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Review

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Microencapsulation and characterization of controlled release intestinal drugs: a critical review

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Abstract: Oral administration has high patient adherence, as it is not only the route for primary site absorption of nutrients and peptide drugs but also important for treating intestinal diseases. However, traditional oral intestinal drugs are limited by drug degradation and inactivation particularly for proteins and peptides, due to gastric acid and digestive enzymes, making it difficult to effectively deliver drugs to target sites. To address these challenges, controlled release microencapsulated intestinal drugs (MeID) have been developed and have attracted attention for their ability to protect drugs from degradation by gastric acid and digestive enzymes. In this study, we systematically reviewed research articles on the preparation methods, release mechanisms, and evaluation strategies of MeID using Google Scholar, Web of Science and Science Direct databases. Our findings show that the preparation of emulsions, microencapsulation methods, shell material selection, and drug properties need to be considered comprehensively for MeID development. In addition, we found that coating, micro/nano carriers, and absorption enhancers can be combined to enhance microcapsule performance. Beyond focusing on drug loading efficiency and microcapsule morphology, we also found that cell models, animal models, and spectroscopic analysis techniques can be used to evaluate drug biocompatibility, stability, and efficacy. Finally, the literature showed that optimizing the preparation process can regulate drug release kinetics. For future research, we suggest studies should focus on low-cost methods for producing monodisperse microcapsules, developing dynamic responsive shell materials, and using organ-on-a-chip technology for precise evaluation, as part of the theoretical support towards the development of microencapsulated drugs and targeted drug delivery.

Key words: Controlled release; Microencapsulated intestinal drugs; Microencapsulation methods; Oral drugs; Response mechanisms

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1 Introduction

Oral drug administration is the main and preferred mode of drug delivery due to its comfort, non-invasiveness and convenience (Drucker, 2020; Chu and Traverso, 2022). The intestinal system is the main absorption site for drugs due to its large absorption surface area and long residence time (Pinto, 2010; Duran-Lobato et al., 2020). In addition, drugs for treating intestinal diseases, such as inflammatory bowel disease (IBD), need to be locally released in the intestine to target the disease site (Duran-Lobato et al., 2020). Similarly, the absorption of vitamins like vitamin B_{12} relies on binding to specific intestinal receptors, and anticancer drugs target M cells in the intestine to reduce systemic toxicity (Xu et al., 2020). However, traditional oral intestinal drugs, especially proteins, enzymes, and antibody-based drugs (Brown et al., 2019; Ahadian et al., 2020), are susceptible to degradation by gastric acid and digestive enzymes, and their penetration through

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mucus and cellular barriers is limited, resulting in low bioavailability (Xu et al., 2020; Ouyang et al., 2023b). To address this problem, encapsulating oral intestinal drugs within biocompatible shell materials with microencapsulation technologies can improve the stability, targeting capability, and bioavailability of oral drug delivery. This is because the shell from the encapsulation protects the drugs from degradation by enzymes and gastric acid leading to targeted release in the intestine (Chen et al., 2019; Yao et al., 2020). Also, specific microencapsulation technologies can mask drug bitterness (Sarabandi et al., 2018), enhance intestinal absorption and adhesion, and reduce negative effects on other parts of the digestive system (Dos Santos et al., 2021).

Smart drug microcapsules can respond to specific physiological characteristics or disease biomarkers to achieve smart, controllable and targeted drug release. The release and targeting mechanisms are commonly designed for encapsulation by different shell materials in microcapsules with appropriate thickness and structure (Wang R et al., 2022; Wang X et al., 2022; Zhang et al., 2023b). Microencapsulated drugs can also regulate the intensity and duration of action, avoid adverse reactions caused by peak blood drug concentrations, and reduce the frequency of administration (Hoffman, 1998; Baek et al., 2016; Daly et al., 2020). Therefore, based on the customization of microcapsules, personalized drug therapy can be achieved with a specific release rate and residence time (Araujo et al., 2015; Dos Santos et al., 2021).

Currently, the preparation methods for microencapsulated intestinal drugs (MeID) include traditional methods for polydisperse particles and microfluidic technologies for monodisperse microcapsules. Traditional preparation methods, such as mechanical stirring and spray drying, are commonly used for industrial production. However, they are limited by poor control of particle size, shell thickness, encapsulation stability and efficiency, and chemical residues (Daly et al., 2020; Zhao et al., 2023). These limitations have been shown in the literature to lead to non-uniform drug distribution within the microcapsules, unstable release rates, and challenges in the stability and release kinetics of the drug microcapsules (Xu et al., 2009; Xuan et al., 2024). Microfluidic technologies have been shown to alleviate these shortcomings through the preparation of monodisperse emulsions as templates by solidifying monodisperse microcapsules through ionic crosslinking, solvent evaporation, and free radical polymerization methods (Li et al., 2018; Jo and Lee, 2020). During the fabrication of phycocyanin (hydrophilic drug) microcapsules (Wang et al., 2022c), monodisperse microcapsules have achieved an encapsulation efficiency (EE) as high as 98%, as well as a uniform particle size with a coefficient of variance (CV) of < 3% (Li et al., 2018) and a controllable structure (Zhao, 2013; Jo and Lee, 2020). With monodisperse microcapsules, the drug release rate can be controlled by the drug content, surface area, volume, diffusion distance, or degradation rate of the microcapsules (Dos Santos et al., 2021). Therefore, the preparation of monodisperse MeID can provide insights into the release mechanism and pharmacokinetics of microcapsules (Xu et al., 2009; Wang et al., 2014).

To analyse and evaluate the current development of MeID, as well as future preparation and evaluation methods, we systematically reviewed literature on the preparation methods and techniques for MeID development (See S1. review methods and Fig. S1). The review also focused on the various methods for evaluating the biosafety, stability, response mechanisms, pharmacokinetics and drug release kinetics, in vitro action mechanisms, and in vivo pharmacological effects of drug microcapsules.

2 Preparation of microencapsulated intestinal drugs

The preparation of MeID is accomplished by using emulsion droplets as templates. The microencapsulation procedure typically includes two steps: 1) emulsification, in which the drug-containing solution is dispersed into another immiscible solution to form an emulsion, and 2) microencapsulation, where the droplets in emulsion serve as templates to form microcapsules through physical or chemical methods (Zhao, 2013).

2.1 Preparation of emulsions

The droplets in emulsions directly affect the size, morphology, and internal structure of the microcapsules (Wang et al., 2014). Fig. 1 shows various methods for preparing microdroplets or emulsions using different devices.

