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Review

Nanoemulsions for drug delivery

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ABSTRACT

Emulsions are liquid–liquid dispersions with one liquid phase dispersed in the other liquid phase as small droplets. Nanoemulsions are nano-sized emulsions with sizes ranging from tens to hundreds of nanometers, and have great potential applications in pharmaceutics, foods and cosmetics due to their attractive properties, such as small sizes, high surface area per unit volume, improved dispersion of active hydrophobic components and enhanced absorption. The article provides an overview of nanoemulsions for drug delivery, starting with an introduction of emulsion types, nanoemulsion preparation and nanoemulsion stability. Surfactants play critical roles in producing and stabilizing nanoemulsions. Different types of surfactants are summarized including small molecule surfactants, particle surfactants, phospholipids, peptide and protein surfactants. Then the applications of nanoemulsions as nanomedicine in drug delivery are presented. Finally, clinical applications of nanoemulsions are discussed.

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Introduction

Emulsions are biphasic liquid systems where one liquid phase known as the internal or dispersed phase is dispersed as small droplets through the second liquid phase known as the external or continuous phase. They are of great interest for the production of food, pharmaceuticals and cosmetics as it can help to mix non-polar and polar molecules, change the texture/taste/smell of products and improve the efficacy of medical treatments (Grumezescu, 2016; Ohshima & Makino, 2014; Sakamoto, Lochhead, Maibach, & Yamashita, 2017). Emulsions have enormous potential in range of industries and can be readily tuned to any number of formulations to meet the specific needs of a product or a process such as dispersing an oil in water or water in oil. However, considerations must be taken when working with emulsions as they are thermodynamically unstable systems and will rapidly separate into two discrete phases unless surface active molecules, also called emulsifiers, are added to the mixture to stabilize the droplets.

Nanoemulsions are nano-sized emulsions with sizes ranging from several tens to several hundreds of nanometers. Surfactants work at the interface between the two immiscible phases to lower the surface tension and act as a barrier to emulsion coalescence which is driven by the system attempting to reach a state of minimal Gibbs free energy (Kabalnov, 1998). Nanoemulsions have great potential as potent nanomedicines as they can readily solubilize hydrophobic drugs, reduce severe adverse effects and be readily modified into next generation smart nanomaterials (Sutradhar & Amin, 2013). This article provides an overview of bioinspired nanoemulsions for drug delivery, including introducing different emulsion systems, discussing their preparation and stability, presenting different types of surfactants and considerations when choosing a surfactant and finally introduce emulsion nanomedicine and clinical applications.

Nanoemulsions

Types of emulsion

Emulsions in their most simple form are composed of a hydrophilic phase and a hydrophobic phase where one is dispersed through the other (Fig. 1). Thus, these emulsions are known as oil-in-water (O/W), where small oil droplets are dispersed through water, or conversely when water droplets are dispersed through oil, water-in-oil (W/O) emulsions. However, more complexity can be added to these simple systems by encompassing an emulsion within an emulsion known as a double emulsion forming water-in-oil-in-water (W/O/W) or oil-in-water-in-oil (O/W/O) emulsions. Traditionally, the formation of double emulsions is a two-step process requiring the formation of the initial internal emulsion that is then encompassed by forming a second emulsion surrounding the initial emulsion. Double emulsions have additional challenges to be formed and stabilized such as protecting the integrity of the initial emulsion when forming the second emulsion, requiring both a lipophilic and hydrophilic surfactant to stabilize each oil–water interface and are more prone to degradation and coalescence due

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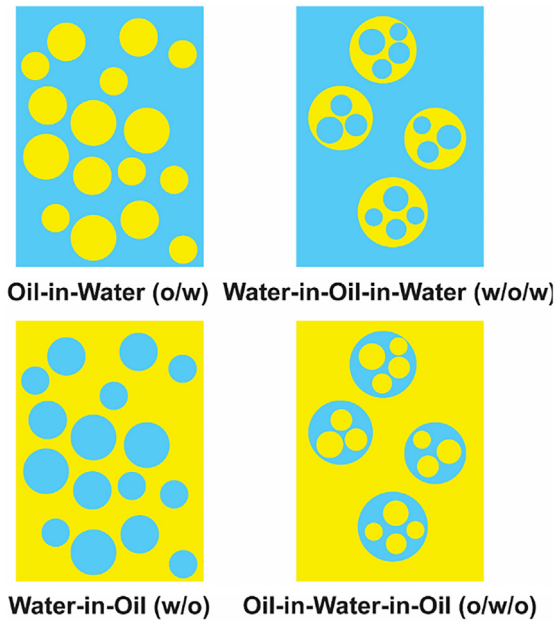

Fig. 1. Emulsion types classified by their dispersed and continuous phases. Oil-in-water emulsions are ideal to deliver hydrophobic drugs loaded into the oil core. Water-in-oil-in-water double emulsions are suitable for loading both hydrophilic and hydrophobic actives in the water and oil phases, respectively.

to diffusion between each phase (Leister & Karbstein, 2020). Uniform double emulsions generated using microfluidic devices have received renewed research interest for applications in food science and as microreactors or templates for particle synthesis (Ran et al., 2017; Zhao & Middelberg, 2011; Zhao, 2013).

Pickering emulsions are O/W or W/O emulsions stabilized by very small particles at the oil–water interface that present a steric barrier to coalescence, rather than small molecules that alter the interfacial tension, first reported by Pickering in 1907 (Pickering, 1907). Particles that can stabilize Pickering emulsions are dually wettable, that is can be wetted by both phases of the emulsion, this allows them to sit at the oil–water interface and in general impart greater stability to the emulsion than traditional surfactants (Xiao, Xu, Zhang, & Hong, 2018; Yang, Wibowo et al., 2017; Yang, Fang et al., 2017). Novel applications for Pickering emulsions are derived from the ability to use the particles on the surface of the droplet for additional functions such as catalysis or antimicrobial functions (Huang & Yang, 2015; Wang, Chen, Hsieh, & Tseng, 2017).

High internal phase emulsions (HIPEs) have an internal phase greater than 74.05% which differs from most emulsions where the internal phase composes a very small percentage (<10%) of the total emulsion volume (Pulko & Krajnc, 2012). HIPEs have been used as templates for porous polymer monoliths by polymerizing monomer dissolved in the continuous phase and more recently as templates for tissue repairing porous hydrogels (Krajnc, Leber, Štefanec, Kontrec, & Podgornik, 2005; Nalawade et al., 2016). Unfortunately, polymer monoliths produced using HIPE template can be very fragile due to high surfactant content and thin polymer walls, however, further work is exploring medium internal phase emulsions (MIPEs) as an alternative template for stronger polymer monoliths (Wu, Menner, & Bismarck, 2013).

Nanoemulsions are emulsions with droplet size ranging from 10 to 1000 nm. Typical nanoemulsions consist of oil, water and a surfactant. The selection of the surfactant is critical for forming and stabilizing nanoemulsions. Nanoemulsions are thermodynamically unstable but kinetically stable. In other words, phase separation of nanoemulsions occurs when given sufficient time. Nanoemulsions have been developed for various applications in pharmaceuticals, food, cosmetics. For all these applications, they need to be biocompatible with no toxic effects. Therefore, the choice of oil and surfactants are important. Biocompatible oils and surfactants are desirable for example vegetable oils or pharmaceutical grade oils. Proteins and lipids have also been widely used as surfactants to stabilize nanoemulsions.

Nanoemulsion preparation

Emulsions form when two immiscible phases are exposed to a shearing mechanical force that separates the dispersed phase into miniscule droplets. Sources of shear force to generate emulsions can be separated into high and low energy methods that is they use either a device to apply the shear force (high energy) or induce emulsion formation by understanding how the physical properties of each liquid phase and chosen surfactants change from the thermal or chemical energy in the system (low energy). To generate nanoemulsions, a two-step process is required where coarse emulsions are firstly formed followed by high-pressure homogenization or ultrasonication to break the big droplets into nano-size, thus forming nanoemulsions.

