Aggregating particles on the O/W interface: Tuning Pickering emulsion for the enhanced drug delivery systems

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1 INTRODUCTION

Recent developments in drug delivery systems have opened up new opportunities for the efficient delivery of drugs, including hydrophobic molecules for anti-tumor therapy, antigens for vaccines, or the engineered cells for adoptive cellular immunotherapy.1-4 It is expected to strategically load different bioactive cargos, pass through the multiple barriers, accumulate inside the target organs or cells, and subsequently release the drugs in a controlled manner, resulting in greater bioactivities and fewer side effects.

During drug delivery, bio-interactions mostly occurred at the bio-interfaces.5 It as particles aggregate on the interface of two immiscible liquids, particle-stabilized emulsion (Pickering emulsion) can offer high stability, an oily core for high-content encapsulation, and the interfacial particulate surface for robust drug release and bio-interactions. It was first proposed by Ramsden and Pickering in 1900s.6,7 But, Pickering emulsions were existed naturally for the numerous years, such as milk (lactoprotein-granule-stabilized emulsion), and the chylomicron (enzyme-or-lipoprotein-granule-stabilized emulsion) during the digestions. In the late 1970s, substantial work had been performed to understand the formation and stability of particle-stabilized interfaces, as well as the mathematic model to illustrate the thermodynamics and kinetics.8-10 With the advancing in low temperature...
field emission scanning electron microscopy and confocal imaging, the interfacial structures of emulsions stabilized solely by solid particles were first made visible in 2002.\textsuperscript{[11]} With the focus on biocompatibility of the particle stabilizers, the use of Pickering emulsions in biomedical applications also extensively focused on drug encapsulation and delivery (such as topical drug delivery and oral drug delivery).\textsuperscript{[12,13]} In 2018, Xia et al. exploited the immunogenicity of Pickering emulsions to function as vaccine adjuvant and delivery systems, which demonstrated the unique softness and fluidity of Pickering emulsion in cellular interactions and the immense potential in the biomedical applications.\textsuperscript{[14]} In fact, it is the aggregation of particles on the interface that determined the bio-properties of Pickering emulsions, such as multi-level structure, high specific area, selective permeability, and stimulus-responsiveness. To start with, Pickering emulsion processes hydrophobic oil core, the adsorbed particles, and the oil/water (O/W) interface. The multi-valent structures paved the way for the efficient loading of bioactive components, including the macromolecular proteins, antigens, or the hydrophobic drugs. Additionally, due to its deformability, Pickering emulsions were expected to deform and pass-through the cellular gaps, thereby facilitating the enhanced tissue dispersion. High specific surface area favored for the higher cellular affinity, and led to the increased cellular internalizations. Moreover, by tuning the particle properties, the permeability and stimulus-responsiveness could be tailored for controlled release of the delivered component.

Despite the promising potentials, the applications of Pickering emulsions in drug delivery were still limited. To start with, previous attempts centered around the applications in petroleum separation, catalysis, or cosmetics, involving the large droplets with limited biocompatibilities, which could hardly be employed in biomedical applications.\textsuperscript{[15–17]} To address this, Pickering emulsions should be re-designed with the approved or biocompatible particles or dispersion phases for the biodegradation and safety profile. Furthermore, as the self-assemble droplets, stability is vitally important, which demands for the optimal particle, energy input, as well as the dispersion and continuous phase conditions. And, the close-packing of particles also left gaps on the interface, which may lead to the pre-leakage of the cargos. Thus, to harness the efficient delivery and controlled release of the loaded drugs, it is required to gain a deep understanding of what the interfacial particle aggregation would bring, and rationally tune the sizes, charges, shapes, and stimuli-responsiveness, accordingly.

To unravel the relationship underlying the physiochemical properties and the delivery process, we reviewed the recent development of Pickering emulsions in drug delivery. Strategies to stabilize the emulsions, as well as the thermodynamic deductions were illustrated according to the particle properties, energy input, and the choice of continuous and dispersion phases. In addition, we also discussed the tunable specific surface area, permeability, softness, and multi-level structures of Pickering emulsions as well as the bio-effects in strategic loading, efficient delivery, and controlled release for the potential applications in parenteral and mucosal drug delivery, vaccines, and cell-based therapies.\textsuperscript{[18–20]} Through rationally controlling the particle aggregation on the O/W interface, Pickering emulsion may shed light on the development of novel, safe and efficient drug delivery systems.

2 | AGGREGATING PARTICLES TO STABILIZE PICKERING EMULSIONS

Under the action of mechanical force, the dispersed phase is mixed with the continuous phase, and formed the droplets. Meanwhile, solid particles in the continuous phase are adsorbed on the interface to prevent the combination of droplets and stabilize the emulsion, indicating the importance of the interfacial attachment to the O/W interface of solid particles.

During the formation of Pickering emulsions, particles are adsorbed between the O/W interface. As the immersion of the particle, it came into partial contact with both phases, which occupied a circular portion of the interfacial region (S\textsubscript{ad}, see the yellow area in Figure 1). Thus, the “removed” area reduces interfacial energy, constituting a thermodynamic drive for colloidal adsorption.\textsuperscript{[21]} In this case, the interfacial adsorption of a single particle can be considered as the interactions among the particle–water (p–w), particle–oil (p–o), and oil–water (o–w) interfaces. The corresponding energy (\(\Delta E\)) can be expressed as:

\[
\Delta E = S_{p-w} \times \gamma_{p-w} + S_{p-o} \times \gamma_{p-o} - S_{ad} \times \gamma_{o-w} \quad (1)
\]

where \(S\) and \(\gamma\) represent the corresponding interfacial area and the interfacial tension, respectively. For the indicated oil phase, water phase and particle, \(\gamma_{p-w}, \gamma_{p-o}\), and \(\gamma_{o-w}\) can be considered as constant value. Accordingly, to achieve the minimum energy, particles were expected to occupy more interfacial areas. In this case, particle wettability, characterized by the three-phase (the disperse phase, continuous phase, and the solid particles) contact angle (\(\theta\)), is a crucial parameter for the adsorption of particles at the O/W interface, and is commonly defined by the Young equation:\textsuperscript{[22]}

\[
\cos \theta = \frac{\gamma_{p-o} - \gamma_{p-w}}{\gamma_{o-w}} \quad (2)
\]

If the particle is considered as a sphere with the radius of \(R_{ad}\), the attachment energy of a colloidal particle at a liquid–liquid interface in Equation (1) can be expressed as:\textsuperscript{[23]}

\[
\Delta E = \pi R_{ad}^2 (1 - |\cos \theta|)^2 \times \gamma_{o-w} \quad (3)
\]

where \(\gamma_{o-w}\) is the interfacial tension between the oil and water (disperse and continuous phase, respectively), \(R_{ad}\) is the radius of the spherical particle and three-phase contact angle \(\theta\) is the surface wetting properties (also known as the wetting contact angle). Thus, thermodynamically, stable Pickering emulsions depend on the particle properties (particle size, shape, and wettability), as well as the interfacial tension between oil/water phases.

Considering the above factors, the properties of solid particles and oil/water phases are the main factors determining the formation of Pickering emulsion, and the preparation method for the enough energy input.\textsuperscript{[24–27]} In the following subsections, strategies were discussed in regard to tuning the
FIGURE 1 Schematic illustration on particle adsorption on the oil/water interface, where $S_{ad}$ represents the particle “occupied” interfacial area. $\gamma_{p-w}$, $\gamma_{o-w}$, and $\gamma_{p-o}$ represent the interfacial tension between particle and water, particle and oil, and oil and water phases, respectively. $\theta$ expresses the equilibrium position of the particle in terms of contact angle

particle properties, the continuous and dispersion phases, as well as the preparation methods.

2.1 Tuning the particle properties

To stabilize Pickering emulsion, particle properties were the main governing parameters to be considered. The impact of the particle properties, such as size, shape, wettability, and charge on the droplet formation was summarized in Figure 2, and discussed as below.

In regard to the particle sizes, larger particles required higher energy to adsorb on the O/W interface, which in turn impeded the stability of the formed emulsions.\textsuperscript{[28]} In most cases, smaller particles allowed for a more stable particle-aggregated droplet. In a recent study, poly(D,L-lactic-co-glycolic acid) (PLGA) particles were prepared with various sizes.\textsuperscript{[29]} It was found that smaller particles (330 nm) favored for the dense array at the droplet interface, which prevented the droplet coalescence. But, larger particles (620 and 1150 nm) resulted in the increased interparticle gaps and higher interfacial tension, impeding the stability of the droplets.