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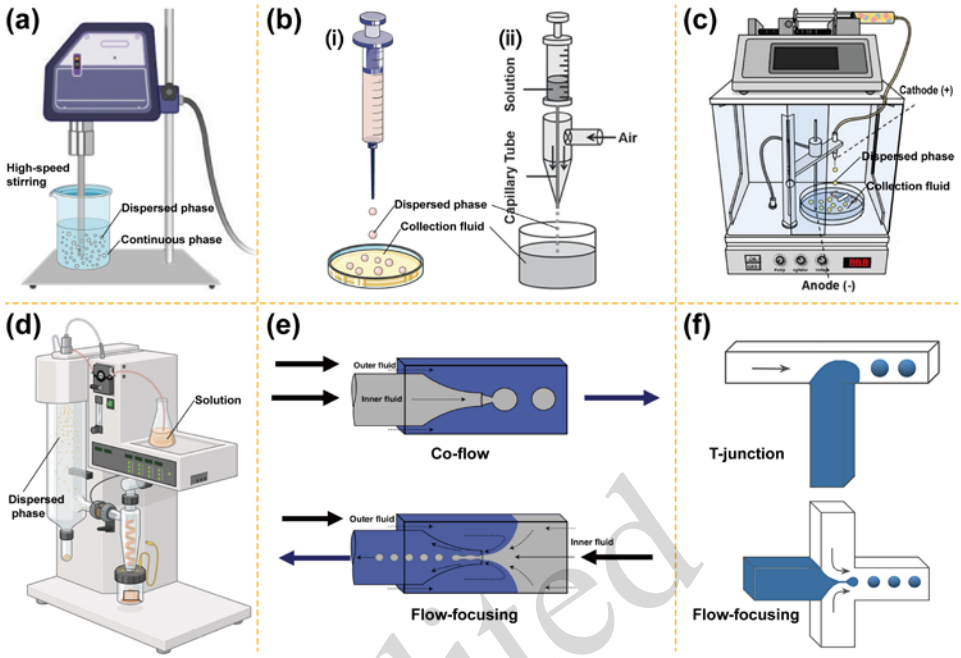

Fig. 1 Dispersion methods for the preparation of microdroplets or emulsions. (a) Mechanical stirring. Adapted from Sastri et al. (2020) by permission of Journal of Applied Pharmaceutical Science. (b) Extrusion technique (i) and gas shearing technique (ii) Adapted from Ghaffarian et al. (2016) by permission of John Wiley and Sons. (c) Electrospray technique. Adapted from Yang et al. (2023). Copyright 2023, with permission from Elsevier. (d) Spray drying. Adapted from Meola et al. (2024) by permission of John Wiley & Sons. (e) 3D capillary microfluidic device. Reprinted from Utada et al. (2007) with kind permission from Springer Nature. (f) 2D poly(dimethylsiloxane) (PDMS) microfluidics device. Reprinted from Su et al. (2021) with kind permission from Taylor & Francis.

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2.1.1 Mechanical stirring

Mechanical stirring can produce high shear stress and impact stress when a dispersed phase is dispersed into another immiscible continuous phase as tiny droplets, such as demonstrated in Fig. 1a (Daly et al., 2020; Sastri et al., 2020). The size and dispersity of the droplets are influenced by the stirring speed and time, emulsifier type and concentration, material properties, and core-shell ratio (Wu et al., 2019; Daly et al., 2020). Mechanical stirring is a simple process with high efficiency in the generation of emulsion, but it leads to a non-uniform and polydispersed droplet size distribution (Li et al., 2018). Additionally, during a vigorous stirring process, large molecular drugs (such as protein drugs) may be damaged and become inactivated by ultrasonic waves or mixed fluid shear stress (Ma, 2014; Li et al., 2022).

2.1.2 Extrusion and gas shearing techniques

The extrusion technique involves dissolving the drug in the shell material solution, and then extruding the emulsion droplets through a syringe or pipette (Fig. 1b (i)) (Uyen et al., 2020; How et al., 2022). The droplet size can be adjusted by the solution concentration, needle diameter and extrusion speed (Uyen et al., 2020). This method does not need complex equipment, high temperature or pressure, and hence is commonly used to produce large droplets in the range of hundreds of micrometers to millimeters (Uyen et al., 2020) with a slow preparation speed (Uyen et al., 2020; How et al., 2022; Zhu et al., 2023). Also, the core-shell structure emulsion droplets can be prepared with a coaxial nozzle for extrusion, in which the core and shell materials are extruded through independent inner and outer feed channels, respectively (How et al., 2022).

Like the extrusion technique, the gas-shearing technique replaces the outer liquid phase with gas in a coaxial nozzle to form emulsion droplets (Fig. 1b (ii)) (Ghaffarian et al., 2016; Tang et al., 2019). With this technique, the droplet size and production rate can be controlled by the gas and the internal solution flow rates (Xuan et al., 2024). This method does not need surfactant (Tang et al., 2019; Liu et al., 2021) or washing after

solidification. Compared to the extrusion technique, the gas-shearing technique can produce much smaller droplets with a high production rate (Wang et al., 2022a).

2.1.3 Electrospray technology

Electrospray technology extrudes the polymer solution (microcapsule precursor) through a syringe at a high voltage at the needle tip (Alfatama et al., 2024). The droplet deforms into a 'Taylor cone' due to the voltage. When the voltage exceeds a critical threshold, the electric field force overcomes the surface tension of the liquid phase at the needle tip allowing the jet to break into tiny droplets before the droplets finally attach to the collection substrate (Fig. 1c) (Daly et al., 2020; Zhang et al., 2022b; Yang et al., 2023). The size and dispersibility of the droplets depend on the operational parameters (i.e. applied voltage, flow rate, needle diameter, and distance between the needle and the collection reservoir) (Alfatama et al., 2024) and the physical properties of the solution (i.e. viscosity, density, and conductivity) (Mehta et al., 2017). The uniformity of droplets (CV range 6-23%) and the EE by this method are better than those of mechanical stirring (Qayyum et al., 2017).

2.1.4 Spray drying

Spray drying involves atomizing a solution containing the drug into small droplets and then drying the droplets using hot air (Fig. 1d) (Ozkan et al., 2019; Xu et al., 2023; Meola et al., 2024). Usually, the droplet size, drying speed and core material activity can be adjusted by the liquid feed rate, inlet and outlet drying air temperature, and gas flow rate during spray drying (Gharsallaoui et al., 2007; Estevinho et al., 2013). This method has advantages of low cost, high production efficiency, and good polydispersity powder characteristics (Xu et al., 2023). However, the high temperatures associated with this process may lead to degradation or damage of bioactive substances in the core material. Also, this method is unable to handle high-viscosity liquids (Jiang et al., 2022; Zhao et al., 2023).

Probiotic microcapsules can be prepared using coaxial spray drying where sucrose is used as a protective agent to effectively protect Lacticaseibacillus rhamnosus GG (LGG) during spray drying, to successfully achieve a survival rate of 10^9 CFU/g (Tan et al., 2022). In other cases, low temperature nitrogen circulating spray drying has been used to prevent insulin degradation during the fabrication of microcapsules (Sun et al., 2016).

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2.1.5 Microfluidic methods

Microfluidic emulsification (Zhao et al., 2020) uses two-dimensional (2D) or three-dimensional (3D) microfluidic devices to introduce the dispersed liquid phase into another immiscible continuous phase liquid, to generate shear forces to form monodisperse droplets at the junction of two microchannels (Zhao, 2013; Xuan et al., 2024). This process leads to the balanced interaction of the inertial, viscous and interfacial tension forces in the flow of the two immiscible fluids (Li et al., 2018). The droplet size resulting from this process can be controlled by the geometry of the microfluidic device and the relative flow rates between the continuous and dispersed phases (Zhao, 2013; Daly et al., 2020). However, microfluidic emulsification cannot be used to disperse high-viscosity liquids (Daly et al., 2020; Jo and Lee, 2020). Additionally, the hydrophilicity or hydrophobicity of the internal microchannel walls significantly affects the generation of droplets (Lee et al., 2016). For instance, to prepare water-in-oil (W/O) droplets, the walls should preferably be hydrophobic, whereas for oil-in-water (O/W) droplets, the walls should be hydrophilic (Li et al., 2018). Detail information of 2D and 3D microfluidic devices See S2.