High energy emulsification technologies require input large amounts of mechanical force into the system to generate monodisperse droplets; methods include high-pressure homogenization (HPH), microfluidization and ultrasonication. HPH is a homogenization process where high pressure (>100 MPa) is applied to a coarse emulsion system prepared before HPH (Echeverri et al., 2020; Li et al., 2018). The coarse emulsion is forced to flow through narrow passages (nozzles or valves) to reduce droplet size under high shear stress and high turbulences. To make nanoemulsion with desired sizes, the HPH process is normally repeated multiple times until droplet size is constant. Microfluidization uses the concepts of HPH to prepare nanoemulsions at high oil phase contents using scalable microfluidic technology (Ganesan, Karthivashan, Park, Kim, & Choi, 2018). Microfluidizers come in single- or dual-channel systems. Single channel microfluidizers pass a coarse emulsion through a HPH system to generate nanoemulsions with smaller size and better dispersity than traditional techniques. The need for a coarse emulsion feedstock limits the scalability of the method as it relies upon poorly scalable mechanical homogenizers. In contrast, dual-channel microfluidizers form a coarse emulsion in situ by colliding oil and water phases then passing them through a HPH system and are able to generate nanoemulsions at 50% oil phase content (Bai & McClements, 2016; Luo et al., 2017). Ultrasound produces finely dispersed nanoemulsions due to the high acoustic energy exerted on the oil phase and has been routinely used for research into nanoemulsions (Maa & Hsu, 1996; Modarres-Gheisari, Gavagsaz-Ghoachani, Malaki, Safarpour, & Zandi, 2019). Ultrasonication works by producing amplified sound waves that lead to rapid cavitation bubbles from the oscillating ultrasound probe, the acoustic energy pulls and tears at the dispersed phase finely shearing it into droplets (Abdou, Kandil, & El Miniawy, 2017; Gupta, Shea, Scaife, Shurlygina, & Rapoport., 2015). Ultrasonication can produce well dispersed nanoemulsions however the technique is poorly scalable due to the high heat generation, ultrasounds can struggle with viscous solutions and short working distance of the probe head.

Low energy emulsification methodologies exploit special chemical, physical, thermal and/or solvent conditions induce the formation of a nanoemulsion; methods include solvent displacement emulsification, phase inversion temperature method, self-emulsification and polymorphic phase transition. Solvent displacement emulsification is performed by adding a small amount of miscible dispersed phase to continuous phase containing the surfactant and then subjecting the mixture to rotary evaporation to remove excess dispersed phase resulting in a nanoemulsion

(Guerrero et al., 2018; Ye & Chi, 2018). The phase inversion temperature method involves mixing two immiscible phases in the presence of a surfactant at high temperature to form a W/O emulsion that upon cooling inverts to form an O/W emulsion (Anton & Vandamme, 2009; Friberg, Corkery, & Blute, 2011; Ren et al., 2019). This process is stabilized by the hydrophilic–lipophilic balance of the surfactant, surfactant concentration, ratio of water to oil and difference between ambient temperature and the phase transition temperature. Depending on the specific temperature requirements of the system this can also be considered a high energy method. Self nanoemulsification has attracted great interest for oral drug delivery applications and can significantly improve the bioavailability of poorly soluble drug (Kumar, Bishnoi, Shukla, & Jain, 2019). A specific mixture of lipid oil, drug, surfactants and co-surfactants spontaneously forms a nanoemulsion in physiological media that can protect the cargo and control the drug release (Alshahrani et al., 2018; Chaudhary, Aqil, Sultana, & Kalam., 2019; Kanwal et al., 2019). The ease of synthesis and high drug content of the self nanoemulsification mixtures makes this technique readily scalable for industrial manufacture, however, the high surfactant content required is a regulatory challenge. Lastly, a new low energy method to prepare nanoemulsion from frozen lipid coarse emulsions with low surfactant and high drug content has been reported (Cholakova, Glushkova, Tcholakova, & Denkov, 2020). After freezing, the solid lipid undergoes a gel to crystal phase transition that spontaneously disintegrates into solid lipid particles. After thawing the lipid particles monodisperse nanoemulsions with sizes <100 nm were reported. The low surfactant content (<2%) and high drug loading (50%) of this method demonstrates a new strategy for scalable emulsification technology.

The translation of emulsion science to applications that have significant impact on society and human being requires industrial techniques that can meet demand. The scalability of a technique is a measure of limit of the amount of product that can be produced before the properties are distorted by the size of the vessel (A matter of scale, 2016). High-pressure homogenization which involves passing the mixture through a narrow channel under pressure that creates a high shear force to form nanoemulsion have been used for large scale production such as homogenizing animal milk (Hayes & Kelly, 2003; Schultz, Wagner, Urban, & Ulrich, 2004). Low energy emulsification such as solvent displacement and phase inversion temperature methods offer improved energy efficiency compared to previous methods and performed with minor changes to existing reactor infrastructure. However, they are currently not widely used in industrial production due to poor understanding of the underlying process, mechanisms and lack of food or medical grade solvents/surfactants.

Nanoemulsion stability

Nanoemulsions are thermodynamically unstable systems that trend towards separation into two discrete phases over time. When stabilized by surfactants this time frame is extended essentially rendering emulsions kinetically stable such that a well-designed emulsion can have prolonged shelf life retaining its original properties for months or years. This is important for the translation of nanoemulsion into various applications but especially emulsion nanomedicine, where changes to a formulation over time may have a serious impact on patient health. Thus, it is paramount to understand the mechanisms of emulsion destabilization and stabilization to ensure that nanoemulsions are properly prepared and stored. The DLVO theory, as proposed by Derjaguin and Landau and, Verwey and Overbeek, describes emulsion stability as a combination of two independent forces, attractive van der Waal's interactions and repulsive electrostatic double layer forces (Derjaguin & Landau, 1993; Verywey & Overbeek, 1948). As the two forces are presumed

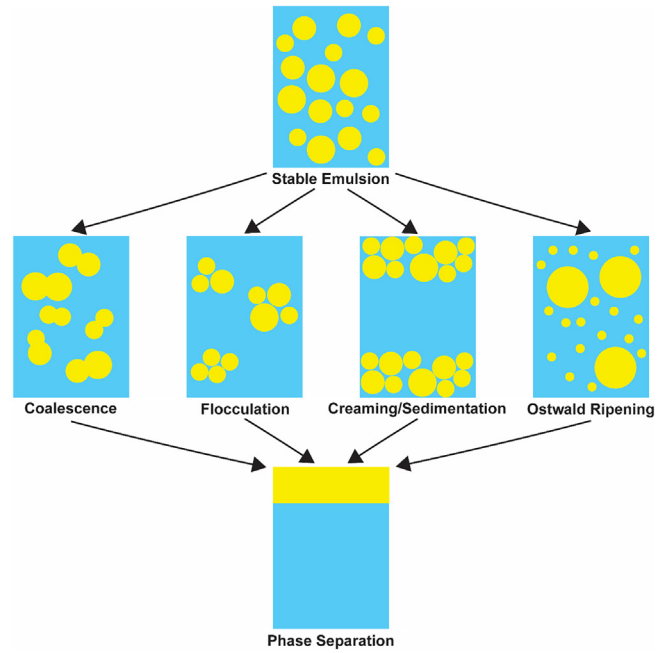

Fig. 2. Illustration of emulsion destabilization mechanisms and pathways. Coalescence is the merging of two or more droplets to reduce interfacial energy. Flocculation is clustering of nearby intact droplets into aggregates. Creaming and Sedimentation involves droplets rising or falling in solution in response to density gradients. Ostwald Ripening is the growth of larger droplets at the expense of smaller droplets. These pathways can occur simultaneously but are inhibited by sufficient electrostatic and steric stabilization that are essential for drug delivery applications.

to be independent, simple addition of the sum of the attractive force (F_A) and repulsive force (F_R) gives the total energy of interaction (F_T) at specific distances and gives good estimation of stability to around 5 nm (Eq. (1)) (Adair, Suvaci, & Sindel, 2001).

$$F_T = F_A + F_R \tag{1}$$

When droplets are far apart the repulsive forces dominate the interaction energy such that the system favors colloid stability, however, as droplets approach one another the attractive forces begin to dominate and destabilization occurs. The DLVO theory describes that when the attractive forces dominate, the emulsion undergoes colloidal destabilization. This occurs by four main pathways (Fig. 2): coalescence, flocculation, creaming/sedimentation and Ostwald ripening.