Besides the sizes, particle’s shape also played a vital role in emulsion stability. In recent years, non-spherical particles such as fibers, rods, and cubes have been employed in Pickering emulsions, leading to the versatile morphology and multiple functions.\textsuperscript{[30–32]} According to Equation (1), the adsorption of particles could be considered as the occupation on the liquid-liquid interface. Particles of diverse shapes (cubic, disc, rod, etc.) occupied varied interfacial areas, as demonstrated in Figure 2ii). Thus, with the well-designed shapes, particles that occupied the maximum surface areas may lead to stable emulsion droplets. To illustrate this, Madivala et al.\textsuperscript{[33]} synthesized a series of elliptical polystyrene particles with different aspect ratios (Ratios of length and width of the particles), by stretching the semi-solidified microspheres within the polyvinyl alcohol film. By occupying larger areas on the interface, the microrods with higher aspect ratio resulted in the droplets with the increased stability. In a similar fashion, Li et al.\textsuperscript{[34]} prepared needle-like cellulose nanocrystals (length: 200 nm, width: 16.4 nm) and ellipsoid cellulose nanocrystals (length: 18.8 nm, width: 10.9 nm) to stabilize the droplets. With higher aspect ratio, the needle-like nanocrystals formed more stable emulsion droplets than the ellipsoid ones. But, particles with much higher aspect ratio may impact the interfacial arrangement, impeding the surface coverage. In this manner, particles of lower aspect ratios could align side by side through the attractive forces, which covered the enlarged interfacial area. On the other hand, due to the geometry restrictions, particles with much higher aspect ratios may result in the reduced surface coverage. In a recent work, Isabelle et al.\textsuperscript{[35]} prepared cotton cellulose nanorods (CCN) and Cladophora cellulose nanorods (ClaCN) with similar wettability and charges but varied aspect ratios from 13:1 to 160:1. Then, CCN- or ClaCN-stabilized emulsions were prepared using hexadecane and 50 mM NaCl with an oil/water ratio of 30/70. Results showed that a high coverage (100%) was obtained with the shorter CCN (with the aspect ratio of 13) and only 40% of coverage with ClaCN (with an aspect ratio of 160). Notably, in the case of ClaCN, although the interface was only covered by 40%, the long nanorods formed interconnected networks on the O/W interface and led to the enhanced stability (Figure 3A). Thus, the impact of the optical aspect ratio and surface coverage should be considered case by case. The stability of Pickering emulsion was only achieved after the optimizations on a series of parameters, such as particle size, charge, surface textures, or the surface arrangement.

In order to change the wettability of particles, it is commonly used to modify the surface with hydrophobic or hydrophilic groups. A typical example was hydrophobic
FIGURE 2 Schematic illustration on the particle properties that dictate the formation of Pickering emulsion: (i) Size: impacting the dense array at the droplet interface. Smaller particles favor for the higher surface coverage. (ii) Shape: impacting the surface morphology and coverage of the droplet. (iii) Wettability: influencing the affinity of interfacial particles towards oil and water phase. The contact angle ($\theta$) indicated the amphiphilicity to prepare stable Pickering emulsions. (iv) Charge: the electrostatic attraction between positive-charged particles and oil-water interfaces facilitates the interfacial assembly (above). The electrostatic attraction between the oppositely charged particles aid the emulsion stability (bottom).

FIGURE 3 (A) Schematic illustration on the influence of particle’s aspect ratio on stability mechanisms of Pickering emulsion. Lower aspect ratio (CCN) align side by side on the oil/water interface, while interconnected networks of droplets are formed by higher aspect ratio (CuCN). Reproduced with permission.[35] Copyright 2013, Royal Society of Chemistry. (B) SEM images of Janus particles with varied wettability, which were prepared by tuning the mass ratios of tripropylene glycol diacrylate (TPGDA)/methacryloxypropyl dimethylsiloxane (DMS): (i) 1/10, (ii) 1/4, (iii) 1/1, and (iv) 2/1. Reproduced with permission.[42] Copyright 2017, American Chemical Society.

modification by adsorption of octyl, propyl- and methyl-based organosilanes on the surface of silica granules, while hydrophobic surface was obtained by functionalization of hydrophilic methoxy-poly(ethylene glycol). Specifically, the contact angle of the modified silica particles was determined as 90°, indicating the appropriate wettability. Na et al.[37] modified the starch granules with short-chain fatty acid acylation, which replaced the hydrophilic OH groups with the hydrophobic acyl groups of short-chain fatty acids. The prepared emulsions were proved to harbor the increased stability over the 50-day storage.

To achieve amphiphilicity against the continuous and dispersed phases, Janus particles, possessing both hydrophilic and hydrophobic hemispheres, were developed to stabilize the emulsion droplets.[38] Furthermore, by tuning the hydrophobic and hydrophilic regions, the adsorption state of the particles on the O/W interface could be easily modified, enabling the various types of emulsions.[39,40] Using microfluidic techniques, Janus particles of different sizes were synthesized by adjusting the inner-phase flow rate with adjustable hydrophobicity. It was discovered that synthetic hydrogels had a hydrophilic side, which better enhanced particle adsorption at the O/W interface because they interacted better with the aqueous phase.[41] In this way, Pickering emulsions were observed to remain stable over months. In another interesting study,[42] Janus particles were prepared using Janus emulsions as templates with varied morphologies and surface properties. Janus emulsions prepared by the one-step vibrational emulsification were suitable templates for the batch-scale fabrication of the Janus particles. Two polymerizable monomers of tripropylene glycol diacrylate (TPGDA) and methacryloxypropyl
dimethylsiloxane (DMS) were used as oil phases, and the surfactant aqueous solutions were used as continuous phases, and they were mixed with different mass ratios using the Vortex-Genine 2. After UV irradiation, the polymerization for both monomers of TPGDA and DMS occurred in situ (Figure 3B). Accordingly, the highly controllable morphologies of the Janus particles were able to be achieved by several factors, such as changes in the mass ratio of TPGDA and DMS to tune the volume fractions of each lobe, altering the mixed surfactants to tune the surface coverage of one phase to the other, and, more importantly, adjusting the input emulsification energy to achieve the wide size range. In this manner, the biphasic particles caused phase inversion of the emulsion droplets, which offered the potential to engineer the shapes and functions of Pickering emulsion for the enhanced bio-applications. But, the fabrications of Janus particles were too complex, which made it difficult to translate to the large-scale production of Pickering emulsion for drug delivery. Additionally, the modification materials, such as TPGDA or DMS, can hardly be degraded, which may impede the safety profile of the prepared Pickering emulsion. Thus, future efforts were expected in the biosafety of the Janus particles, as well as the facile and efficient fabricating methods.

In addition to the wettability, the charges also impact the emulsion stability.\[43,44\] By strengthening the electrostatic attraction between particles and oil-water interfaces, particles were prone to adsorb on the interface, and thus reducing the energy barrier for the stable droplets. For most oil phases, the oil droplets possessed a negative charge on the surface when dispersed in water.\[45\] Hence, the use of cationic nanoparticles as stabilizers of Pickering emulsions could improve the stability due to the affinity of the particles to the O/W interface. Under these circumstances, Caroline et al.\[46\] prepared cationic nanofibrillated cellulose and used them as stabilizers on the oleic acid/water interface of Pickering emulsions, they found cationic cellulose was more efficient in partitioning to the O/W interface compared to their anionic analogous. The electrostatic attraction between the positively charged trimethylammonium groups and the negatively charged deprotonated oleic acid reduced the interfacial tension and improved the colloidal stability of oil-in-water Pickering emulsions. In recent reports, the emulsion stability could be enhanced by using the oppositely charged colloidal particles. Based on this electrostatic effect, Binks et al.\[47\] used a mixture of two types of particles with opposite charges (Positively charged and negatively charged polystyrene latex particles functionalized by amidine and sulfate groups respectively) to stabilize Pickering emulsions with good long-term stability being obtained without the need for any electrolyte.