Compared to the other dispersion methods, microfluidic emulsification can be used to fabricate droplets with precisely controllable structure and composition (Li et al., 2018; Jo and Lee, 2020). It also has the advantages of high EE, low reagent consumption (Araujo et al., 2015), and good reproducibility (Li et al., 2018; Jo and Lee, 2020; Wang et al., 2022b) for the preparation of monodisperse droplets (with CV < 3% (Li et al., 2018; Jo and Lee, 2020)) in the size range of 5-500 μm (Daly et al., 2020). However, microfluidic devices are difficult to clean for re-use (Nabavi et al., 2017), and the low production efficiency for single channel microfluidic devices limits their large-scale application. Some researchers have designed parallel multi-channel microfluidic devices for mass production of monodisperse droplets, i.e. 128 cross-junctions or 128 co-flow microfluidic (Nisisako and Torii, 2008) and 15 dropmaker units (Romanowsky et al., 2012) integrated on a chip.

2.1.6 Comparison of different emulsion methods

Double emulsion can be used to encapsulate a single/multi droplet within another droplet (Utada et al., 2005). Double emulsions serving as templates for microcapsules can achieve more complex functionalities and reduce cross-contamination of different materials (Zhao, 2013), see S3 and Fig. S2.

As summarized in Table 1, mechanical stirring, spray drying, and electrospray methods are low-cost, highly efficient, and suited for large-scale emulsion production. However, there are challenges with the non-uniform particle size distribution and low EE, particularly for the preparation of droplets with complex internal components. The extrusion technique needs only very simple equipment, as it is a low-cost emulsification method for large particle size droplet production. Gas shearing technology on the other hand does not require surfactants. Microfluidic-based dispersion technology has greater advantages for small-batch, high-value drugs, producing good monodisperse droplets, controllable structures, good batch-to-batch reproducibility, and high EE, especially for multilayer structures or specific morphologies. However, microfluidic emulsification faces challenges in large-scale production and costs. Therefore, it is necessary to consider the objectives of the microcapsules and the characteristics of the drug when selecting an emulsification method. Theoretically, the smaller the particle size of the microcapsules, the larger the surface area-to-volume ratio, hence higher molecular diffusion (Wang et al., 2022a). But to prepare monodisperse microcapsules of a diameter of 10 μm or less presents challenges (Champion et al., 2007; Zhao et al., 2021).

Table 1 Summary and comparison of various droplet dispersion methods

Dispersion methods	Principle	Advantages	Disadvantages
Mechanical stirring	Mechanical shear and impact stress.	The process is simple and highly efficient.	Droplet size is difficult to control; EE is low.
Extrusion	Mechanical force extrudes solution into emulsion droplets.	Easy operation with a simple equipment.	Preparation speed is slow with large droplet size, CV%=15-40% (Freitas et al., 2005).
Gas shearing	Shear force generated by gas to liquid phase.	Does not need surfactant.	Droplets are polydisperse.
Electrospray	Voltage causes droplets to form and disperse.	High production efficiency.	Non-uniform droplet size, CV=6-23% (Qayyum et al., 2017).
Spray drying	Atomizing droplets and drying with high-temperature airflow.	Low cost, high production efficiency.	Polydisperse droplets; high temperatures not suited for heat-sensitive biological materials.
2D PDMS microfluidics	Shear forces generated by two immiscible phases at junctions of 2D microchannels.	High EE with controllable size and structure, CV＜3% (Li et al., 2018).	Incompatible with organic solvents, difficult for multiple emulsions, complex and costly.
3D capillary microfluidic	Shear forces at junctions or aperture of immiscible phases.	Easy for multiple emulsion preparation, CV＜2% (Yang et al., 2024).	Manually assembled, difficult to reproduce and massive produce.

CV: coefficient of variance; EE: encapsulation efficiency; 2D: two-dimensional; 3D: three-dimensional.

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2.2 Microencapsulation methods

Microencapsulation methods for MeID are based on the physical and chemical properties of shell materials, which include ionic crosslinking, solvent evaporation, and free radical polymerization, see Fig. 2.

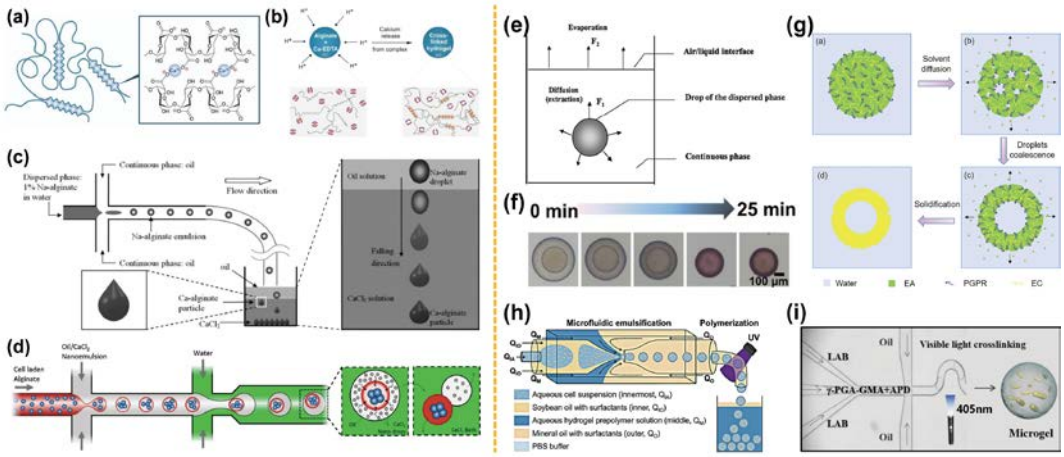

Fig. 2 Microencapsulation methods. (a) Ca^{2+} crosslinked with sodium alginate. Reprinted from Jo and Lee, (2020) by permission of John Wiley & Sons. (b) Internal gelation. Adapted from Utech et al. (2015) by permission of John Wiley & Sons. (c) External gelation. Adapted from Lin et al. (2013) by permission of John Wiley & Sons. (d) Double emulsion microfluidic improved external gelation. Adapted from Samandari et al. (2019). Copyright 2019, with permission from Elsevier. (e) Schematic diagram of solvent evaporation. Reprinted from Li et al. (2008). Copyright 2008, with permission from Elsevier. (f) Particle size changes during solvent evaporation. Reprinted from Jiang et al. (2021) by permission of The Royal Society of Chemistry. (g) Hollow microcapsules fabricated by solvent evaporation. Reprinted from Song et al. (2022). Copyright 2022, with permission from Elsevier. (h) Initiation by UV light. Reprinted from Kim et al. (2022). Copyright 2022, with permission from Elsevier. (i) Initiation by low-energy visible light. Reprinted from Wang et al. (2022b) by permission of John Wiley & Sons.

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2.2.1 Ionic crosslinking

The ionically crosslinked gelation process is widely used for the preparation of biomedical microcapsules. Among the mild and simple ionic crosslinking gelation processes, sodium alginate with high biocompatibility is the most common shell material for the preparation of pH-responsive microcapsules (Gombotz and Wee, 2012; Lee and Mooney, 2012). Sodium alginate is a naturally occurring anionic polymer composed of β-D-mannuronic acid and α-L-guluronic acid (Smidsrod and Skjak-Braek, 1990; Gombotz and Wee, 2012; Lee and Mooney, 2012; Aguero et al., 2017). The ionic crosslinking of sodium alginate connects different polymer chains to form an egg-box structure due to the exchange of sodium ions in guluronic acid with divalent cations, as shown in Fig. 2a (Gombotz and Wee, 2012; Lee and Mooney, 2012; Jo and Lee, 2020).

