmful environment for probiotic cells. Because alginate hydrogels have been proven to be suitable materials for increasing the survival rate of probiotics in acidic gastric conditions, their use has been investigated to protect bacteria against the low pH present in juices. Furthermore, it seems possible to prepare efficient hydrogels for such applications by combining alginate with other non-polysaccharide biopolymers. As seen from the examples above, alginate-based hydrogels have proven to be suitable materials for oral delivery systems of probiotic bacteria. The possibility of efficient applications of polysaccharide hydrogels as probiotic delivery systems has been proven in numerous studies. These hydrogels can improve the survival of encapsulated probiotic strains in the conditions of the digestive tract and also during storage at different temperatures or during heat treatment. The following tables provide a summary of the types of tested hydrogels, probiotic strains and research conditions. (Ding et al, 2007)

Challenges of using probiotics

While the aim of this study was to focus on the use of polymeric carriers for enhanced delivery of probiotics, we would like to emphasize the importance of considering the intrinsic characteristics of the bacterial strain intended for delivery. Some researchers have questioned the effectiveness of probiotics in general for exerting health benefits, but the general consensus is that probiotics complement the flora of the gut and genitourinary tract, which in themselves have functional health benefits. There are large individual differences between commercially available and next-generation probiotics. Also, the mode of action, efficacy and colonization ability of a given bacterial strain along with its potential to resist the encapsulation process are important. The use of polymeric carriers for enhanced and targeted delivery of probiotics is currently a hot topic. (Anselmo et al, 2016) The use of polymers either to coat encapsulated probiotics (with the aim of protecting the stomach) or as a carrier for the capsule. Direct cultivation of live microorganisms should be done with methods that allow high survival of the bacteria to lead to the desired final properties. Several studies have appeared evaluating the use of polymeric carriers to enhance the delivery of probiotics. Recently, more attention has been drawn to better adhesion to the intestinal mucosal layer, leading to gradual release and site-specific delivery of probiotics. As mentioned earlier, the formulation of

commercial probiotics usually includes some polymeric carrier. Such polymer additives are usually considered as "excipients" and the details about their production method or the role of additive polymers in achieving the results are not fully studied (Shen et al, 2014) to obtain more efficient probiotic formulation design. , biomaterials/chemical engineers are needed. In this way, it can be ensured that 1. the most specific/effective probiotic strains are selected, 2. the polymer carriers used to make the product are optimized according to the natural characteristics and sensitivity of the selected strain, and 3. the formulation It has been improved based on in vitro, in vivo and preclinical optimization methods. Among the more than 2000 clinical trials that have focused on the use of probiotics, the essential role of polymeric carriers in the efficient delivery of probiotics has not been covered much. We suggest that more attention should be paid to the application of polymeric carriers for enhanced or targeted delivery of probiotics. This also opens new doors for the clinical use of probiotics (Panigrahi et al, 2017).

Discussion

The main goal of this review is to clarify the potential of using polymeric carriers and polysaccharide hydrogels to improve the performance of probiotics as health supplements and disease treatment. Polymeric carriers increase the viability of probiotics during manufacture, storage and passage through the acidic stomach. In polysaccharide hydrogels, adding the second component as a coating reduces the pore size and minimizes the protection of probiotic strains against harmful environments. Finally, two-component polysaccharide-based hydrogels are considered suitable probiotic delivery systems. We conclude that the correct selection of polymer carrier(s) according to the desired strain, manufacturing method, storage conditions and target delivery location improves the performance of probiotics and is expected to pave the way for more use of probiotics in clinical applications. smooth out

We suggest that further research should be focused on evaluating the performance of different combinations of polysaccharide hydrogels and polymeric carriers for probiotic delivery. Comparative studies should investigate the effect of different conditions and probiotic strains on the efficiency of delivery systems. In addition, the development of innovative techniques for the controlled release of probiotics in specific areas of the gastrointestinal tract should be explored. These efforts will contribute to the development of improved probiotic delivery systems with greater viability and performance, and ultimately pave the way for treatment by probiotic-based supplements.

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