### 2.2 Regulating the dispersion and continuous phase

Apart from modifying the particles, adjusting oil/water phase and oil/water phase ratio is another strategy to stabilize the emulsion.\[48,49\] According to Equation (2), the three-phase contact angle is directly linked to the oil used through the interfacial tensions. As a result, the choice of oil, especially the oil polarity, may dictate the contact angle on the interface. Binks et al.\[50\] investigated the effect of the polarity of the oil phases. They showed that nonpolar oils (e.g., hydrocarbons) favored the formation of oil-in-water emulsions whereas polar oils (e.g., esters and alcohols) favored water-in-oil emulsions. Such influence of the polarity of the oil phase on aggregating polystyrene latex stabilized emulsions was observed, showing that low polarity oils such as \textit{n}-dodecane led to oil-in-water emulsions, whereas relatively polar oils such as 1-undecanol led to water-in-oil emulsions.\[51\] In addition, it was demonstrated that for a constant amount of iron particles and a constant amount of emulsification time, the emulsified oil volume increased as the viscosity of the oil (dispersed phase) decreased. By slowing the diffusion and adsorption of particles at the O/W interface, oil viscosity dampened particle anchoring at the O/W interface. Thus, there existed a dispersed phase viscosity limit for a given agitator time beyond which emulsification became impossible. In case of silicone-oil-based emulsions, stable glass-bead-stabilized droplets were prepared when the oil viscosity was lower than 486 mPa\cdot s. Alternatively, the higher viscosity led to the dramatic increase in sizes. The increase in droplet sizes may be attributed by the limited particle diffusion and high energy input that required for the highly viscous oil.\[52\]

By regulating the ionization state of the particles, the continuous phase conditions (such as pH and ion concentrations) may impact the emulsion formation. For instance, the surface charge of the cellulose nanocrystals was too high to adsorb on the interface between oil and pure water (surface charge density greater than 0.03 e/nm$^2$). But, by adding higher amount of the electrolytes, such as NaCl or KCl in the continuous phase, the electrostatic forces were shielded for the increased droplet stability.\[53\] In case of silica-based Pickering emulsion, electrostatic repulsions between the charged silica particles acted against the adsorption at high pH and low ionic strength. Emulsion stability was ensured by decreasing electrostatic repulsions by lowering the pH from 9 to 3 of the continuous phases. Even at much lower silica concentrations, monolayer of silica particles were stabilized on the O/W interface, with the surface coverage of 54%, indicating that regulating the continuous phase may offer a robust way to control the stability of the droplets.\[54\]

### 2.3 Optimizing the energy input and preparation methods

Because of the high interaction energy of the interfaces, Pickering emulsions demand for an external force to overcome the high energy barrier at the particle-adsorbed interface. According to the energy input and droplet forming mechanism, the droplet-forming methods were discussed into two categories (Figure 4). Top-down approaches, such as homogenization and sonication, that employ high energy-input to disrupt large oil droplet into small ones in bulk scale. On the other hand, the bottom-up approaches can induce the interfacial attachment of the particles in a droplet-by-droplet manner, for instance, the microfluidic techniques. Here, the approaches to produce Pickering emulsions were summarized in Table 1.

As commonly employed bulk methods, high-pressure homogenization and sonication can break down the large oil droplets into small ones and offer sufficient energy input for the formation of Pickering emulsions (Top-down). In
case of high-pressure homogenization, Li et al.\textsuperscript{[59]} prepared Pickering emulsions via tea water-insoluble protein nanoparticles. They found that either increasing homogenization pressure (0–80 MPa) or increasing homogenization times (under 40 MPa) can decrease the emulsion sizes. For ultrasonication, the droplet sizes could be tuned by changing the ultrasound frequency and the emulsification time. He et al.\textsuperscript{[60]} observed that the increased sonication time led to the reduction of droplet size and polydispersity. When the mixture was sonicated for only 1 min, broad size distributions were observed. Meanwhile, average diameter of the emulsion droplets was decreased to around 5 μm when the sonication time was expanded to 7 min. Collectively, the high mechanical shear provided by ultrasonics and homogenization facilitated the breakdown of energy barriers during the emulsion formation. The higher energy input typically led to smaller sizes of the prepared droplets.

But both of these devices produced a high droplet dispersion due to unevenly distributed power and duration under fluidic conditions. As a result of such inhomogeneities, the prepared emulsions were more prone to agglomerating and even breaking, which limited the biological applications that required narrow droplet sizes. What’s more, fabricating Pickering emulsion with smaller sizes (below 1 μm) was rather challenging. High mechanical shear to produce smaller droplets could also destabilize or deform fragile particles or aggregates during the emulsification. In case of microgels, Destribats et al.\textsuperscript{[61]} found that emulsification energy changed the morphology of the microgel. A high energy supply made the microgels flatten at the droplet interface, while the low energy input resulted in the spherical-shape microgels that adsorbed on the O/W interface.

In order to obtain uniform-size droplets and avoid deforming fragile particles, membrane emulsification was employed to prepare uniform-sized Pickering emulsions. The techniques were evolved with direct membrane emulsification (DME) and premix membrane emulsification (PME) processes. For DME, the dispersed phase was pressed or injected through the uniform-pore membrane into the continuous phase under the critical trans-membrane pressure. For PME, the dispersed phase and continuous phase were mixed to obtain the pre-emulsion, and then pressed through the membrane for the uniform-distributed Pickering emulsions. As one of the pioneers in this area, Prof. Ma’s group\textsuperscript{[62]} heralded membrane emulsification technique to prepare uniform-sized Pickering emulsions. In this way, chitosan-coated alginate particles-stabilized emulsion was prepared with a coefficient variation value of 23.2% using the PME.
technique. Afterward, via the polymer deposition method, the particle-covered droplets were further solidified for insulin encapsulation (96.7%). The colloidosomes were proved to retain stable in the acidic solutions, but burst release of insulin at the simulated intestinal fluid (pH = 6.8), indicating the potential in oral delivery. To better tune the emulsification process and the droplet properties, microfluidic techniques were emerged. Additionally, through a microfluidic device, the droplets were formed drop-by-drop and dispersed individually into the continuous phase, which allowed for more precise control over the droplet properties. Capillary microfluidic device and mesostructured reactor, offered the tailor-made ways to control over the droplet size and morphologies. Moreover, the miniature devices such as chip-type microfluidic devices, introduction designs of solutions, and capillary-type microfluidic devices, were used to produce lipid nanoparticles as drug delivery carriers, which may shed light on the preparation of Pickering emulsions. The formation of the emulsion can be achieved by introducing an oil phase and a dispersed phase containing particle-stabilizers solution into the microfluidic device. In this manner, the properties of droplets can be easily tuned by the flow rate, flow rate ratio (the rate ratio of the aqueous and the oil solution), mixing rate, and particle concentrations.

With the well-designed devices, delicate fabrications of Pickering emulsion can be achieved. For the bulk preparation methods, excess fluidic particles in the continuous phase were considered inevitable, which may impede the biofunctions or safety of the prepared formulations. Recently, with a flow-focusing emulsification junction and a series of triangular posts, the excess particles were removed by a new microfluidic device. After emulsification, the emulsions and excess particles can travel to a series of triangular posts, where a pure solvent stream was attached to carry the emulsion droplet (CPca). Due to a variation of pinched flow fractionation, the prepared emulsion droplets were forced to “climb” along the triangular posts with the high-speed flow of CPca, which allowed for the separation from the external continuous phase (CPex) streams. Then CPex was flowed into a waste port to collect the excess particles (Figure 4B-iii).

Notably, due to the limitations from the membrane pore size and energy input, the prepared emulsion droplets may not smaller than the high-energy emulsification methods, such as sonication and homogenization. New techniques for nanosized Pickering emulsions were demanded.

In summary, the stability of Pickering emulsions depended on solid particle’s size, shape, wettability, charge, oil/water ratio, pH, ion concentration, and the sufficient energy input. Therefore, in the preparation of Pickering emulsions, the selection of particles with suitable properties, the appropriate oil/water ratio, and conditions, and the energy input methods should be considered. Furthermore, as drug delivery system, the biocompatibility of the particles and the oil phase is vitally important. As shown in Table 2, the clinical-approved materials for parenteral administration are still limited. Future efforts are demanded to exploit the structures and properties of the approved materials, such as PLGA, squalene, or alum hydrogel, for the enhanced stability and delivery efficiency of the prepared Pickering emulsions.

### 3 AGGREGATING PARTICLES TO DICTATE THE DELIVERY PROCESS

By tuning the particle assembly on the O/W interface, Pickering emulsions were designed with high specific surface areas, permeability, softness, and multi-level structures. To illustrate the vast bioactivities in drug delivery, recent development of Pickering emulsions in drug delivery was reviewed in this section, and summarized in Table 3. Additionally, the structural-effect relationship was discussed with respect to the strategic loading, efficient delivery, and controlled release.