When sodium alginate is used as the shell material, its performance is influenced by the type of cation, the proportion of G units (α-L-guluronic acid), the crosslinking agent, and the crosslinking temperature (Smidsrod and Skjak-Braek, 1990; Gombotz and Wee, 2012), see S4. Ionic crosslinking can be accomplished by internal or external gelation (Qiao et al., 2024). Internal gelation usually involves the addition of water-insoluble calcium carbonate ($CaCO_3$) nanoparticles or calcium-EDTA into the sodium alginate solution as a source of Ca^{2+}. Emulsion droplets are crosslinked by the release of Ca^{2+} triggered by acid (Fig. 2b) (Tan and Takeuchi, 2007; Lee and Mooney, 2012; Utech et al., 2015). However, internal gelation may lead to the aggregation of gelling droplets resulting in channel clogging in microfluidic devices (Samandari et al., 2019).

For external gelation, the sodium alginate droplets are collected in calcium chloride solution, and the Ca^{2+} ions are diffused into the sodium alginate droplets to accomplish the crosslinking (Pawar and Edgar, 2012; Ghaffarian et al., 2016). Usually, the tailed-shape microcapsules, as shown in Fig. 2c (Lin et al., 2013; Samandari et al., 2019), result from uneven calcium gelation at the oil/water interface (Ling et al., 2019). However, this principle can be used to prepare microgels of different geometries by controlling the size, impact force, and concentration of the sodium alginate droplets (Hu et al., 2012). Samandari et al. (2019) improved external gelation by preparing W/O/W emulsion droplets by adding calcium chloride nanodroplets into the middle oil phase, which successfully avoided the formation of tailed-shape particles and did not require the use of acids or a chelator reagent (Fig. 2d). Song et al. (2013) compared the differences between internal and

external gelation and found that microcapsules prepared by internal gelation achieved more regular morphology and narrower particle size distribution.

2.2.2 Solvent evaporation

Solvent evaporation for micorencapsulation preparation (Fig. 2e) involves: 1) dissolving organic shell material in a volatile organic solvent, 2) emulsification to form tiny droplets, 3) diffusion of the solvent within the emulsion droplets slowly into the external continuous phase or evaporation into the surrounding air, 4) increasing the concentration of the shell material and finally solidification as microcapsules (Freitas et al., 2005; Li et al., 2008; Lee et al., 2016).

The evaporation rate of the solvent determines the morphology, encapsulation and release behavior of the microcapsules (Rosca et al., 2004; Sawalha et al., 2011). Compared to other microencapsulation methods, this technique requires the emulsion droplets to have high stability due to long curing periods (Lee et al., 2016). A rotary evaporator can be used effectively to shorten the evaporation time. Moreover, during the solidification process, the volume of particles shrinks significantly, as shown in Fig. 2f (Jiang et al., 2021). At the same time, the drug usually accumulates on the surface of the microcapsules, resulting in burst release at the initial decomposed stage (Rosca et al., 2004). Nevertheless, organic solvents may cause drug inactivation (Wang et al., 2022c).

Song et al. (2022) used ethyl acetate as a solvent to dissolve ethyl cellulose to prepare microcapsules with a hollow structure (Fig. 2g). They found that the interfacial mass transfer rate between ethyl acetate and water could be successfully controlled by adjusting the concentration of polyglycerol polyricinoleate in the oil phase.

2.2.3 Free radical polymerization

Free radical polymerization usually adds shell monomers or oligomers to the dispersed phase to form emulsions (Li et al., 2018; Zhang et al., 2022b). The thermal or photo initiators decompose to produce free radicals by heat or ultraviolet light, which initiates the polymerization and curing of the shell monomers to form microcapsules (Lee et al., 2016). The concentration of the initiator directly affects the solidification time and the structure of the microcapsules (Wang et al., 2022b). Thermal initiation polymerization may damage the bioactivity of drug molecules and the stability of emulsions due to high temperatures, leading to coalescence of particles (Lee et al., 2016). The reaction speed of photoinitiated polymerization is relative high, while emulsion stability shows less negative effects (Li et al., 2018; Zhang et al., 2022b). This method can be used to fabricate microcapsules with response mechanisms and high drug loading capacity (Ma et al., 2023), but the generated free radicals may cause biological toxicity (Zhang et al., 2022b).

To address the potential biocompatibility issues of radical polymerization, Kim et al. (2022) prepared triple emulsion droplets using a microfluidic approach as microcapsule templates, with polyethylene glycol diacrylate as the shell material, solidified through UV-induced free radical polymerization. The inner phase of the emulsion droplets was a cell solution, the outer phase was a prepolymer solution containing the photoinitiator and shell material, and the intermediate oil layer protected the cells from direct exposure to the photoinitiator (Irgacure 2959, 1%) and UV light during polymerization, achieving more than 98% cell viability within the microcapsules (Fig. 2h). In contrast, Wang et al. (2022b) grafted methacrylate groups (C=C double bond groups) onto the main chain of poly-γ-glutamic acid through an epoxy ring-opening reaction, giving it the capability to polymerize. The study used N,N-(2-amino-1,4-phenylene) diacrylamide as a crosslinker, initiating polymerization to solidify the shell under low-energy visible light (405 nm) irradiation. This method effectively reduced the negative impact of ultraviolet light on cell viability and achieved a cell loading density as high as 6.0×10^8 cells mL^{-1} (Fig. 2i).

2.2.4 Other microencapsulation methods

Phase change material as a shell can be used to form microcapsules by changing the temperature, i.e. by solidification of the shell material (Li et al., 2018). Fu et al. (2024) used dissolved gelatin in water at 37 °C, then slowly dropped it into liquid paraffin with an emulsifier at 60 °C. The gelatin droplets were transferred and cooled down at 4 °C to form microcapsules.

The combination of different microencapsulation methods to prepare MeID can improve their physico-

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chemical properties. For example, Li et al. (2017b) fabricated microcapsules sequentially through photopolymerization and solvent evaporation using W/O/W droplet templates (Fig. S3a). During the experiment, gelatin methacrylate was used as the core material to load hydrophilic drugs, and poly(lactic-co-glycolic acid) (PLGA) solution was used as the shell material to load hydrophobic drugs. By controlling the shell thickness, they achieved the effects of synergistic drug delivery and programmed drug release rates.

Gan et al. (2022) initially prepared sodium alginate microparticles using ionic crosslinking before forming a gelatin outer shell layer of the microparticles by freezing to construct core-shell structured microcapsules. The experiment required heating to maintain the fluidity of the gelatin before collecting the gelation solidification in an ice bath (Fig. S3b).

Li et al. (2022) modified sodium alginate as the microcapsule shell material by using W/W/O emulsion droplets as a template. The microcapsules were sequentially crosslinked through ionic and covalent crosslinking (Fig. S3c). This double crosslinking strategy reduced the mesh size of the solidified hydrogel, resulting in an elastic modulus roughly five times higher than that of the original sodium alginate gel.

2.2.5 Comparison of microencapsulation technologies

The emulsion dispersion method, microencapsulation method, shell material selection, and drug characteristics are interrelated. When selecting a microcapsule shell material, the hydrophilicity and solubility of the drug must be considered. For drugs with good hydrophilicity and water solubility, sodium alginate is suitable. In contrast, for drugs that are soluble in organic solvents, it is more appropriate to prepare shell materials using a solvent evaporation method, as this helps to enhance the drug's loading capacity and EE.

Different microencapsulation methods also vary in their compatibility with different emulsion dispersion methods. As shown in Table S1, ionic crosslinking is suitable for most emulsion dispersion methods, while solvent evaporation tends to favor mechanical stirring, spray drying, and microfluidic methods, as these facilitate solvent diffusion and evaporation. Note that due to the incompatibility of PDMS microfluidic devices with organic solvents, solvent evaporation is typically used for 3D glass capillary microfluidic devices. Free radical polymerization tends to favor microfluidic methods, where the precise control of droplet structure helps to avoid the potential toxicity of substances that may be generated during the free radical polymerization process.