Coalescence is the merging of two or more small droplets in close proximity into a single larger droplet. This occurs when droplets approach and deform leading to the formation of a thin film of continuous phase. The film thinning is governed by the flow of the continuous phase and surfactant along the film. Fluctuations in the thickness of film can lead to rupture when the film thins to a critical point (Dickinson & Miller, 2001; Ivanov, Danov, & Kralchevsky, 1999). Film thinning can be attributed to the Marangoni effect that describes mass transfer along an interface in relation to interfacial tension gradients (Mackay & Mason, 1961). Upon merging the total surface area of the droplets decreases reducing the interfacial energy of the system leading to a reduction in the Gibbs free energy.

Flocculation is the aggregation of dispersed discrete unruptured droplets as clusters in solution separated by a thin film of the continuous phase. The stability of the film slows coalescence of the emulsion by electrostatic repulsion, however, stability against coalescence must be considered separately to stability against flocculation such that emulsions may readily flocculate then slowly coalesce and vice versa (Hubbard, 2002). Flocculation can be a reversible or irreversible process depending on the strength of the

attractive forces governing the interaction. Once an emulsion has flocculated, coalescence is more likely to occur due to the close proximity of the droplets (Ivanov et al., 1999).

Creaming and sedimentation of emulsion droplets arise when droplets of different density to the dispersed phase separate to the top or bottom of the solution (Oprea & Grumezescu, 2017). Similar to flocculation, creaming/sedimentation increases the likelihood of droplet coalescence by raising the probability of interaction between droplets. Controlling this process is very significant for the food industry costing billions each year and can be slowed by the addition of viscous polysaccharides (Robins, 2000). Creaming can also result from the aggregation of droplets into a large floc network, occurring spontaneously or induced by polysaccharide bridging, due to enhanced separation kinetics (Dickinson, Golding, & Povey, 1997; Robins, 2000).

Ostwald ripening is the process by which larger droplets grow at the expense of smaller droplets by diffusion of dispersed phase through the continuous phase (Taylor, 1998). This process is a result of the Kelvin effect, that says that particles with a smaller diameter have greater solubility in solution, and reduces the total surface area of the dispersed phase resulting in a reduction in the Gibbs free energy (Davis, Round, & Purewal, 1981; Kabalnov, Pertzov, & Shchukin, 1987). Ostwald ripening can be inhibited by ensuring a monodisperse size distribution of droplets and using a dispersed phase with very low solubility in the continuous phase (Mitra, Cholkar, & Mandal, 2017).

According to the DLVO theory, emulsion stabilization is enhanced by two different pathways that act to repel or inhibit droplet interaction reducing the occurrence of the destabilization pathways. The first is electrostatic repulsion where like-charge droplets exert a repulsive force on each other as they approach. The second is steric stabilization where droplet interaction is obstructed by a thick surface layer that provides a strong energy barrier to coalescence.

Emulsions coated with ionic surfactants recruit counter ions from solution that generate an electrical double layer to enable electrostatic stabilization by repulsive forces (Fig. 3) (Verwey, 1940). An electrical double layer is made of three distinct parts: (1) the charged emulsion surface, (2) the Stern layer of strongly bound counter ions and (3) a diffuse layer of high concentration loosely bound ions and counter ions (Park & Seo, 2011). The point at which the diffuse layer no longer stays in motion with the emulsion droplets is called the slip plane (Delgado, González-Caballero, Hunter, Koopal, & Lyklema, 2007). The charge at the slip plane is the point used to determine the zeta-potential of an emulsion where very positive or negative emulsions are more stable; an important measure to determine the stability of emulsion (Delgado et al., 2007; Wooster & Augustin, 2006).

Steric hindrance acts as a thermodynamic barrier to droplet interaction, a requirement for destabilization as described by the DLVO theory, by increasing the thickness and complexity of the surface layer (Fig. 4) (Tambe & Sharma, 1994). This can be done by using non-ionic surfactants with long side chains, Pickering particles or conjugating/coating polymers to the surface of the droplet (Claesson, Kjellin, Rojas, & Stubenrauch, 2006; Tadros, Vandamme, Levecke, Booten, & Stevens, 2004). When droplets approach there is an increase in the Gibbs free energy as the polymers and side chains interact, which acts as a barrier to the attractive van der Waals forces (Napper & Netschey, 1971).

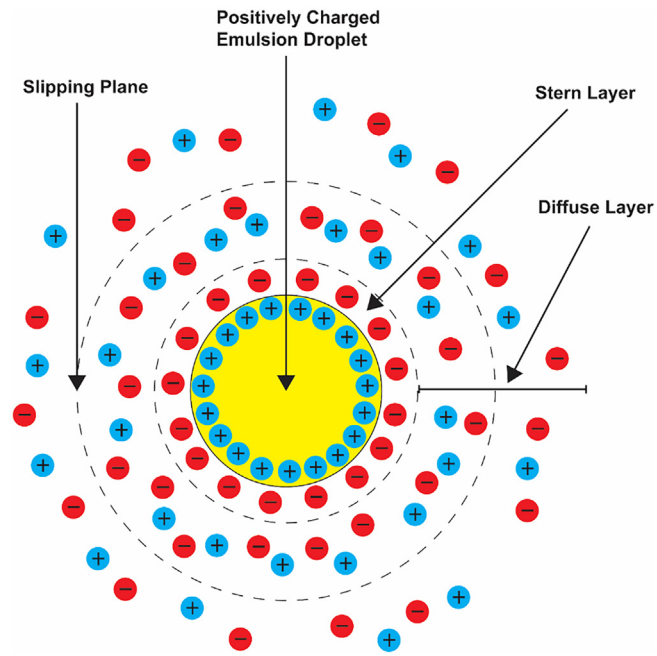

Fig. 3. Illustration of the electrical double layer surrounding charged emulsion droplets. The high charge surrounding the droplet repel droplets as they approach providing electrostatic stabilization. Electrostatic stabilization is typically insufficient for biological applications due to the high salt concentration screening the charge leading to rapid coalescence. Highly charged droplets also promote the adsorption of serum proteins to the charged surface leading to destabilization and opsonization.

Surfactants

Small molecule

Small molecule surfactants are the most abundantly utilized emulsifiers as they are cheap, readily synthesized with well understood surface properties (Fig. 5). Small molecule surfactants have a head–tail morphology, a hydrophobic tail with a hydrophilic head. The respective hydrophobicity and hydrophilicity of the head and tail groups of surfactants can be expressed by their hydrophilic–lipophilic balance (HLB) (Greth & Wilson, 1961). A HLB value <10 describes a surfactant that is oil soluble and >10 a surfactant that is water soluble and is used to determine whether a surfactant can form W/O or O/W emulsions, respectively (Zheng et al., 2015, chp 8). Small molecule surfactants are categorized by the charge of the head group into anionic, cation and non-ionic surfactants.