#### 3.1 On-demand loading

As shown in Figure 5, Pickering emulsions were produced by mounting the interfacial-aggregated particles on the oil droplet, allowing large amounts of hydrophobic components to be encapsulated within ample oil nuclei. Additionally, the particulate O/W interface presented high specific surface areas for the efficient adsorption of a series of macromolecules, such as antibodies, antigens, or therapeutic
### Table 2. The biodegradable and clinical-approved components to prepare Pickering emulsions

| Materials          | Component         | Biodegradable | Clinical approved | Properties                                                                                                                                                                                                 | Ref. |
|--------------------|-------------------|---------------|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Aluminum hydroxide | Particle/hydrogel | −             | +                 | Aluminum hydroxide adjuvant is approved for using in parenteral human and veterinary vaccines. The positively charged microgel possesses high specific area and deformability to better absorb on the oil/water interface, which is beneficial for the stability of Pickering emulsions. | [67,68] |
| PLGA               | Particle/polymer   | +             | +                 | Clinical approved for parenteral and oral administrations. The degradation rate and ratio of hydrophilic and hydrophobic groups can be tuned by varying the PLGA molecular weight or its lactide/glycolide ratio. | [69–71] |
| Albumin            | Protein granule/particle | +       | +                 | Clinical approved for parenteral and oral administrations. Low dosage and the small sizes of albumin (∼6 nm) may result in the droplets with smaller sizes (∼300–500 nm). | [72] |
| Starch             | Particle          | +             | +                 | Clinical approved for oral administration. The deformability of starch particles can be tuned by heating, which facilitates the adjustable permeability of Pickering emulsion. | [73] |
| Cellulose          | Particle          | +             | +                 | Clinical approved for oral administration. Natural amphiphilic characteristic, high aspect ratio, large elastic modulus, and CNCs-based complexes generating by physical adsorption interactions (e.g., hydrogen bonding and electrostatic attraction interaction) to an easier approach to adjust hydrophobicity and interfacial tension of CNCs. | [74,75] |
| Alginate           | Particle/hydrogel | +             | +                 | Clinical approved for oral and topical administrations. Alginate is a natural anionic polymer that possesses pH-sensitivity. Modifications (e.g., linking with alkyl groups or chitosan) were demanded to tune the hydrophilicity for the stable emulsions. | [76,77] |
| Titanium dioxide   | Particle          | −             | +                 | Clinical approved for oral and topical administrations. Titanium dioxide displays UV-induced tailored wettability and controlled release by demulsification due to the hydrophilic conversion of the particles. | [78–80] |
| Cyclodextrin       | Particle          | +             | +                 | Clinical approved for topical administration. Good emulsifying effect due to cyclodextrins’ amphiphilic properties. Cyclodextrins have large hydrophobic cavities that can encapsulate natural bioactive components. | [81–83] |
| Squalene           | Oil phase         | +             | +                 | Squalene is a substance naturally found in the human body, as well as in animals and plants. Squalene, which is derived from the liver of sharks, has been used in over-the-counter medicines, dietary health supplements, and vaccines. | [84] |
| MCT                | Oil phase         | +             | +                 | Clinical approved for parenteral, oral, and topical administrations. Absorption enhancement, stability against oxidation, nontoxicity, nonirritation, and noninhibition of skin respiration. | [85,86] |
| Soybean oil        | Oil phase         | +             | +                 | Clinical approved for parenteral, oral, and topical administrations. | [87–90] |
| Corn oil           | Oil phase         | +             | +                 | Clinical approved for parenteral, oral, and topical administrations. | [91] |
| Olive oil          | Oil phase         | +             | +                 | Clinical approved for oral and topical administrations. | [92] |
| IPM                | Oil phase         | +             | +                 | Clinical approved for topical administration. IPM is used as a penetration enhancer for transdermal formulations, and may increase bioavailability in topical and transdermal applications. | [90] |

**Note:** Here, biodegradable materials are referred to the materials that can be dissolved, decomposed or digested by the human body, which do not mean the biocompatibility or the toxicity of the particles.

**Abbreviations:** PLGA, poly(lactic-co-glycolic acid); CNC, cellulose nanocrystals; IPM, isopropyl myristate; MCT, medium-chain triglycerides; + = approved for clinical usage or biodegradable; − = negative for biodegradation or clinical application.
| Application                  | Route | Particle stabilizer | Drug                        | Emulsion type | Delivery perspective                                                                 | Ref. |
|------------------------------|-------|---------------------|-----------------------------|---------------|--------------------------------------------------------------------------------------|------|
| Vaccine delivery system      | i.m./s.c. | Albumin            | Lipopeptide ovalbumin       | O/W           | Albumin-stabilized emulsions can pass through the cellular gaps, which favored the direct lymph-node transfer. | [93] |
|                               | i.m.  | Alum                | Recombinant RBD of the      | O/W           | The hydrophobicity of the oil/water interface allowed a higher affinity towards the cellular membranes. | [94] |
|                               | i.m./s.c. | PLGA                | Ovalbumin                   | O/W           | Particle aggregation amplified the protonation effect and allowed for the lysosome escape of antigen. | [14] |
| Anti-tumor drug delivery     | Oral  | SBNP                | Curcumin and betanin        | W/O/W         | Co-encapsulation of betanin and curcumin by SBPN-stabilized W/O/W emulsions induced synergetic anti-tumor effect. | [95] |
|                               | s.c.  | Nanogels polymerized by NIPAM, MAA, SBMA and BAC | Doxorubicin and ICP inhibitor HY19991 | O/W           | The multi-level structure of Pickering emulsion allowed for the delivery of different drugs to tumor sties. | [96] |
|                               | i.v.  | PNA                 | Doxorubicin                 | O/W           | The special tumor cell-surface molecule markers coupling on nanocapsules via Pickering emulsion was proposed as a nanocarrier for targeted delivery. | [97] |
| Anti-inflammatory and analgesic drug delivery | Topical | Cyclodextrin         | Bupivacaine                 | W/W           | Regulated the skin penetration depending on various types of oil.                         | [98] |
| Oral                         | HSA-CS | Ibuprofen           | O/W                         | The pH-responsiveness enabled the controlled release of ibuprofen, achieving zero content in SGF and 88.37% content in SIF. | [99] |

Abbreviations: i.m., intramuscular administration; s.c., subcutaneous administration; i.v., intravenous administration; PLGA, poly(lactic-co-glycolic acid); P(NIPAM-co-AAC), poly(N-isopropylacrylamide-co-acrylic acid); SBNP, sugar beet pectin-bovine serum albumin; NIPAM, N-isopropylacrylamide; MAA, N-methylallylamine; SBMA, betaine-based zwitterionic sulfobetaine methacrylate; BAC, N,N′-bis(acryloyl) cystamine; ICP, immune checkpoint inhibitor; PNA, poly(N-isopropylacrylamide-co-acrylic acid) nanogel; EHL/COS, lignin/chitosan; HSA-CS, chitosan-hydrophobic alginate nanocomposite; SGF, simulated gastrointestinal fluid; SIF, simulated intestinal fluid; W/O, water-in-oil; O/W, oil-in-water; W/W, water-in-water; W/O/W, water-in-oil-in-water.

**Figure 5** Hierarchical structures of Pickering emulsion for the strategic loading of multiple drugs: (i) oil core: encapsulating hydrophobic drugs within the oil core, or encapsulating hydrophilic drug through W/O/W (water-in-oil-in-water) double emulsion, (ii) Interparticle gaps: conferring the high-specific surface area to adsorb therapeutic proteins, antigens, and target molecules within the gaps, (iii) Surface particles: exploiting with the tunable physiochemical properties to load various components via chemical linking, electrostatic interactions or encapsulated within the hollow or multi-shell nanostructures.
Furthermore, the densely packed particles could be exploited with the hierarchical structure (hollow or porous particles), as well as the tunable surface properties. These unique features enabled the strategic drug loading via the hydrophobic interactions, chemical linking, or electrostatic adsorptions. For example, via the cashew gum nanoparticle-stabilized droplets, hydrophobic indomethacin was encapsulated within the oil core, with the encapsulation efficiencies up to 52%. In addition, Han et al. achieved up to 94% curcumin encapsulation in the inner oil (medium chain triglyceride) by using a chitosan/gum Arabic nanoparticle-stabilized oil-in-water Pickering emulsion.