Furthermore, the morphology and response mechanisms of the microcapsules need to be considered when choosing a microencapsulation method. Microcapsules prepared by ionic crosslinking typically have a wrinkled surface structure, while those prepared by solvent evaporation have a smooth surface. Due to the limitations of biocompatible shell material types, the response mechanism of MeID with a solid homogeneous structure is generally based on physiological mechanisms in the human body, while microcapsules prepared by free radical polymerization allow for more flexible shell material designs that can respond to various release mechanisms.

Microcapsules prepared by composed microencapsulation multiple methods can exhibit a core-shell structure and can simultaneously load drugs with different hydrophilicities. However, the order of solidification of the core-shell materials needs to be considered to achieve different structures and shell materials for microcapsules, which directly affect the drug release behaviors and mechanisms, such as diffusion-control, swelling- control, and erosion-control (Arifin et al., 2006).

2.3 Performance improvement of drug microcapsules

Conventionally prepared MeID face issues such as low drug loading, low EE, inadequate gastric protection, poor targeting, and uncontrollable drug release profiles. However, by incorporating coating methods, micro/nano carriers, and absorption enhancers, these limitations can be effectively addressed, significantly improving the therapeutic efficacy of the microcapsules.

2.3.1 Coating methods

Fig. S4a shows a microcapsule coating method where the microcapsules are immersed in a polymer solution with opposite charges and then alternately polycations and polyanions are absorbed on the microcapsule surface to form a protective coating (Sato et al., 2011).

Yang et al. (2022) formed a coating on sodium alginate microcapsules and increased the drug loading capacity of the coated microcapsules from 9.2 ± 2.3% (w/v) to 18.0 ± 2.5% (w/v) (Fig. S4b). However, the microcapsules showed insufficient protection in the stomach, releasing about 50% of the drug within 2 h during

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an SGF experiment. Ling et al. (2019) added chitosan to calcium chloride collection solution and simultaneously finished the chitosan coating with ionic crosslinking. This increased the retention efficiency of the loaded nanoparticles by 35% (Fig. S4c). Ghaffarian et al. (2016) also stabilized a chitosan shell by crosslinking the amino groups of chitosan with genipin after coating the microcapsules with chitosan, to successfully reduce the drug initial burst release by 25% within one hour in SIF (pH=7.8). Hu et al. (2025) alternately deposited five layers of negatively charged sodium alginate-silver nanoparticles and positively charged aminocellulose-silver nanoparticles on the surface of chitosan microcapsules, forming a multilayer rigid coating that enhanced the mechanical stability and adsorption capacity of the microcapsules.

In addition to using electrostatic interactions to form a coating on the surface of microcapsules, Xu et al. (2025) used an oxidative polymerization method to coat the surface of porous PLGA microcapsules with pH-responsive polydopamine, achieving targeted delivery to disease sites. Additionally, Zhu et al. (2023) used a solvent evaporation-induced coating method by applying drug-containing sodium alginate droplets in an ethanol solution containing zein and calcium chloride. After the ethanol evaporated, the solubility of zein was decreased and it precipitated on the surface of the microcapsules to form a zein coating. Note that secondary growth of the coating can be achieved by repeating this process (Filippidi et al., 2014).

The coating of MeID can enhance their stability, drug loading capacity, and drug release time in low pH environments (Aguero et al., 2017; Yang et al., 2021). The stability and performance of the coating are influenced by the pH of the solution, temperature, adsorption time, and the charge density and molecular weight of the polyelectrolytes (Borges and Mano, 2014).

2.3.2 Micro/nano carriers

Low-cost and biocompatible natural bio-micro/nano particles have been highlighted for drug delivery in microcapsules (Iravani and Varma, 2021; Zhang et al., 2022a; Seol et al., 2024). Microalgae cells are suitable for loading various drugs due to their surface containing carboxyl, amino, phosphate groups, and negative charges (Zhang et al., 2023a; Zhang et al., 2024). Also, the fluorescence characteristics of the internal chlorophyll in microalgae cells are particularly suitable for drug distribution monitoring (Zhong et al., 2021). Polymers are also used to prepare drug-loaded nanoparticles, such as the commonly used PLGA, where the degradation rate of PLGA can be controlled by adjusting the ratio of lactic acid and glycolic acid (Rezvantalab and Keshavarz Moraveji, 2019).

Nanoparticle carriers in microcapsules also can simultaneously load multi-type drugs with different physicochemical properties. The micro/nano co-delivery system combines the advantages of nano- and micron-scale particles (Araujo et al., 2015; Ahadian et al., 2020; Dos Santos et al., 2021). The use of nanoparticles as drug carriers dispersed and encapsulated within microcapsules can promote drug absorption (Zhang et al., 2014; Ghaffarian et al., 2016; Ling et al., 2019), enhance targeting of the intestines (Yang et al., 2021) and disease sites (Fu et al., 2024). The interaction with biological systems can be enhanced by modifying the surface functionalization of nanocarriers (Zhang et al., 2014; Sun et al., 2022), thereby improving mucosal adhesion (Zhang et al., 2014; Liu et al., 2021) and promoting drug absorption (Araujo et al., 2015; Sun et al., 2022), see S5.

In recent times, Janus-structured magnesium-based micro/nano self-propelled motors have been proposed as drug delivery carriers for MeID (Mundaca-Uribe et al., 2023a; Chen et al., 2024). Magnesium (Mg) particles are initially placed on a glass slide as a template, and then layers of a catalytic metal (or TiO_2) or drug are deposited alternately layer-by-layer on the surface of the magnesium particles using sputtering deposition technology, ultimately forming an onion-like layered structure (Fig. S5g) (Maric et al., 2022; Dutta et al., 2023; Chen et al., 2024; Ren et al., 2024). After microencapsulation, a tiny opening is formed at the particle-glass interface (Dutta et al., 2023), creating an asymmetric structure. The reaction of magnesium with water in the intestine then generates hydrogen bubbles that act as a motor to propel drug release (Chen et al., 2016; Dutta et al., 2023), deliver to the target intestinal region (Mundaca-Uribe et al., 2023b), and extend the retention time by adhering to the intestinal wall (Wu et al., 2019). However, the lifespan of magnesium-based micromotor microcapsules is relatively short, and the drug can be completely released within 3 min (Maric et al., 2022). Therefore, the dissolution time required for the polymer layer needs to be controlled by adjusting the thickness of the enteric coating, thereby regulating the travel distance in the intestine before activation (Li et al., 2016).

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Wei et al. (2019) prepared microcapsules using magnesium-based micromotors, and managed to double the cellular uptake of antigen carriers compared to static microcapsules (Fig. S5h).

However, it is important to note that micro/nano carriers occupy a certain amount of space in microcapsules and hence decrease drug loading capacity (Sabu et al., 2019; Sun et al., 2022; Zhang et al., 2023b). Also, defatting cytoplasm (Wang et al., 2024), chemically etching (Liu et al., 2014; Li et al., 2017a), and electrochemical anodizing (Zhang et al., 2014) micro/nano carriers increases the complexity of the microencapsulation process.