Anionic surfactants are commonly used in soap, cosmetics and cleaning products as they can be prepared from cheap source materials such as waste cooking oil and well understood chemistry e.g. saponification and sulfonation (Jin et al., 2016; Martins & Dias, 2019, chp 27). Anionic surfactants have good foaming ability and foam stability compared to non-ionic and cationic surfactants (Williams, 2007, chp B.1.II). The negatively charged polar head functional group is typically a sodium salt of a carboxylate or sulfate ester derivative such as sodium oleate or sodium dodecyl sulfate (Delample, Jérôme, Barrault, & Douliez, 2011; Singer & Tjeerdema, 1993).

Cationic surfactants make good surface coatings due to their electrostatic attraction to negatively charged surfaces such as cell membranes, hair and fabric fibers (Rhein, 2007, chp 3). This property makes cationic surfactants ideal for applications as hair conditioners and fabric softeners as the head group attaches to the charged surface presenting the hydrophobic tail giving fibers a softer feel (Puchta, 1984; Ran, Zhang, Song, Wang, & Cao, 2009). The positively charged polar head functional group is typically made from charged quaternary amines salts such as cetylpyridinium chloride, an antibacterial used in mouthwash, and cetrimonium bromide, used for purifying DNA extracts (Porebski, Bailey, & Baum, 1997; Radford, Beighton, Nugent, & Jackson, 1997).

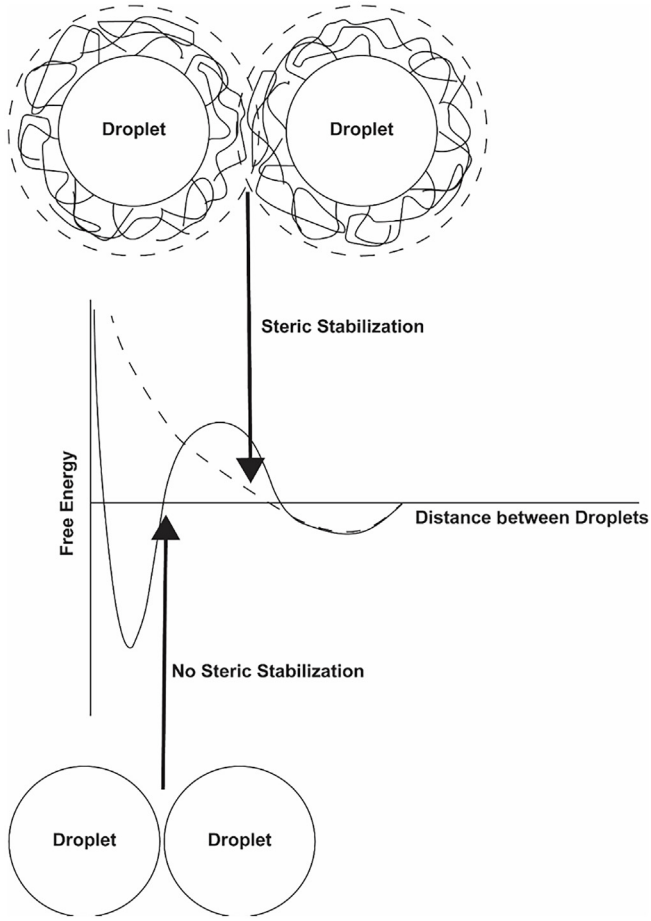

Fig. 4. Illustration demonstrating the energy of interaction between droplets with steric stabilization versus droplets without steric stabilization. As droplets with steric stabilization approach the energy of interaction increases acting as an energy barrier to coalescence and protein adsorption. Bare droplets have no such energy barrier to their interaction as they approach, and there is a small increase in interaction energy to overcome interfacial energy that quickly dissipates as the droplets merge.

Non-ionic surfactants have uncharged polar head functional groups, typically composed of PEG, combined to a fatty acid tail (Sonia & Sharma, 2014, chp 5). There are a wide variety of non-ionic surfactants divided into different classes such as sorbitan esters, polysorbates and Brij polyoxyethylene alkyl ethers among others (Peltonen, Hirvonen, & Yliruusi, 2001; Ribeiro et al., 2012; Vasanthan, Venkitasamy, Sandrine, Philippe, & Jayakrishnan, 2018). The differentiating factor between different groups of non-ionic surfactants are their HLB values, cost and most importantly, toxicity. Non-ionic surfactants used for biological applications result in fewer adverse effects than cationic or anionic small molecule surfactants (Pulce & Descotes, 1996, chp 28; Sonia & Sharma, 2014).

Toxicity is an important consideration for any surfactant that is to be used in food, cosmetics or pharmaceuticals. Many small molecule surfactants are not well tolerated by living organisms and can cause acute toxicity, organ damage and allergic reactions. Surfactants including the non-ionic surfactants Cremophor EL and Tween 80 are non-ionic that are routinely used as excipients for the administration of poorly water-soluble drugs such as paclitaxel, diazepam and vitamin K (Amédée-Manesme, Lambert, Alagille, & De Leenheer, 1992; Burton, Lenz, Thomas, & Midda, 1974; Sparreboom, van Tellingen, Nooijen, & Beijnen, 1998). The pharmaceutical industry is looking for an alternative to these surfactants due to the adverse effects experienced by patients and regulatory limitations on surfactants content in food and drug products.

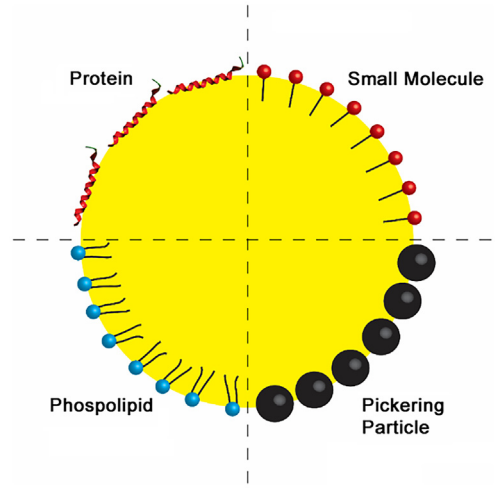

Fig. 5. Different types of surfactants stabilizing an emulsion droplet. Top left: protein surfactants typically sit along the oil–water interface acting as a steric barrier to destabilization and their unique structures can be readily modified with additional functionalities. Top right: Small molecule surfactants have a head–tail morphology and are characterized by their head group as either anionic, cationic or non-ionic. Bottom right: Pickering particles are dually wettable particles that preferential sit at an oil–water interface and provide a stronger steric barrier to coalescence than small molecule surfactants. Bottom left: Phospholipid surfactants have a head tail morphology and are derived from animal or plant sources making them biocompatible.

Particles as surfactants

Particle surfactants have become increasingly popular in recent years as they produce extremely stable emulsions, are versatile and cheap. There is an extensive variety of particles that have been found to have surface active properties including silica, clay, iron oxide, hydroxyapatite and graphene oxide (Ashby & Binks, 2000; Lin, Yang, Petit, & Lee, 2015; Lin, Zhang, Li, & Deng, 2016; Rodríguez et al., 2019; Tikekar, Pan, & Nitin, 2013). Particle wettability can be tailored to a specific system by using surface modification strategies (Xiao et al., 2018). Pickering emulsions have been explored for drug delivery applications, as an alternative to small molecule surfactants, due to their enhanced stability and biocompatibility. Particles stabilize emulsions by adsorbing to the oil–water interface acting as a steric barrier to coalescence. Stabilizing emulsions with particles requires particles with intermediate wettability, that is, partially wetted by the continuous and dispersed phases. Particles with poor wettability in one phase lead to rapid coalescence. The degree of wettability each solvent has on the particle controls whether an O/W or W/O emulsion is formed, with particles wetted more by water forming O/W emulsions and particles wetted more by oil forming W/O emulsions (Kralchevsky, Ivanov, Ananthapadmanabhan, & Lips, 2005). Particle shape, such as spheres, sheets and rods, has a significant impact on the thermodynamic barrier to coalescence by controlling the self-assembly of particles at the interface (Ortiz, Pochat-Bohatier, Cambedouzou, Bechelany, & Miele, 2020). In addition, particle concentration affects the coverage of the steric barrier on the surface impacting the long term stability of droplets and controls the minimum size of the droplets (Tang, Quinlan, & Tam, 2015).