Furthermore, with the multi-level structures, Pickering emulsion can be exploited as potent multi-drug delivery systems. Cocktail therapy, which involves the co-administration of multiple drugs (such as cancer vaccines, photothermal therapy, and chemotherapeutics), has repeatedly been shown to be a promising treatment for tumor regression and multidrug resistance. But, how to spatiotemporally deliver therapy, and chemotherapeutics, has repeatedly been shown to be a promising treatment for tumor regression and multidrug resistance. In addition, Pickering emulsion was designed with a particle-packed shell and oily core, which offered mechanical-sensing deformability. In this manner, the droplets can attach to and deform between cells, allowing them to pass through endothelial spaces (20–100 nm) and penetrate to the target sites. In a recent work of our group, we developed a deformable albumin-stabilized emulsion. After the intramuscular injection, the droplets (~ 350 nm) could hitchhike on the interstitial flow, and were attached and spread between the cells and adjusted themselves to pass through the endothelial gaps. In this manner, the droplets were passively drained to the surrounding lymphatic vessels (intracellular pathway). Intriguingly, surfactant-stabilized droplets were observed to have a hydrated shell that prevented them from interacting with cells. Thus, limited surfactant-stabilized droplets were accumulated within the draining lymph node (LN). Additionally, some emulsions of larger sizes (above 350 nm) could retain at the injection sites for potent antigen uptake, activation, and LN-tropism, which allowed the vaccines to be transferred intracellularly to the LNs via the antigen-presenting cells (intracellular pathway). Compared with solid albumin particles and antigen, dual LN transfer significantly enhanced antigen accumulation and stimulation of LN drainage, stimulating cellular immune responses, and improving survival of tumor-bearing mice. As shown in Figure 6A, through labeling antigens, the cellular skeleton, and lymphatic endothelial cell membranes with Cy5, FITC-phalloidin, and Fluro@555-VEGFR (an indicator for lymphatic endothelial cells) respectively, the deformable granule-stabilized emulsion passed through the intracellular and intracellular transfers to LN was observed. As a result, antigen accumulations within the LNs (Figure 6B,C), comparable levels of antigen uptake and enhanced CD86 expression, cross-presentation of dendritic cells (DCs), and CD8+ T cell activation (Figure 6D) were achieved in the LNs.

3.2 Advanced delivery

Efficient drug delivery is expected to surpass the multi-scale barriers: (1) Tissue Level: Distributing to the specific tissue or organ for higher precision and lower toxicity, (2) Cellular level: Provoking the internalizations of the delivered cargos for the onset of therapeutic effects, (3) Subcellular level: Escaping from the lysosomes to prevent the enzyme-triggered degradation of the delivered cargos. In this section, the structural effects of Pickering-emulsion-based drug delivery system were discussed in regard to the efficient tissue targeting, cellular uptake, and cytosolic delivery.

3.2.1 Distribution

Penetrating the interstitial barriers to the target tissue has always been a challenging task. With the nano-sized intercellular gaps (10–100 nm), the delivery vehicles with larger sizes and positive charges, such as chitosan microparticles or alum hydrogels, tend to retain at the administration sites. To address this, previous attempts concentrated around engineering the vehicles with smaller sizes and negative-charged surfaces. But, the smaller particles or emulsions also led to the decreased drug payloads, impeding the wide and robust medical efficacy. In fact, immune cells, such as macrophages, monocytes, or dendritic cells, can deform and squeeze into the intercellular spaces under the physiological environment, where they infiltrate into the inflammation site and eliminate the pathogens. By imitating this, Pickering emulsion was designed with a particle-packed shell and oily core, which offered mechanical-sensing deformability. In this manner, the droplets can attach to and deform between cells, allowing them to pass through endothelial spaces (20–100 nm) and penetrate to the target sites. In a recent work of our group, we developed a deformable albumin-stabilized emulsion. After the intramuscular injection, the droplets (~ 350 nm) could hitchhike on the interstitial flow, and were attached and spread between the cells and adjusted themselves to pass through the endothelial gaps. In this manner, the droplets were passively drained to the surrounding lymphatic vessels (intracellular pathway). Intriguingly, surfactant-stabilized droplets were observed to have a hydrated shell that prevented them from interacting with cells. Thus, limited surfactant-stabilized droplets were accumulated within the draining lymph node (LN). Additionally, some emulsions of larger sizes (above 350 nm) could retain at the injection sites for potent antigen uptake, activation, and LN-tropism, which allowed the vaccines to be transferred intracellularly to the LNs via the antigen-presenting cells (intracellular pathway). Compared with solid albumin particles and antigen, dual LN transfer significantly enhanced antigen accumulation and stimulation of LN drainage, stimulating cellular immune responses, and improving survival of tumor-bearing mice. As shown in Figure 6A, through labeling antigens, the cellular skeleton, and lymphatic endothelial cell membranes with Cy5, FITC-phalloidin, and Fluro@555-VEGFR (an indicator for lymphatic endothelial cells) respectively, the deformable granule-stabilized emulsion passed through the intracellular and intracellular transfers to LN was observed. As a result, antigen accumulations within the LNs (Figure 6B,C), comparable levels of antigen uptake and enhanced CD86 expression, cross-presentation of dendritic cells (DCs), and CD8+ T cell activation (Figure 6D) were achieved in the LNs.

Pickering emulsion was also engineered with the enhanced tissue-infiltration capabilities by the incorporation of penetration-enhancing molecules, coupled with its deformability. In intradermal delivery, glycerol or oleic acid was used as the oil phase to distort the stratum corneum structures and tight junctions. Through the particle-stabilized droplet’s pliability, it was easier for the droplet to adhere to and penetrate the skin’s intercellular spaces. Moreover, the particle-aggregated interface conferred a higher adhesion energy, leading to the partial breaking of the
FIGURE 6  Advanced delivery of Pickering emulsion (A) Muscle sections and schematic illustrations of the intercellular and intracellular transfer of Cy5-labeled antigens (red) between muscle cells (blue) or within lymphatic vessels (green). (B) Antigen accumulations within the LNs 24 h after administration. The nucleus and antigens are labeled by DAPI (blue) and Cy5 (red), respectively. Scale bar = 400 μm. (C) The presence of antigens and the corresponding quantitative fluorescence intensity in lymph nodes (LNs) drainage over time. The left picture showed the representative fluorescent images of LNs 24 h after administration. (D) Proportions of CD86+ DCs, SIINFEKL-MHC I-presenting DCs, and CD8+ IFN-γ+ T cells within the LNs 24 h after administration. DASE and SAP are the abbreviations of albumin-stabilized emulsions and solid albumin particles, respectively. Reproduced with permission. [93] Copyright 2021, Wiley. (E) Schematic illustrations on the enhanced cellular internalization: (i) particulate surface: providing high specific surface area to facilitate the landing and grasping of the cellular microtubules; (ii) Partial exposing oil: increasing the hydrophobicity for the enhanced cell affinity; (iii) Softness: deforming during the cellular contact to enlarge the contact area for the increased cellular internalization.

As one of the major advantages of drug delivery systems, targeted delivery offers the potential to reduce the undesirable side effects and increase the therapeutic efficacy by boosting the tissue-specific accumulations of the delivered cargos. With the particle-adsorbed interface, Pickering emulsion can be exploited as efficient targeted delivery system. The particle-adsorbed interface offered the loading sites of target ligands, which were proved to move laterally to trigger the dynamic recognition. Furthermore, with the oily core, Pickering emulsions were granted with deformability, which can attach on the target cells, and enlarge the contact areas to increase the cellular binding of the modified target ligands. In a recent study, doxorubicin (DOX) was encapsulated within the nanocapsule prepared via the solidification of Pickering emulsion. Then, the nanocapsule was cross-linked by redox-sensitive disulfide bond and targeting agent of c(Arg-Gly-Asp-d-Phe-Lys) (cRGD) peptide. As a result, the intracellular concentration of DOX was markedly increased compared with the fluidic DOX, displaying that cRGD targeted delivery increased the cellular uptake. Furthermore, significant tumor growth inhibition was observed in the B16F10-melanoma-bearing mice from day 10 to 22. These data indicated that the redox-sensitive nanocapsule facilitated the DOX release within the intertumoral microenvironment. [97] In a recent study, Jia et al. developed a pH-responsive Pickering nanoemulsion (PNE) by using multi-sensitive nanogels (designated as SNG) for the spatiotemporal delivery of DOX and immune checkpoint inhibitor (HY19991). In regard with the varied fuction sites,
it is expected to deliver DOX intracellularly, and target the extracellular membrane-residing immune checkpoint receptors for the enhanced anti-tumor efficacy. By the polymerizations of thermosensitive N-isopropylacrylamide (NIPAM), pH-responsive N-methylallylamine (MAA), betaine-based zwiterionic sulfobetaine methacrylate (SBMA), and disulfide bond-containing N,N-bis(acryloyl) cystamine (BAC), the nanogels were granted with pH-sensitivity and glutathione (GSH)-responsive properties. Then, nanogel-stabilized emulsion was prepared to encapsulate DOX with the interfacial nanogels, and HY19991 within the internal oil core. After administration, the prepared nanoemulsion remained stable at the normal physiological environment, but disassembled within the tumor microenvironment, due to the pH-triggered wettability reversal of the nanogels. With smaller sizes, the discharged nanogels can penetrate to the deeper zone of the tumor and be internalized by the cancer cells, which delivered high concentrations of DOX intracellularly due to the high GSH concentration. Parallely, the entrapped HY19991 was discharged and then inhibit the PD-1/PD-L1 signaling, resulting in the enhanced T cell immune responses. In this manner, the spatial-temporal targeted delivery synergistically induced the DOX-mediated tumor lysis and immunogenic cell death, which may offer the implications for a novel way of Pickering-emulsion-based targeted delivery.[96]