2.3.3 Absorption enhancers

Enzyme inhibitors and intestinal permeation enhancers in microcapsules can improve the bioavailability of oral drugs (Pereira De Sousa and Bernkop-Schnurch, 2014; Negrulj et al., 2016; Twarog et al., 2019). Enzyme inhibitors can reduce enzyme activity, thereby reducing the degradation of drugs and increasing permeability across the intestinal epithelial monolayer (Liu et al., 2014). Permeation enhancers could increase the transport of peptide drugs by altering the structure and function of the intestinal epithelium (Maher et al., 2016). For example, Araujo et al. (2015) achieved a synergistic therapeutic effect by co-loading peptides and the enzyme dipeptidyl peptidase 4 inhibitor into microcapsules (Fig. S5f). Sun et al. (2016) on the other hand used an ion pairing method to complex insulin with sodium deoxycholate, thereby reducing the accessibility of proteases to insulin to promote its absorption through paracellular and transcellular transport pathways. The bioavailability of the insulin after microencapsulation increased from less than 0.5% to 16.1% compared to non-encapsulated insulin.

However, enzyme inhibitors and intestinal permeability enhancers may lead to negative effects such as damage and dysfunction of the cellular barrier and drug instability. Therefore, it is necessary to consider the concentration of the intestinal permeability enhancers in microcapsules and the recovery time of the cellular barrier for oral drug administration (Bernkop-Schnurch, 1998; Maher et al., 2016).

2.3.4 Sub-summary

During their preparation, coating the microcapsules (Roque-Borda et al., 2023; Zhu et al., 2023), loading micro/nano carriers (Liu et al., 2014; Li et al., 2017a; Jiang et al., 2021), and adding absorption enhancers (Araujo et al., 2015) or combining these methods in an orderly manner (Ghaffarian et al., 2016; Yang et al., 2021; Fu et al., 2024) can improve their controlled release effect, bioavailability, drug half-life, and stability.

Generally, coating can enhance the protective layer of the microcapsules in the stomach and increase their drug loading capacity. Micro/nano carriers within microcapsules can provide a programmed control of drug release rates, retention of absorption advantages of nanoparticles in the intestines, and synergistic drug delivery of the microcapsules. Absorption enhancers address the problems of poor absorption and easy degradation of the original drugs. By combining the above methods, the bioavailability and efficacy of MeID is expected to significantly improve.

3 Methods for evaluating microencapsulated intestinal drugs

Various standard instruments and methods have been used to evaluate the performance of oral drug intestinal microcapsules. For example, drug loading efficiency can be detected by high-performance liquid chromatography (HPLC) (Huang et al., 2024) and ultraviolet spectrophotometry (Wang et al., 2022c; Zhang et al., 2023b). Microcapsule particle morphology and particle size distribution can be measured by optical microscopy (Jiang et al., 2021) and dynamic light scattering (Huang et al., 2024). The detailed morphology and structure of the microcapsules can be analyzed by scanning electron microscopy (SEM) (Li et al., 2017a), atom force microscopy (AFM) (Li et al., 2022), and transmission electron microscopy (TEM) (Zhang et al., 2023b). The components of the microcapsules can be analyzed by nuclear magnetic resonance (NMR) (Wang et al., 2022b), energy dispersive spectroscopy (EDS) (Ouyang et al., 2023a), Fourier transform infrared (FT-IR) spectroscopy (Chen et al., 2022), and X-ray diffraction (XRD) (Deng et al., 2021). In addition, some special evaluation methods are still required for evaluation of biosafety, stability, response mechanisms, pharmacokinetics, and pharmacodynamics.

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3.1 Biosafety

In vitro tests for the biosafety of drug microcapsules are carried out by detecting cytotoxicity (leading to cell death) and cytostasis (preventing cell proliferation) (Liu et al., 2014; Adan et al., 2016; Li et al., 2017a; Wang et al., 2022b; Elmorshedy et al., 2023). For instance, the dye exclusion method is a cost-effective and easy to operate method that allows direct observation of cytotoxic effects in microcapsules (Ouyang et al., 2023a). However, it cannot distinguish between apoptosis and necrosis of cells, has low sensitivity (Adan et al., 2016), and is not suitable for high-throughput screening (Niles et al., 2008). The metabolic activity assay on the other hand assesses cell viability by detecting the activity of reductase enzymes during cell metabolism (Niles et al., 2008). For example, the 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay is commonly used for high-throughput screening but requires dimethyl sulfoxide to dissolve formazan in cells (Niles et al., 2008) and also kills cells. This approach affects subsequent studies and has a long experimental cycle (Adan et al., 2016). In contrast, the cell counting kit-8 (CCK-8) assay does not require a dissolving solvent and it is suitable for continuous monitoring of live cells (Niles et al., 2008). The adenosine 5'-triphosphate (ATP) assay method can detect cell viability based on the reaction between intracellular ATP and exogenous luciferase (Niles et al., 2008; Liu et al., 2014; Adan et al., 2016). It has high sensitivity and speed, but cannot distinguish between cytotoxicity and cytostasis (Adan et al., 2016), as well as being relatively expensive (Niles et al., 2008).

Compared to the limitations of in vitro models, in vivo biocompatibility assessment can provide a more comprehensive evaluation of the potential toxic effects of microcapsules by monitoring a complete physiological response and long-term effects. To assess liver and kidney damage after oral administration of drug microcapsules, Elmorshedy et al., (2023) measured serum biochemical indicators of mice, i.e. alanine transferase (AST) and aspartate transferase (ALT) reflecting liver reaction, blood urea nitrogen (BUN) and creatinine (CRE) reflecting kidney reaction (Kim et al., 2022; Elmorshedy et al., 2023; Du et al., 2024; Lin et al., 2024). After experimentation with drug microcapsules, histological examination of the major organs (i.e. heart, lungs, spleen, liver, and kidneys) of mice allows the visualization of organ toxicity effects (Gan et al., 2022; Fu et al., 2024). Ren et al. (2023) and Du et al. (2024) conducted long-term drug biosafety studies of MeID on mice over 30 d and 50 d, respectively. They observed pathological changes and abnormal inflammation in major organs through blood and biochemical testing, and histological analysis.

From this review, in vitro cytotoxicity tests have been found to have the advantages of being simple to operate and having a low cost and fast screening speed, but they may lead to deviations in toxicity assessment results. In vivo biosafety tests on the other hand can accurately reflect actual physiological changes within the organism, especially in the evaluation of chronic toxicity during long-term treatment. For biosafety screening of microencapsulated drugs, it is therefore recommended to prioritize in vitro tests at the early stages, combined with in vivo tests for in-depth evaluations. To select detection methods, the cell type, interactions, and culture conditions should be considered (Weyermann et al., 2005; Adan et al., 2016).

3.2 Stability

Optical microscopy can be used to observe and compare the morphological changes of MeID during long-term storage to evaluate their storage stability (Gan et al., 2022). This method is simple but cannot assess internal structure or drug activity changes in microcapsules. Thermogravimetric analysis (TGA) can be used to study the anti-erosion of gastric acid stability of microcapsules by measuring the residual weight of the microcapsules after digestion in SGF (Zhu et al., 2023). For probiotic microcapsules, the storage stability can be evaluated by measuring the number of viable cells under different storage conditions (i.e. 4 °C and 25 °C) at different storage times (Deng et al., 2021).

To precisely assess and analyze changes in the structure and bioactivity of protein drug microcapsules during digestion, Wang et al. (2022c) systematically evaluated the release of protein drugs from microcapsules after digestion in SGF and SIF. The released protein drugs retained their original structure according to the electrophoretic analysis of protein bands, FT-IR detection of protein secondary structure, and fluorescence spectroscopic analysis of the position and intensity of fluorescence emission peaks. Additionally, the biological activity of the released protein drugs was confirmed through experiments of free radical scavenging and macrophage activation. To investigate the stability of protein drugs after encapsulation and the release mechanism of microcapsules, Martinez-Lopez et al. (2019) and Ma et al. (2023) analyzed the secondary structure of insulin after release from microcapsules using circular dichroism spectroscopy. They verified that the encapsulated and

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released insulin could maintain structural and biological stability through reduction in blood glucose levels in mouse experiments.