Phospholipids

Phospholipids are the major component of cell membrane and, similar to small molecule surfactants, have a head–tail morphology characterized by a positively charged phosphate head and two fatty acid tails. Phospholipid surfactants are used extensively in food, cosmetics and pharmaceuticals due to their good biocom-

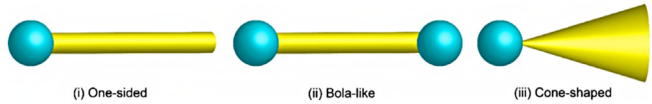

Fig. 6. Structures of end-by-end peptide-based surfactants: (i) one-sided with a polar head (blue) and a hydrophobic tail (yellow); (ii) bola-like with two polar heads at the end (blue) connected by a hydrophobic tail (yellow); and (iii) cone-shaped with a polar head (blue) and a hydrophobic tail (yellow) with an increasing or decreasing side-chain length. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

patibility, are readily sourced from agricultural products such as lecithin from soy beans and egg yolks and impart good long term stability to emulsified mixtures (Pichot, Watson, & Norton, 2013). Phospholipids stabilize emulsions in two ways: first, by providing an electrostatic repulsion between droplets and second, and acting as a steric barrier increasing the thermodynamic energy required to coalesce (Isailović, Todosijević, Đorđević, & Savić, 2017, chp 7).

Bioinspired peptide surfactants

Peptide surfactants consisting of both a hydrophobic and a hydrophilic region, can be split into two categories based on their different design patterns: (1) end-by-end, also known as lipid-like or surfactant like peptides (SLPs), having a similar structure to naturally occurring phospholipids (hydrophilic head and hydrophobic tail); (2) side-by-side, in which the peptide has specifically located hydrophilic and hydrophobic amino acid sequences enabling the formation of α-helices or β-sheets. SLPs have been widely studied in recent years as they can self-assembly into large tertiary structures such as nanotubes or nanovesicles.

End-by-end surfactants

End-by-end peptide surfactants have a head–tail structure consisting of a hydrophobic tail having several hydrophobic amino acids joined in sequence and a hydrophilic head formed by 1–2 polar residues (Fig. 6) (Jiang et al., 2020). Typical examples include the SLPs developed by Zhang's group such as Ac-V_6D−COOH with an anionic aspartic (A) head (Koutsopoulos, Kaiser, Eriksson, & Zhang, 2012), or cationic head Ac-A_6K-NH_2, Ac-A_6K−$CONH_2$, Ac-V_3D−COOH (Castelletto et al., 2010). Many SLPs can self-assemble into vesicles, micelle or nanotube structures to stabilize proteins, chemicals and medicines, thus facilitating their production and functionality (Wang et al., 2011).

Bola-like surfactants have been designed consisting of two like-charge amino acids located at each end of a peptide linked by a hydrophobic amino acid sequence. It was revealed that the bola-like surfactants are more likely to form extended confirmations such as β-sheet due to molecular symmetry (Zhao, Deng, Wang, Xu, & Lu, 2015).

Cone-shaped peptide surfactants have a head–tail structure where the tail consists of hydrophobic residues with increasing or decreasing hydrophobicity or side chain size (Jiang et al., 2020). They have been demonstrated to self-assemble in water forming hydrogels that are biocompatible, tunable and resisting high mechanical stress and high temperature up to 90 °C (Mishra et al., 2011). Several novel designed examples have been reviewed by Wang et al. including Ac-LIVAGD with a cone-shaped structure self-assembling into transitional α-helices that transform into β-turn structure (Hauser et al., 2011; Khoe, Yang, & Zhang, 2009).

Side-by-side surfactants

Side-by-side surfactants generally have an α-helical structure consisting of specifically placed amino acids that enable the formation of α-helix or β-sheets where patterned hydrogen bonding and alignment of hydrophobic and a hydrophilic side chains form interconnected strands (Fig. 7). Zhang et al. discovered an ionic self-complementary peptide Zuotin that could bind to left handed Z-DNA in yeast protein (Zhang, Lockshin, Herbert, Winter, & Rich, 1992). It contains a repeated unit EAK16-II (n-AEAEAKAKAEAEAKAK-c) with an alternative hydrophilicity in amino acids (Zhang, 2017).

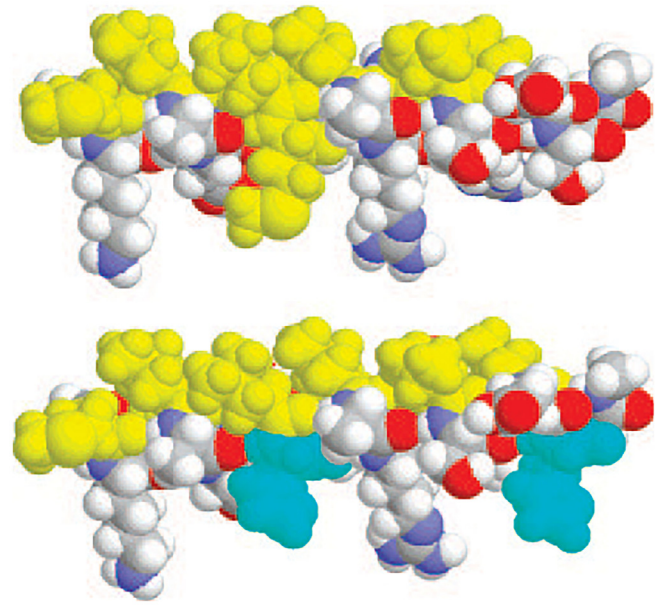

Fig. 7. Surfactant peptides (top) Lac 21 and (bottom) AM1. The peptides are modeled as right-handed R-helices, with hydrophobic residues (methionine, leucine, and valine) shown in yellow and metal-binding histidine residues in blue. Reprinted from ref (Dexter & Middelberg, 2007) with permission from the American Chemical Society. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Another type of side-by-side peptides have been designed with stimuli-responsive property. This type of peptides consists of specifically placed hydrophilic and hydrophobic residues that self-assemble to α-helices or β-sheets with surface activity (Dexter, Malcolm, & Middelberg, 2006). Middelberg's group have reported a 21-residue amphipathic peptide, AM1, designed as a stimuli-responsive surfactant (Dexter & Middelberg, 2007; Zhao, Dwyer, Yu, & Middelberg, 2017). The design of AM1 was originated from bacteria derived amphipathic peptide Lac21 with the substitution of two histidine residues in amino acid sequence at positions 9 and 20 to allow the formation of mediated metal-ion crosslinking between the interfacial peptide surfactants forming an interfacial network. It can be actively switched between a mobile detergent state and a film state by altering pH and metal ions concentrations. This special property confers emulsions or foams with a stimuli-responsive function which is desirable for various potential applications (Li et al., 2016; Wang, Wibowo, Shao, Middelberg, & Zhao, 2017; Wibowo, Wang, Shao, Middelberg, & Zhao, 2017; Yang, Wibowo et al., 2017; Yang, Fang et al., 2017).