3.2.2 Cellular Uptake

Effective delivery systems are necessary to obtain high intracellular concentrations. Particulate vehicles, such as polymeric nanoparticles or liposomes, were engineered with smaller sizes to reduce the energy consumption of cellular wrapping and grasping, as well as the positive charges to increase the electrostatic interactions with the negatively charged cellular membranes, and thus increasing the transmembrane efficiency.[116–118] But smaller particles also led to the decreased cargo payloads, which may diminish the overall intracellular concentrations. Furthermore, the cationic surface may disturb the distribution of the membrane lipid, which may cause cell damage and other side effects, such as local inflammatory responses.

With the particle-aggregated interface, Pickering emulsion may offer alternative strategies for the trans-membrane delivery. To start with, the intensely amplified specific surface area was presented on the particle-adsorbed O/W interface. Furthermore, the interparticle gaps on the surface also exposed partial oil core, increasing the hydrophobicity in the confined space. Because of the hydrophobic lipids among the membranes, the cellular affinity was evidently stimulated. In addition, Pickering emulsions can deform during the cellular contact, converting the cell contact area from “point to point” to “face to face”, and thus increasing the contact area with the membranes (Figure 6E). What’s more, with the antigen embedded in the particle gap, the fluidity of the antigens can trigger danger signaling receptors dynamically, increasing the cellular recognition for the potent endocytosis. Under these circumstances, even with micrometer sizes, and negative charges, Pickering emulsion can be designed to elicit potent endocytosis of the target cells.

To illustrate this, a Pickering emulsion was developed by our group and heralded as vaccine adjuvant. And, the force-dependent deformation that increased the contact area on the cell membranes was illustrated by Quartz Crystal Microbalance on the membrane-coated chips. Furthermore, fluorescence recovery after photobleaching analysis was also employed, which proved the fluidity of the antigen between the particles, suggesting that during the cellular contact, the adsorbed antigens diffused from other parts of the droplets to the contact zone, triggering the multivalent interactions with the antigen presenting cells. As such, cellular uptake was boosted, compared with the nanosized particles and surfactant-stabilized emulsions, the developed Pickering emulsion boosted the antigen uptake by a factor of ~3 ($p < 0.001$, analyzed by one-way analysis of variance (ANOVA).[114] Such enhanced cellular affinity was also observed in our another work. We packed alum on the squalene/water interface, forming an alum-stabilized Pickering emulsion (PAPE). Interaction forces between dendrite-mimicking particles and PAPE, as well as alum in PBS, determined using optical tweezers. DC-membrane-coated particles were constructed, and elongation of the dendrites during cellular encounter was replicated by prodding the membrane-coated particles toward PAPEs. Results showed that PAPE evidently increased cellular affinity. In regard to the dLNs responses, PAPE, comparing with alum, induced approximately threefold higher antigen-internalized DCs and 220% more epitope-presenting cells on days 5.[94]}

3.2.3 Cytosolic delivery

Endosomal degradation of therapeutic agents is one of the major challenges in drug delivery. As intracellular defense mechanism, the delivery systems, treating as the exogenous agents, were subjected to degradation in endo-lysosome vesicles. With the maturation, the early endosomes were acidized, and subsequently fused with the lysosomes, which were abundant with enzymes, resulting in the degradation of the delivery systems, as well as the delivered drugs. To elicit potent therapeutic effect, it is crucial for the vehicles to escape from the early endosomes and achieve the cytosolic delivery. Recently, the ionizable particles were widely employed to avoid lysosome-caused degradation.[119] By capitalizing on the acidic environment ($pH = 5–6.5$) within the endosomes, the amine groups were protonated, demonstrating positive charges, which may destabilize the lysosome membranes to unleash the delivered cargos.[120] Furthermore, the protonation also consumed a large amount of H+, which caused a rapid inward flow of hydrogen and chloride ions, leading to swelling or rupture of the lysosome, and the release to the cytoplasm.

In the case of single nanoparticles, the protonation may not be evident enough. But, for Pickering emulsion, hundreds of particles aggregating on one droplet. Thus, the protonated effect was intensively amplified, which may even allow for the lysosome escape of the less ionizable particles. A typical example was that, the carboxyl groups on PLGA nanoparticles were reported to cause limited cytosolic delivery. By aggregating effect on the O/W interface, PLGA nanoparticles-stabilized emulsion demonstrated
potent charge reversal (anionic to cationic), leading to the destabilization of the lysosomes. Via the confocal images, the co-localization of the delivered antigens and lysosomes was a small degree lower, contrasting with the PLGA nanoparticles-treated cells, indicating the potent lysosome escape of the droplets. Furthermore, higher hemolysis of mouse red blood cells in vitro further validated the enhanced cytosolic delivery of the amplified protonation, which prevented the degradation and allowed for the robust cross-presentation of the antigens.[14]

3.3 Controlled release

In case of Pickering emulsion, controlling the release kinetics is to harness the stability, according to the different target sites or responding to the varied external stimuli. To start with, the interparticle gaps granted the droplets with the permeability, facilitating the release of the oil-encapsulated drugs through the particle gaps. In this way, the payloads were discharged in a diffusion mode, which was driven by the concentration gradient between the inner phase and the outer environment.[121,122] The release kinetics can be further tuned by the sizes, cross-link degree, or the physiochemical properties of the interfacial layer. On the other hand, the encapsulated payloads can also be released by the destabilization of the droplets. By responding to the cues from the external stimuli, such as pH, temperature, and light. The physiochemical properties of the interfacial particles were changed, leading to the rupture of the droplet for the burst release. By balancing the stability and responsive rupture of the droplets, Pickering emulsions can be exploited as the stimuli-responsive delivery systems.

Under these circumstances, Pickering emulsions were engineered in two ways, one is to exploit the tunable permeability for the controlled release, and the other to exploit the stimuli-responsive particles on the surface to dictate the release of the delivered components.

3.3.1 Diffusion controlled release

Controlled-release delivery systems can prolong the drug persistence, protect the drugs from physiological degradation or elimination and reduce the administration frequency, improving patient compliance. Nowadays, FDA-approved polymeric microparticles, such as PLGA are widely employed in the controlled release of insulin, triptorelin, lanreotide, and liraglutide. [123–125] However, they still suffered from the abrupt release due to the degradation of polymer chains. Alternatively, diffusion-controlled release, which exploits the gradient in concentration between continuous and dispersion phases, is expected to demonstrate a slow but constant release profile. Compared to the surfactant-stabilized emulsion, Pickering emulsion with its inter-particle gaps exposed more oil droplet surfaces, which led to an enhanced capability to release the loading components. In a recent study, a model lipophilic drug (di-butyl-phthalate) was encapsulated within the silica nanoparticles stabilized emulsion. The corresponding release kinetics were studied with respect to the interfacial density of the interfacial silica nanoparticles. Sustained release of di-butyl-phthalate was facilitated by the rigid interfacial layers of hydrophobic silica particles, whose half-release time was about 18 h. Meanwhile, the cargos within the uncoated droplet were rapidly discharged. What’s more, the release rate was further retarded in the presence of a thick interfacial particle wall, indicating that the diffusion-controlled release may be impacted by the interfacial gaps and the thickness of the particulate shell.[126]