3.3 Response mechanism of microcapsule shells

To achieve precise drug release at a predetermined location, intestinal microcapsules can be designed to respond to pH variation, microbiota, and intestinal peristalsis in the human body. For example, Liu et al. (2014) used hypromellose acetate succinate (HPMCAS) as the shell material and adjusted the ratio of acetate and succinate to prepare microcapsules with multistage pH responses. Their SGF and SIF experiments (Fig. 3a) proved that the shell can protect the microcapsules in the stomach and release drug in the intestine. Yang et al. (2024) used dextran modified with glycidyl methacrylate and tannic acid as shell materials for microcapsules, with the dextran degraded exclusively by colonic bacteria-secreted dextranase to achieve targeted release in the colonic environment (Lu et al., 2022). Gastrointestinal fluid experiments showed that the microcapsules could remain intact in SGF and SIF, as well as respond to degradation and release in dextranase solution (15 U/mL), simulating the action of dextranase secreted by intestinal microbiota (Fig. 3b) (Yang et al., 2024). Kim et al. (2022) designed a thin-shell microcapsule that can respond to intestinal peristalsis. They used a micropipette aspiration device to simulate mechanical stress in the intestine, and observed the deformation and rupture of the microcapsules in the constriction region (Fig. 3c).

Targeted microcapsules can be designed to respond to abnormal secretions caused by diseases. For example, Regmi et al. (2019) designed microcapsules responsive to reactive oxygen species (ROS) to treat inflammation resulting from a significant increase of ROS, and observed the ROS-dependent in vitro release of the microcapsules in a simulation experiment (Fig. 3d). Wang et al. (2022b) proposed a novel nitric oxide (NO)-responsive poly-γ-glutamic acid hydrogel microcapsule strategy based on a droplet microfluidic technology platform. During intestinal inflammation, the expression of NO significantly increased and auto-oxidated to dinitrogen trioxide (N_2O_3). When the microcapsules were exposed to N_2O_3, the cross-link bonds of the shell were broken down and the microcapsules disintegrated. The researchers also found that as a result of the cytoprotective effects of the microcapsule, there was high viability in SGF (89.67%) and SIF (93.67%) environments, respectively (Fig. 3e).

Fig. 3f shows a photoacoustic computed tomography system capable of real-time in vivo monitoring of microcapsule migration in mouse tissues (Wu, et al., 2019). Wu et al. (2019) developed a gold layer inside microcapsules that effectively converts near-infrared light into heat. They observed that the moment the microcapsules reached the targeted intestinal location, continuous-wave near-infrared (NIR) irradiation triggered the release of the core material of the microcapsule.

Several studies (Kim et al., 2022; Yang et al., 2023; Du et al., 2024) have also shown that after orally administering fluorescently labelled drug microcapsules in mice to directly observe the distribution and retention time of microcapsules using the in vivo PerkinElmer IVIS Spectrum Series imaging system, the stability and release behaviour of microencapsulated intestine drugs can be observed (Fig. 3g).

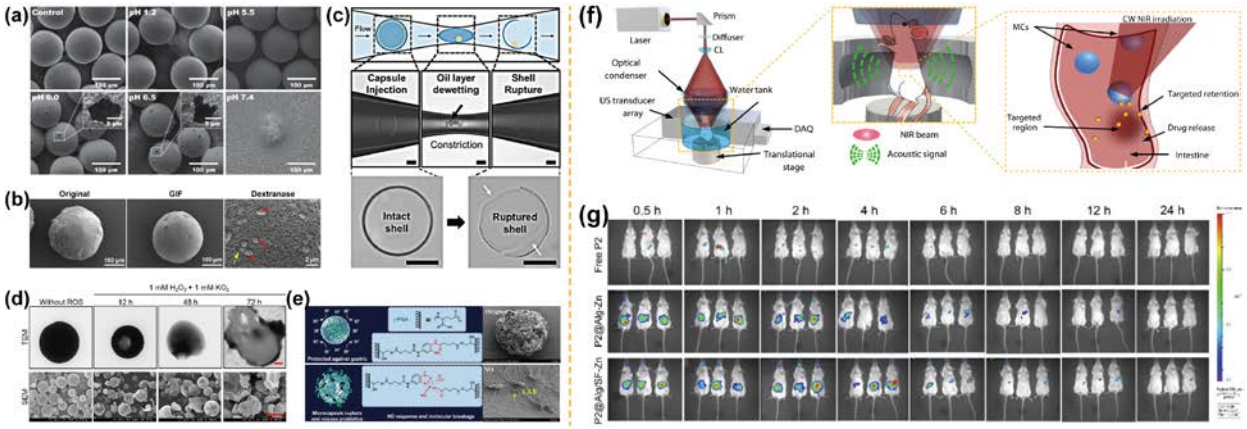

Fig. 3 Response mechanism of the MeID. (a) Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) experiments with pH-responsive microcapsules. Reprinted from Liu et al. (2014) by permission of John Wiley & Sons. (b) Dextranase-responsive microcapsules. Reprinted from Yang et al. (2024). Copyright 2024, with permission from Elsevier. (c)

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Microcapsules with release responsive to intestinal peristalsis. Reprinted from Kim et al. (2022). Copyright 2022, with permission from Elsevier. (d) TEM and SEM images of reactive oxygen species (ROS) responsive microcapsules. Reprinted from Regmi et al. (2019). Copyright 2019, with permission from Elsevier. (e) Comparison of microcapsules before and after release in response to nitric oxide (NO). Reprinted from Wang et al. (2022b) by permission of John Wiley & Sons. (f) Near-infrared (NIR) light induced microcapsule release. Reprinted from Wu et al. (2019) by permission of the American Association for the Advancement of Science. (g) In vivo imaging system of the microcapsule distribution of mice. Reprinted from Du et al. (2024). Copyright 2024, with permission from Elsevier.

3.4 Pharmacokinetics and drug release kinetics

For MeID development, understanding the mechanisms of drug release, absorption, distribution, metabolism and excretion is crucial (Uyen et al., 2020). By combining in vitro and in vivo models in the evaluation system, the drug release and pharmacokinetic properties of microcapsules can be optimized for more accurate performance assessment. Usually, the concentration of the drug in the blood circulation is measured to plot the blood concentration-time curve to obtain parameters from the area under the curve (AUC), as well as peak concentration (C_{max}), peak time (T_{max}) and bioavailability. The other method is to place intestinal microcapsules in SGF and SIF, and then extract samples to plot drug-time release curves (Paarakh et al., 2018; Ouyang et al., 2023a). See S6 Pharmacokinetics and drug release kinetics models.

The drug release kinetics of microcapsules include diffusion-controlled, swelling-controlled, and erosion-controlled systems (Arifin et al., 2006). The rate of release rate of drugs from microcapsules is usually influenced by the shell thickness (Rosca et al., 2004; Wang et al., 2022c), particle size (De La Vega et al., 2013; Zhao, 2013), particle size distribution (Wang et al., 2014), shell material solution concentration (Mandal et al., 2010), swelling properties (Yang et al., 2022; Ouyang et al., 2023b), drug solubility and dosage (Ghaffarian et al., 2016) of the microcapsules. Drug release kinetics models can be used to analyse and optimize the formulation of microcapsules (Huang et al., 2008; Trucillo, 2022), and to guide the design of microcapsule structure and drug loading (Arifin et al., 2006; Paarakh et al., 2018).