Many bifunctional peptides have been designed based on the AM1 peptide by modularizing partial peptide sequence of AM1 with a sequence for different functions (Hui, Wibowo, & Zhao, 2016, 2018; Wang, Wibowo et al., 2017; Wibowo, Zhao, & Middelberg, 2014; Zhao & Liu, 2018). SurSi is a bimodular catalytic peptide where the first module, the Sur module, is a partial AM1 α-helix to stabilize oil–water interface and the second module, the Si module (RKKRKKRKKRKKGGGY), can catalytically generate a silica shell forming a silica nanocapsule (Wibowo, Zhao, Middelberg et al., 2014). Core–shell nanoparticles fabricated using SurSi have excellent drug loading efficiency of 65% compared to traditional drug loading efficiency of 10% or lower and exceptional encapsulation

efficiency of up to 99% (Wibowo, Zhao, Middelberg et al., 2014; Yang et al., 2019). The high drug loading efficiency enables more efficient cellular uptake of hydrophobic therapeutic drugs, thus improved anti-cancer effect and reduced side effects (Yang et al., 2019). AM-S consists two modules with first being the AM1 α-helix and the second module — a hydrophobic tail (GAGAGAGY) that better anchors the peptide at the air–water interface thus improving foam stability (Wang, Wibowo et al., 2017; Wibowo, Wang et al., 2017). A range of peptides have been designed to enhance their functionality, biomineralization, and surface activity. The great diversity in the physiochemical properties, structures and functions of amino acids offers great opportunity in creating extensive number of peptides with unique self-assembly functions.

Bioinspired protein surfactants

Proteins can be used as surfactants to stabilize emulsions due to their amphiphilic nature that is they contain hydrophilic and hydrophobic amino acid sequences that act at the oil–water interface. Compared to small molecule surfactants, proteins adsorb to oil–water interfaces at a slower rate resulting in coalescence of droplets early in the emulsion forming process (Lam & Nickerson, 2013). However, as the proteins adsorb onto the droplet surface the protein surfactant provides a much stronger stabilizing effect with powerful electrostatic repulsion and steric stabilization due to the extensive protein networks that form (Lam & Nickerson, 2013). The tertiary structure of proteins denature into random coils as it rests on the interface with the hydrophobic portions aligning along the interface into the oil phase and the hydrophilic portions forming a diffuse or compact shell based on intermolecular interactions (Wilde, Mackie, Husband, Gunning, & Morris, 2004). However, proteins with specialized surface-active sequences have been discovered and invented such as surfactant proteins A, B, C, and D, which act at the air–water interface in the lung, and DAMP4, an engineered protein used in nanomedicine research (Middelberg & Dimitrijev-Dwyer, 2011; Whitsett & Weaver, 2002).

Protein-based surfactants acting as carriers have been widely applied in hydrophobic drug delivery. Albumin, a heart-shaped protein consisting three homologous domains, is commonly found in blood plasma that act as a plasmic carrier for hydrophobic drug delivery (He & Carter, 1993). Nanoparticle albumin-bound (nab™) technology has been widely used in tumor treatment due to the high accumulation of albumin in tumors (Desai, 2015). This technology has been used in developing nab drugs such as nab-paclitaxel (Abraxane®) approved by FDA in 2005 to treat metastatic breast cancer and non-small cell lung cancer (Desai, 2015). Another example is poractant alfa (CUROSURF®) which was approved by the FDA in 1999 containing 1% non-pyrogenic pulmonary surfactant SP-B and SP-C that is used for surfactant-deficient infants to restore the surface activity of the lungs.

Similar to surface active peptides, protein surfactants can be rationally designed and produced via a recombinant microbial approach with different stimuli-responsive property (Middelberg & Dimitrijev-Dwyer, 2011; Zhao et al., 2017) and multiple functions (Sun, Wibowo, Middelberg, & Zhao, 2018; Sun, Wibowo, Sainsbury, & Zhao, 2018; Wibowo, Zhao, Peters, & Middelberg, 2014; Wibowo, Yang, Middelberg, & Zhao, 2017; Wibowo, Zhao, & Middelberg, 2015; Yang, Wibowo et al., 2017; Yang, Fang et al., 2017). Industrial biotechnology can be applied for the modification and production of biosurfactants, thus providing engineering solutions to address the problems associated with yield, cost and future scale-up (Dimitrijev Dwyer, Brech, Yu, & Middelberg, 2014). Unlike switchable peptide surfactants which are synthesized using chemical methods, protein-based surfactants can be expressed and produced using recombinant DNA technology. However, it often associates with high production costs because of the complicated expression and expensive chromatography-based purification and separation (Dimitrijev Dwyer et al., 2014). Middleburg's group have invented the first non-naturally occurring protein-based surfactant, DAMP4, that connects four repeated AM1 peptides (Dimitrijev Dwyer et al., 2014). DAMP4 can resist high salt concentrations and high temperature up to 95 °C. This special feature allows a simple "bake-to-break and precipitate" process for the biosurfactant precipitation and purification that extensively reduces the cost of production. Multiple fusion proteins have been developed based on the DAMP4 protein. An antimicrobial protein DAMP4-Pexiganan was designed comprising the surface active DAMP4-modified variant with an antimicrobial peptide pexiganan. Similarly, the $DAMP4_{VAR}$-Pexiganan can be purified using the low-cost chromatography free technology (Zhao, Dwyer et al., 2015). Also, the D4S2 protein was designed having a biosilicification-active peptide (RKKRKKRKKRKKGGGY) that can form silica nanocapsules for applications in biomedical and agriculture industry (Wibowo et al., 2015).

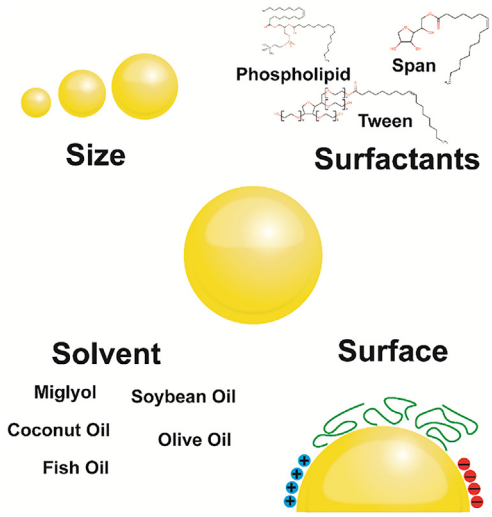

Fig. 8. Physicochemical properties of emulsion nanomedicine governing their efficacy as drug delivery vehicles. Size governs the available surface area and the adsorption of serum proteins. Surfactants for nanoemulsion drug delivery must be non-toxic and effectively stabilize the oil-water interface in biological media. The solvent excipient to solubilize drug must also be non-toxic and FDA approved oils are generally derived from plant or animal sources. The surface charge and coatings of the emulsion droplet govern bio-nano interactions and the formation of the protein corona.

Emulsion nanomedicine

Considerations for emulsion nanomedicine

A multitude of physicochemical factors have been identified that affect the behavior of emulsion nanoparticles in vivo (Fig. 8). Novel emulsion nanomedicines must be controlled for these factors to successfully treat disease and meet stringent regulatory requirements. Size is an important physical parameter that influences the cellular response to nanoparticles. Nanoparticles with a size of ~50 nm have the greatest cellular uptake with slightly larger, >60 nm, and smaller, <20 nm, particles being rapidly cleared by the renal and reticuloendothelial systems (Jiang, Kim, Rutka, & Chan, 2008). The cellular uptake pathways utilized change as the size of nanoparticles increases with small nanoparticles <200 nm up taken by pinocytosis pathways, ~250 nm by phagocytosis and large micron particles by non-receptor mediated micropinocytosis (Foroozandeh & Aziz, 2018). Size distribution is a measure of the different populations of each size and is becoming increasingly

important for FDA approval of nanomedicine. Formulations with monodisperse emulsion droplets have reproducible and expected biological behavior compared to polydisperse droplet systems (Danaei et al., 2018). This highlights the importance of emulsion stability against coalescence.