Furthermore, the permeability can be regulated for the tunable release rate. If the drugs were uniformly presented within the oil core, the transport to the continuous phase can be assumed only occurred at the O/W interface. Accordingly, the permeability (P) of the droplets was correlated with the diffusion coefficient (Ds), of compound through oil droplets:[127]

$$P = \frac{R}{DC_i} \left\{-D_s \frac{\partial C}{\partial r}\right\}$$

where $D_s$ is the diffusion coefficient of compound through the shell material, $C_i$ is the concentration at the interface between the core and the shell, and $\left(\frac{\partial C}{\partial r}\right)$ is the concentration gradient in the shell. As aforementioned, $D_s$ is in a close relation with the density and sizes of the interparticle gaps. Thus, the permeability can be adjusted by increasing the particle concentration regulating the sizes or deformability of the particles, or the thickness of the particulate layers. In a recent work, Pickering emulsions were stabilized by gliadin/sodium caseinate (Gli/CAS) nanoparticles. According to the rheological curve, an increase in the particle concentration resulted in a denser packaging of the surface film, which impeded the drug leakage.[128] More recently, in the context of layer-by-layer deposition, multi-layered polyethyleneimine/laponite stabilized Pickering emulsions were prepared by alternating adsorption of anionic sodium alginate and cationic chitosan. The permeability of the microcapsules was controlled by tuning the adsorption layers according to the particular final purpose. By using ibuprofen a model drug, additional polyelectrolyte layers coated were prepared to investigate the effect of different adsorption double layers on ibuprofen release. As expected, with increasing polyelectrolyte double layers, the release rate was obviously reduced and the remaining ibuprofen increased correspondingly, indicating that additional deposited layers could result in a greater obstacle for the release channel of ibuprofen.[129]

Besides engineering the particles, solidification of the O/W interface is another method of controlling the permeability of the droplets. Through cross-linking the particles, the colloidosomes were formulated with tunable shell thickness, which were employed as efficient control-release system. In a recent study, CTAB–functionalized polydopamine (PDA) nanoparticles (PDA@CTAB) were prepared to stabilize chloroform-in-water Pickering emulsions. Subsequently, PDA nanoparticles assembled on the surface of an oil droplet were cross-linked by thiol–isocyanate click reaction conducted in the oil phase to form the colloidosomes. With the large inner cavity, hydrophobic cargo of 10-hydroxycamptothecin (HCPT) was encapsulated in the core, and further applied for drug release. The release behaviors of the encapsulated cargo differed with different shell thicknesses. For example, with ~100 nm shell thickness, about 40% of the encapsulated HCPT has been released within 3 h. The release yield was ~60% within 13 h. However, with ~130 nm shell thickness, the release yield was ~31% within 3 h, and ~56% after 13 h.[130]
3.3.2 Stimuli-responsive release

Stimuli-responsive release may shed light on the “smart” release and remote control over the delivered drugs. As nanoparticle-assembled systems, Pickering emulsion inherited distinct physiochemical properties from the adsorbed particles. When exposed to the external stimuli, such as the changes in pH, temperature, or light, the stimuli-responsive particles would change in the wettability, charges, or sizes, disrupting the interfacial arrangement for the emulsion breakage. In this way, Pickering emulsions can be manipulated to release the “cargo” in a controlled manner.

pH-responsiveness is widely used mechanism in drug delivery systems, which can leverage the pH variations of the target tissue (acidic environment in stomach or neutral condition in intestines), or the specific cell or subcellular organelles (acidic tumor cells or the acidic lysosomes). In regard to the pH-responsiveness, it is important to retain the emulsion stability, until the exposure to the target sites. For instance, Mao et al.\textsuperscript{99} prepared pH-sensitive chitosan/hydrophobic-stability, until the exposure to the target sites. For instance, (acidic tumor cells or the acidic lysosomes). In regard to the pH-responsiveness, it is important to retain the emulsion stability, until the exposure to the target sites. For instance, Mao et al.\textsuperscript{99} prepared pH-sensitive chitosan/hydrophobic-modified alginate nanocomposites (HSA-CS), and subsequently formed a HSA-CS-stabilized emulsion to encapsulate and release the hydrophobic drug (ibuprofen) in a controlled manner. As pH increased from 1.5 to 12.0, the charges of HSA-CS nanocomposites changed from 25.3 to $-42.9 \text{ mV}$ due to the chitosan-amino groups and alginate carboxyl groups. The emulsion property was affected due to the electrostatic interactions between the nanocomposites and the negatively charged O/W interface, which influenced the release performance. Under the continuous pH variation, the shrinkage and swelling of the HSA-CS nanocomposites at the interface manipulated the drug-diffusing process (Figure 7A). Under the gastric-fluid-simulated environment, the droplet remained stable for over 3 h (Figure 7B). Alternatively, at higher pH (simulated intestinal fluid), 88.37% of ibuprofen was discharged (Figure 7C), indicating the potential of oral delivery to release at the intestines but retain stable at the stomach. Another pH-responsive Pickering emulsion was stabilized by hydrolysis lignin/chitosan oligosaccharide (EHL/COS) nanoparticles. Hydrophilic drug, cytarabine was encapsulated within EHL/COS, meanwhile curcumin (hydrophobic) was loaded in the inner soybean oil. The involved materials, lignin, and chitosan not only possessed biocompatibility but also can functioned as the pH-responsive switch. At the neutral pH (pH = 7.4), 40% of cytarabine was released from the particles after 5 h. But, at lower pH (pH = 6.0), the amino groups of EHL/COS became protonated, which caused the partially disassembly of the nanoparticles, and thus led to 97% release of the cargos. Meanwhile, the pH-responsiveness of EHL/COS led to the destabilization of the emulsions, and discharged the inner curcumin, with 93% release after 15 h. The therapeutic effect against cancer cells in vitro using leukemia cell line as a tumor model indicated that the pH-triggered co-delivery of the anti-cancer drugs realized the synergistic cancer therapy.\textsuperscript{131}

Changes in temperatures may also serve as the stimuli for drug release. The constant temperature in human body allows for the drug release at around 37°C, but retain stable at lower or higher temperature during the storage and transfer. By varying the temperatures, poly(N-isopropylacrylamide) (pNIPAM) experienced coil-granule transitions, which changes in particle sizes, hydrophobicity, and the interparticle gaps on the interface, which have been widely employed in the thermal-sensitive drug delivery systems. In case of thermally sensitive Pickering emulsion, lignin nanoparticles, which modified with pNIPAM was prepared for the droplet formations. Owing to the abundant UV chromophoric groups (aromatic groups and double bonds) on lignin, the light stability of the oil-encapsulated trans-resveratrol was significantly improved. Furthermore, with the increasing on the temperatures (45°C), pNIPAM chains experienced the transition from coil to granule, which “shrunk” the sizes of the particles and the assembled droplets, reducing the release behavior of trans-resveratrol. On the other hand, when the temperature was cooling down to 25 °C, the thermal-sensitive side chains return to normal sizes, which opened up the interparticle gaps for the evident drug release (84.0%), indicating the immense potential in thermal-sensitive delivery systems.\textsuperscript{132}

As an alternative strategy, magnetic-responsiveness allows for the remote control of drug release via an applying external magnetic field. In case of the delivery of curcumin, magnetic nanoparticles (Fe$_3$O$_4$@cellulose nanocrystal)-stabilized Pickering emulsion (MCNC-PE) was prepared. Under the magnetic field, MCNCs were attracted and concentrated around the magnetic source, and subsequently detached from the O/W interface. Accordingly, more surface areas of oil droplets were exposed, leading to the rapid release of the encapsulated curcumin (Figure 7D). As shown in Figure 7E, the curcumin content released was observed to be 14.59 ± 3.66 and 53.30 ± 5.08% for MCNC-PE without and with external magnetic fields respectively. The results showed an increased release rate of approximately 39% of curcumin from the MCNC-PE in the presence of external magnetic fields, suggesting the possibility of using a magnetically responsive vehicle for remote controlled drug delivery.\textsuperscript{133}

Another remote control over the drug release could be achieved by electrochemical-responsive Pickering emulsions. By applying the redox-active microgels, the formation and deformation of the microgel can be regulated by the external potential, thus endowing the Pickering emulsion with controlled behavior. In a recent study, cyclodextrin and ferroene-functionalized 8-arylpoly(ethylene glycol) were employed to form potential-responsive microgels, and used such microgels to form Pickering emulsion. Due to the ferroene-functionalized 8-arylpoly(ethylene glycol) being oxidized at the potential of +0.80 V, the microgels were disassembled, leading to the breakage of the emulsions for the controlled release of the cargos.\textsuperscript{134} The electro-sensitive microgels have been used in the Pickering emulsion-based biocatalysis, which open up new possibilities for controlled drug delivery systems.