The mathematical models of drug release from microcapsules, which can be used to predict drug release behaviour based on the parameters of the diameter, shell material, drug loading and solubility in microcapsules (Siepmann and Peppas, 2012), see Table S2.

However, the drug release kinetics of microcapsules is still affected by the uniformity of the particle size and the drug distribution in the microcapsules. To this end, there is the need to explore drug release kinetics, the structure-activity relationship between the drug and the shell material, and the theoretical design of various monodisperse microcapsules.

3.5 Pharmacodynamics

3.5.1 In vitro simulation

In vitro simulation models constructed by cell culture technology are commonly used to study the absorption and mechanisms of MeID. For instance, mice intestinal epithelial cells (IEC-6) have been used to simulate intestinal epithelial cells to study the release of insulin from microcapsules and the endocytosis of the released insulin (Ren et al., 2023). Ren et al. (2023) used human colon cancer cells (Caco-2) and mouse macrophages (RAW264.7) to simulate M cell-mediated endocytosis and macrophage phagocytosis, to verify the drug absorption ability of microcapsules. Ma et al. (2023) used the Transwell model to simulate the small intestinal epithelium using Caco-2 cells. They analysed the transmembrane mechanism of the microcapsules by pre-treating with betaine or L-tryptophan to block PAT1.

In recent years, organ-on-chip technology based on cell culture and microfluidic technology has shown great potential due to its high physiological relevance (Macedo et al., 2023; Xu et al., 2024). This technology provides new possibilities for in vitro simulation of MeID and is expected to significantly advance the drug development process (Ahadian et al., 2020; Shin et al., 2020). In vitro models can quickly and cost-effectively help in the evaluation of toxicity and efficacy of drug microcapsules. However, they cannot fully simulate the complex physiological processes of drugs in the human body, especially where there are significant differences in pH, transit time, mucus, and intestinal fluid volume as a result of various disease states (Hua, 2020).

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3.5.2 Animal models

Animal disease models are expensive, although they can accurately provide evaluation of the actual effects and biological responses of drugs (Yao et al., 2020). Disease models for mice can be induced using some specific methods. For instance, streptozotocin (STZ) is used to induce a diabetic mouse model (Ren et al., 2023), and 1,2-dimethylhydrazine (DMH) to induce a mouse colon cancer model (Elmorshedy et al., 2023). An arthritis (AIA) rat model can be established in rats by induction with Complete Freund's Adjuvant (CFA) (Zhang et al., 2023b). Using the unilateral ureteral obstruction (UUO) rat model, renal fibrosis can be simulated (Sun et al., 2022), while a mouse metabolic syndrome (MetS) model can be induced by a high fat high sugar (HFHS) diet (Zhao et al., 2021).

The evaluation of drug performance in animal disease models is usually carried out by the comparison of detecting biomarkers (Elmorshedy et al., 2023) and histopathology (Li et al., 2022). For instance, a dextran sulfate sodium (DSS) solution can be used to induce a colitis model to simulate IBD treatment (Kim et al., 2022; Zhu et al., 2023). Therapeutic effects are then evaluated through a disease activity index (DAI), colon length, histopathology, intestinal tissue repair (Ouyang et al., 2023a), and levels of inflammatory factors (Du et al., 2024). In a murine model of colitis, Liu et al. (2021) used orally administered microcapsules loaded with thiolated hyaluronic acid for treatment. Immunohistochemical analyses revealed that, in the treatment group, the proportion of myeloperoxidase-positive neutrophils was significantly reduced, indicating that the microcapsules effectively inhibited the infiltration of inflammatory cells. Simultaneously, the expression level of proliferating cell nuclear antigen was significantly elevated, suggesting that the proliferation of intestinal epithelial cells was enhanced, thereby promoting the repair of intestinal tissue. Du et al. (2024) treated subjects with orally administered microcapsules containing bornyl acetate. Immunofluorescence staining results showed that this treatment significantly restored the expression levels of MUC-2 mucin and the tight junction protein ZO-1 in the colonic tissues, indicating that the microcapsules were capable of repairing the mucus layer and the tight junctions between epithelial cells, thereby reestablishing colonic barrier function. Ouyang et al. (2023a) used microcapsules containing orally administered selenium nanoparticles modified with hyaluronic acid for treatment. Immunofluorescence staining results showed that the fluorescence intensity of pro-inflammatory factors IL-6 and IL-1β in mouse colon tissues was significantly reduced, effectively inhibiting the inflammatory response.

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4 Conclusions and outlook

This review has covered in-depth and critical analysis of the development, application and progress of various MeID. It shows that the controlled drug release of microcapsules offers significant support for precision medicine. The outcomes are expected to provide support for the development of MeID, precise control of drug dosage, and delivery and evaluation methods.

For the preparation of MeID, drug hydrophilicity and solubility provide the basis for selecting shell materials which should also be compatible with specific microencapsulation methods. In addition, modifying the emulsion dispersion process enhances the monodispersity, size, and structure of the microcapsules. Furthermore, the review has shown that coating, micro/nano carriers, and absorption enhancers can be used to achieve multifunctional and intelligent release of microcapsules.

Beside standard methods used to evaluate microencapsulated drugs, biosafety and efficacy were found to be evaluated using in vitro cell and animal models. Structural stability and drug activity on the other hand were found to be typically analyzed using microscopic and spectroscopic techniques. Response mechanisms, however, were found to be investigated under simulated physiological conditions, with fluorescence tracing used to study the in vivo distribution of microcapsules.

From the review, it appears critical that preparation processes are optimized to regulate drug release kinetics and enhance therapeutic efficacy. This is because the current preparation of MeID faces challenges including the high cost of monodisperse microcapsules, difficulties in large-scale production, and suboptimal targeted release performance. In addition, existing in vitro cell models lack dynamic physiological simulation, and studies on in vitro–in vivo correlation of drug release kinetics remain insufficient. Therefore, future research should focus on developing low-cost, scalable methods for producing monodisperse drug microcapsules.

Also, to achieve active and precise targeted release, it is necessary to explore novel shell materials or

carriers with excellent biocompatibility and dynamic responsiveness (e.g., dual responses to disease biomarkers or enzymes) and to integrate these with functionalities for biological targeting and external stimuli (e.g., magnetic fields or infrared radiation). Meanwhile, organ-on-chip technologies have been proposed for evaluating the biocompatibility and pharmacological efficacy of microcapsules, which is expected to accelerate drug development and reduce associated costs. Furthermore, monodisperse drug microcapsules can be used as standardized research objects to establish a database of structure-activity relationships between drugs and shell materials, enabling rapid screening of suitable materials and processes, to accelerate drug development.

It is our view that through interdisciplinary collaborative innovation, the field of MeID will transition from laboratory technology to clinical application in the future, thereby advancing the development of personalized precision medicine.

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Author contributions

Weiguang SU: conception, supervision, acquired funding, performed the investigation and formal analysis, validated the data, and original draft of the manuscript. Dawei WANG: the original draft writing, performed data curation, investigation, and visualization. Siegfried YEBOAH, Jinshen WANG, and Chonghai XU: review and editing of the manuscript. Li WANG: supervision, acquired funding, review and editing of the manuscript. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

All authors declare that they have no conflict of interest. This review does not contain any studies with human or animal subjects.

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Supplementary information

Table S1-S2; Figs. S1-S5

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