The surface properties of emulsions regulate their stability against coalescence but also their interactions with biological tissues. It is known that nanoparticles' surfaces when administered into the body are coated with a layer of extracellular proteins. The constituents of this layer, called the 'protein corona', are determined in large part by the specific surface properties of the nanoparticle. Surface charge plays an important role in modulating the protein corona and subsequent interactions with cells. Anionic nanoparticle surfaces are not readily taken up by cells due to electrostatic repulsion and generally rapidly cleared by liver and macrophage cells (Longmire, Choyke, & Kobayashi, 2008). Cationic nanoparticle surfaces strongly bind to the negatively charged cell surface promoting uptake, however, still faced increased clearance due to opsonization and uptake by macrophages (Longmire et al., 2008). Neutral nanoparticle surfaces with low to no charge show reduced opsonization as there is less electrostatic attraction between particles and proteins and thus, reduced clearance (Longmire et al., 2008). Coating nanoparticles with hydrophilic uncharged polymers, such as PEG, has a similar effect by providing a steric barrier to protein adsorption and reducing electrostatic attraction (Fam et al., 2020).

Selection of an appropriate emulsion excipient requires careful consideration of the specific needs of a nanomedicine formulation. The excipient must readily solubilize the drug of concern, be non-toxic, biocompatible and form monodisperse stable emulsion droplets to meet FDA guidelines. The most common excipients for pharmaceutical use are sourced from plant based oils, containing long chain and/or medium chain triglycerides, as they require little assistance to solubilize drugs, have good biocompatibility and can be easily purified to remove toxic trace compounds (Strickley, 2004). Similarly, pharmaceutical surfactants must be biocompatibility, have low toxicity and good surface activity. There are a variety of common FDA approved surfactants used to deliver compounds of pharmaceutical interest including lecithin phospholipid surfactants sourced from egg or plant sources and non-ionic small molecule surfactants such as Span, Brij and Tween surfactants (Tharwat, 2005).

Emulsion formulations are routinely used to administer drugs as they enhance the solubility of hydrophobic compounds, improve the pharmacokinetic profile and reduce adverse effects experienced by patients. Emulsions can be administered to patients through several routes that will be briefly introduced (Fig. 9). Locoid Crelo® is an emulsion containing hydrocortisone butyrate stabilized by the non-ionic surfactant cetostearyl alcohol that is administered by topical application to the skin (Pieérard, Lachapelle, Frentz, Schopf, & Stolz, 1996). The properties of the emulsion including emulsion type, drop size, surfactant, emollients affect the dermal delivery of drug (Otto, Du Plessis, & Wiechers, 2009). Diprivan® is a general anaesthetic emulsion containing propofol stabilized by egg lecithin that is intravenously administered to sedate patients (McCulloch & Lees, 1985). Intravenous delivery of biologically active compounds is ideal for drugs where the effects are required straight away as the drug enters circulation immediately or the bioavailability of the drug through the gut is poor due to slow absorption or degradation (Bardal, Waechter, & Martin, 2011, chp 2). Restatis® and Gengraf® are ciclosporin A emulsions, administered via ocular or oral routes respectively, for suppressing the immune system in patients who have inflammatory/autoimmune disease and/or organ/tissue transplants (Mah et al., 2012; Qazi, Forrest, Tornatore, & Venuto, 2006). Oral route administration is the preferred method due to the high patient compliance to drug regimens (Shahiwala, 2011). Drug-emulsion administration via intraperitoneal and intramuscular methods are under active research for applications in insulin delivery and as vaccine adjuvants, respectively (Haggag et al., 2018; Tegenge et al., 2016).

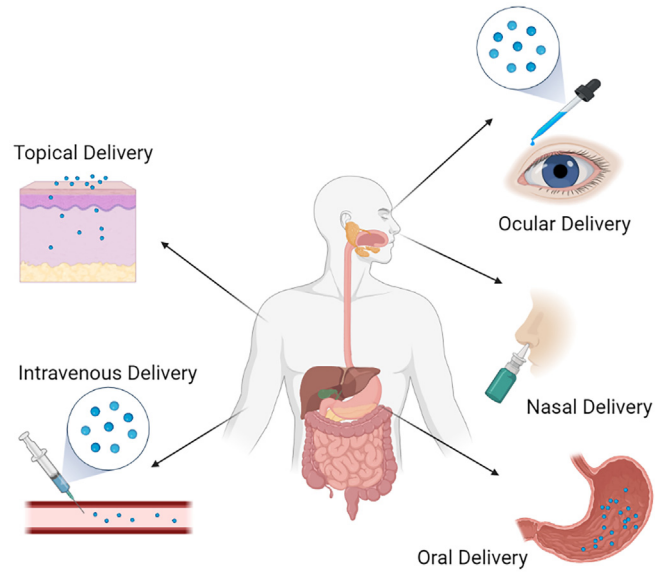

Fig. 9. Different routes of administration for nanoemulsion drug delivery.

Despite the clear advantages of nanoemulsions as a platform technology for drug delivery the system faces some significant challenges to clinical translation. Scaling up the preparation of nanoemulsion to meet industrially relevant production is major challenge. Traditional techniques to prepare nanoemulsions such as ultrasonication and rotor stator homogenization lack industrial scalability due to incredible heat generation and variable high shear environments producing polydisperse material. Whereas, high throughput techniques that are readily scalable such as high-pressure homogenization and microfluidization are now regularly used to prepare but require high energy investment and can incur significant costs from wastage. Secondly, non-ionic surfactants are routinely used in the clinical setting as they are well-tolerated, however they are not readily modified with additional functionalities. The addition of functional moieties and targeting ligands requires expensive and lengthy chemical modification of existing surfactants. In addition, they still face strict regulatory restrictions on their total content in pharmaceutical products. These factors limit their application as next generation nanoemulsion drug delivery system. The development of flexible surfactant libraries that can be readily adapted with multiple functionalities and readily scalable emulsification technology is paramount to future clinical success.

Strategies for therapeutic and diagnostic emulsion nanomedicine

Emulsion nanomedicine has distinct advantages over free drug including improved bioavailability, stability against degradation, reduced clearance and unique biological interactions. However, nanomedicines have struggled to improve the efficacy of treatment in the clinical setting. Cancer is a diverse disease and it has been identified that specific cancers require unique nanomedicine strategies to significantly improve treatment (Chauhan & Jain, 2013). In recent years strategies have been developed to prepare next generation emulsion nanomedicine with greater efficacy and safety for a variety of cancers (Fig. 10).

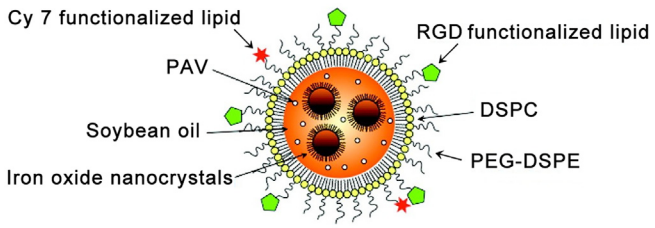

Fig. 10. Schematic of a next generation nanoemulsion platform stabilized with DSPC phospholipid surfactant that is externally modified with Cy 7 fluorescent dye for diagnostic imaging, the stealth polymer PEG and the arginylglycylaspartic acid (RGD) targeting peptide. The soybean oil core is loaded with the hydrophobic drug prednisolone acetate valerate (PAV) and hydrophobic iron oxide nanocrystals for MRI imaging applications Adapted with permission from (Gianella et al., 2011) with permission of the American Chemical Society.