Light-responsive materials, such as TiO$_2$, azobenzene, and coumarin-based groups, can change in their wettability upon irradiations, which may offer an alternative strategy to control the release of the delivery cargos. For example, the wettability transition of TiO$_2$ nanoparticles can be reversibly converted by UV/dark actuation due to the adsorption and desorption of hydroxyl groups on their surfaces. By exploiting this, TiO$_2$-nanoparticle-stabilized Pickering emulsion was prepared with Astragalus polysaccharides (functioned as immunopotentiator) encapsulated. When exposed to the UV light at 254 nm, the TiO$_2$ nanoparticles become hydrophilic due to the adsorption of hydroxyl groups, leading
FIGURE 7  (A–C) pH-responsiveness for the potent oral delivery. (A) Schematic illustration of chitosan/hydrophobic-modified alginate nanocomposites (HSA-CS) stabilized Pickering emulsions and their stimuli-responsive release behavior (From top to bottom, pH, magnetic, photothermal). (B) Release of the loaded ibuprofen from under the simulated gastric fluid. (C) Release of ibuprofen under the simulated intestinal fluids. Reproduced with permission.[99] Copyright 2020, Elsevier. (D,E) Magnetic-triggered release. (D) Illustration on the curcumin (CUR)-loaded Fe₃O₄@cellulose nanocrystal (MCNC)-stabilized Pickering emulsions. (E) Release profile of CUR release with or without external magnetic field (EMF). (F,G) Near-infrared irradiation (NIR), temperature-controlled release. Reproduced with permission. [133] Copyright 2019, Elsevier. (F) Schematic illustration on the polydopamine- and poly(N,N-diethylacrylamide)-modified silica nanoparticles (SiO₂-PDA-PDEAA), formation of Pickering emulsion (top), and the loading and NIR- or temperature-controlled release of doxorubicin hydrochloride (DOX). (G) The accumulative drug release efficiency of SiO₂-PDA-PDEAA under near-infrared irradiation at different intensities of (a) 2.0 W/cm² (blue), (b) 1.0 W/cm² (red), and (c) 0.5 W/cm² (black). Reproduced with permission. [136] Copyright 2020, Elsevier.

Moreover, the use of N-doped TiO₂ particles extended light-triggered release to the visible range. [135] But, the wide applications of UV-light-responsive release may be hindered by the limited transdermal efficiency of the irradiations.

As an alternative external light source, near infrared responsive (NIR), with the biological friendly wavelength range (650–950 nm), can penetrate the tissues deeply, which seems promising for the noninvasive stimuli-responsive drug delivery. NIR-responsive materials, such as polydopamine, phosphorus, and gold nanoparticles, can exploit the light irradiations to produce heat, offering the photothermal-responsiveness. In a recent work, silica nanoparticles (SiO₂) were successively modified by polydopamine(PDA) and poly(N,N-diethylacrylamide) (PDEAA). And, doxorubicin was loaded within the SiO₂-PDA-PDEAA particles (whose loading efficiency reached up to approximately 70%) (Figure 7F). NIR photothermal effect brought by PDA decreased the viscosity of PDEAA, which facilitated the doxorubicin release. And the increase in temperature boosted the hydrophobicity of the PDEAA, leading to the phase inversion from O/W to W/O. The results showed that, an increase in the intensity of illumination from 0.5 to 2.0 W/cm² resulted in the enhanced drug release from 11.8% to 43.2% (Figure 7G), indicating the NIR-mediated drug release. [136] Such response paved a feasible way for PDA-based NIR-responsive drug carriers.

Recently, multi-responsive Pickering emulsion opened up opportunities for the precise control over drug delivery. In a recent study, photothermal agent (indocyanine green, ICG) and chemotherapeutic drug (doxorubicin, DOX) were loaded within the hydrophobic core of the Pickering-emulsion-based nanocolloidosomes. By modifications of galactose on the
surface, the colloidosomes can specifically bind with the asialoglycoprotein receptors (ASGPRs) on the tumor cells, and achieve tumor-targeted delivery. Furthermore, the co-delivery of ICG and DOX enabled the synergistic effect of photothermal and chemotherapy, which induced evident tumor lysis in vivo.\textsuperscript{[137]} In case of sequential release, Wu et al. developed dual-responsive microcapsules based on silica nanoparticle-stabilized emulsions. Mesoporous silica nanoparticles were grafted with pNIPAM inside the mesopores to increase thermal sensitivity. Pickering emulsion was then formulated by mixing the pH-responsive monomer-encapsulated 1-hydroxycyclohexyl phenyl ketone solution with thermal-sensitive silica nanoparticles. Upon UV irradiation, pH-responsive monomers were polymerized along the interior surfaces of the nanoparticles, and formed the dual responsive colloidal microcapsules. Afterward, Nile red and 5(6)-carboxyfluorescein diacetate were loaded in the particles and the oil phase, respectively. In this manner, the release can be triggered independently by the variations in pH or temperature, implying the potential for the programmable payload release of different drugs.\textsuperscript{[138]} Thus, the multi-responsive systems offered the controlled delivery of the multiple drugs. With the development in interfacial property control, stimuli-responsiveness and the novel functions of the particles, novel Pickering-emulsion-based systems can be expected, such as the spatial-temporally assembled droplet, stimuli-responsive engulfment droplets, and droplet-based micro-robots.\textsuperscript{[139–141]} These may aid the multi-stimuli responsive Pickering emulsions, and offer the enhanced synergistic treatment and the programmable payload release.

Collectively, Pickering emulsion can be employed as potent and controllable drug delivery systems. Notably, the encapsulation and release of the delivered cargos depended on the rupture of the prepared Pickering emulsions. To some extent, the aforementioned strategies centered on the particle rupture, melting, or deformation, which may hamper the mechanical and stability of the emulsions. Thus, future effort was demanded in balancing the stability and the potent release behavior of stimuli-responsive Pickering emulsions. Additionally, the efficacy and the robustness of Pickering-emulsion-based drug delivery systems may sometimes be impeded by the uniformity of the particle. It was still difficult to control the heterogeneity of the interparticle gaps, stimuli-responsiveness, and impact the drug release behavior from batch to batch. Thus, it is expected to precisely control over the uniformity of the structures and physiochemical properties of the particles.

4 | CONCLUSION

Although, Pickering emulsion has been proposed for over a hundred years, the biomedical applications are still limited. As most biological reactions took place at the interface, optimizing the interfacial assembly of the particles and studying the interfacial effects was a significant step towards Pickering-emulsion-based drug delivery systems. With the up-coming biodegradable materials, such as poly(lactic-co-glycolic acid), ionizable lipids, and PEGylated co-block polymers, Pickering emulsion may be evolved with the enhanced safety profile. Additionally, high-end techniques, including cryoelectron microscopy, super-resolution confocal imaging, and the emerging biomechanical characterization techniques paved the way for the single-droplet manipulation and analysis for the thorough bioactivity studies. It is high time to exploit the multi-level structures, mobility, and mechanosensing nature of Pickering emulsion for the enhanced drug delivery systems. Here, by reviewing the recent development in Pickering emulsion and their applications in drug delivery, we pointed out the strategies to stabilize the emulsion, as well as the mechanisms underlying the enhanced drug delivery efficiency. Furthermore, the structure-effect relationship of Pickering-emulsion-based drug delivery systems was also discussed, such as the multi-level structure for effective loading, flexibility, and permeability for enhanced delivery, and the stimuli-responsiveness for the controlled release of the drugs. Through exploiting the enhanced bioactivities, Pickering emulsion showed great promise in the delivery of anti-tumor drugs, vaccines, mucosal delivery, and cell therapy. Through the rational designed the interfacial effects, substantial momentum would be gained in the next-generation drug delivery systems.

It is worth noting that particle properties, including size, charge, shape, and responsiveness, not only play a vital role in the stability of the droplets, but also dictated the delivery process of Pickering emulsions. Sometimes, tailoring the interfacial properties for one aspect may contradict with another. For example, increasing the wettability with the interface can increase droplet stability, but may interfere the permeability for drug release. Positive charges facilitated the cellular uptake of the droplets, but also reduced the tissue distribution by the electrostatic interactions with the cells at the injection sites. Thus, to achieve higher efficacy, future effort in Pickering emulsion was expected for a more balanced design, which comprehensively integrates the whole delivery process, including the droplet formation, tissue targeting, cellular internalization, and drug release.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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