Efficient delivery of active cargo to disease sites using nanomeulsions can utilize two methods of drug loading: encapsulation and/or conjugation. Encapsulation involves loading the hydrophobic drug into the oil phase of the emulsion. Encapsulation can be performed prior to emulsification leading to high encapsulation efficiency and minimal wasted drug (Qadir, Faiyazuddin, Talib Hussain, Alshammari, & Shakeel, 2016). Many other nanoparticle vectors load the drug using post-synthetic process (Liu, Yang, Jin, Xu, & Zhao, 2020) such as by passive diffusion that have poor encapsulation efficiency, take many hours/days and incur high waste (Han et al., 2015; Lu, Liong, Zink, & Tamanoi, 2007). Preloading drug gives high drug loading efficiency, however, the drug may be susceptible to heat or harsh reaction conditions used for post-modification of the nanoemulsions. Whereas, conjugation involves attaching the drug to the surface of the emulsions by covalent bonds. Conjugation drug loading efficiency is related to the ratio of reactive groups on the drug and emulsion and the reaction kinetics (Yousefpour, Atyabi, Vasheghani-Farahani, Movahedi, & Dinarvand, 2011). The drug molecules displayed on the surface remain susceptible to oxidation and chemical degradation as they are not protected within the core (Pasut, Scaramuzza, Schiavon, Mendichi, & Veronese, 2005).

Conjugation can also be used to modulate the surface interactions of emulsion nanomedicine with biological tissues. Hydrophilic uncharged polymers such as PEG, polyvinyl alcohol and polysulfoxides are commonly conjugated to the surface of nanomedicine as they reduce opsonization and have low toxicity (Suk, Xu, Kim, Hanes, & Ensign, 2016). These stealth polymers form a hydrophilic extension of the nanoparticle surface that, when compressed by opsonin proteins, act as a thermodynamic barrier to protein adsorption (Walkey, Olsen, Guo, Emili, & Chan, 2012). This hydrophilic barrier alters the type and density of opsonin proteins adsorbed to the particle surface and decreases the phagocytic clearance of nanoemulsion (Pozzi et al., 2014). Nanoemulsion stealth properties can be controlled by altering the polymer(s), its length and branching which influence the binding of opsonin proteins.

Targeted drug delivery utilizing emulsion nanomedicine is superior to non-targeted methods as it improves biodistribution and reduces non-specific uptake in healthy tissues. Passive targeting exploits the pathways that preferentially distributes nanoparticles in specific tissues throughout the body such as the tumor, liver, spleen, kidneys and reticuloendothelial system. This can be used to delivery large payloads of therapeutic agents to these sites. In the treatment of cancer passive targeting has struggled to achieve widespread clinical success due to the heterogeneity of the EPR effect exhibited within a tumor, between different cancers and different patients (Rosenblum, Joshi, Tao, Karp, & Peer, 2018). However, the EPR effect may prove useful in promoting tumor accumulation when used with combinatorial EPR enhancing therapies (Golombek et al., 2018). Active targeting uses targeting ligands conjugated to the surface of the emulsion droplet (Yoo, Park, Yi, Lee, & Koo, 2019). Many diseases are characterized by the upregulation of certain receptor proteins on the surface of unhealthy cells (Afzal, Shareef, Dinesh, & Kishan, 2016; Kim et al., 2008). The ligand–receptor binding promotes internalization of the nanoparticle into the cell where the drug can be released. This results in higher uptake of ligand bearing nanoemulsion into the diseased tissue reducing non-specific uptake and toxicity (Ganta et al., 2016).

Clinical applications of nanoemulsions

Nanoemulsions are an attractive platform to develop effective nanomedicines for drug delivery. The facile preparation and well understood properties of emulsions and surfactants provide a flexible framework to develop a range of nanomedicines. Their small size allows them to penetrate deep into tissues, prolong their circulation and have unique bio-nano interactions. While the oil core can be loaded with a variety of hydrophobic cargo such as drugs, photosensitizers and contrast agents. Loading into the core structure protects sensitive materials from degradation, increases the bioavailability of poorly adsorbed or soluble drug and more effectively delivers cargo to the disease site. Additionally, by decorating the surface with hydrophilic polymers, targeting moieties, monoclonal antibodies and/or diagnostic markers nanoemulsions can have potent physiological activity. Rational design of the internal and external architecture taken together with their small size makes nanoemulsion systems an ideal platform for clinical pipeline research.

Emulsions have enormous potential in the clinical treatment and diagnosis of disease. Lipid emulsion formulations have been routinely prescribed by physicians in the past two decades to administer hydrophobic drugs such as corticosteroids, anaesthetics and immunosuppressants for transplants. An extensive library of potential emulsion nanomedicines have been reported in the literature to deliver a variety of medications including anticancer, antimalarial, antipsychotic, antiglaucoma and cholesterol lowering drugs (Bali, Ali, & Ali, 2010; Boche & Pokharkar, 2017; Huang et al., 2013; Laxmi, Bhardwaj, Mehta, & Mehta, 2015; Morsi, Ibrahim, Refai, & El Sorogy, 2017). In recent years, novel treatments for cancer, autoimmune and chronic disease, including immunotherapy, vaccines and photo/thermal therapies have been developed that may be improved by utilizing the unique properties of nanoemulsions as a delivery vector. Immunotherapy is a promising new method to indirectly treat cancer by activating a pro-inflammatory antitumor phenotype immune cells and an immunogenic tumor microenvironment. Nanoemulsions are an attractive adjuvant to enhance the immunogenic response as they have been widely utilized in the clinical setting and have good pharmacokinetic profiles and safety. Kim et al. demonstrated that encapsulation of TLR 7/8 agonists within a nanoemulsion can successfully reverse immunosuppression at the tumor site, promote and prolong a pro-inflammatory immune environment activating various immune cell populations and recruiting tumor infiltrating lymphocytes to suppress tumor growth (Kim et al., 2019). Vaccines have great potency at containing and prevent disease such as influenza epidemics that cause significant economic and emotional damage (Vesikari et al., 2011). Building on these concepts Zeng et al. reported a PEGylated antigen-Clec9A nanoemulsion that exploited traditional targeted nanoemulsion drug delivery without the need for additional adjuvants to promote uptake in dendritic cells and further enhance immunogenic responses in tumor specific CD4+ and CD8+ T-cells and consequently suppress tumor growth (Zeng et al., 2018). Finally, Pellosi et al. have reported the synthesis of a nanoemulsion system containing magnetic γ-maghemite nanoparticles and chlorin e6, a partially hydrogenated porphyrin

photosensitizer, for dual magnetic hyperthermia and phototherapy treatment (Pellosi, Macaroff, Morais, & Tedesco, 2018). In vitro assessment of the dual action nanoemulsion revealed that the emulsion system protected the chemically vulnerable photosensitizer from deactivation and upon activation with an alternating electromagnetic field and targeted laser the nanoemulsion significantly reduced the viability of MCF-7 breast cancer cell lines compared to NHI-3T3 mouse fibroblast cell lines.

Diagnostic nanoemulsions may provide clinicians with tools to guide investigation where traditional diagnostic techniques and contrast agents fail. They do so by transporting hydrophobic contrast agents to disease sites in diagnostically relevant concentrations, prolong circulation for multiple scans and reduce toxic adverse effects.

Wallyn et al. recently developed a dual diagnostic imaging nanoemulsions by encapsulating two different hydrophobic contrast agents iron oxide nanoparticles to enhance MRI contrast and vitamin E conjugated to an iodinated benzoyl group to enhance X-ray images; MRI and X-ray are two of the most common diagnostic tools utilized by clinicians (Wallyn et al., 2019). Incorporating the multiple contrast agents into a nanoemulsion, reduces the need for multiple injections, improve the biodistribution and can increase the concentration in the tissues of interest improving the resolution gain (Wallyn et al., 2019). Likewise, Kennedy's group has investigated nanoemulsions containing perfluorinated carbon species, combined with anticancer drugs for the theranostic treatment of cancer (Rapoport, Gao, & Kennedy, 2007, 2011). Perfluoropentane emulsion undergo a droplet to bubble transition when exposed to ultrasound simultaneously selectively releasing drug and the bubbles provide enhanced contrast at the tumor site for several days (Rapoport et al., 2007). However, perfluoropentane (boiling temp. 29 °C) can be difficult to work with due to thermally induced droplet to bubble transition at physiological conditions and readily foams with incorrect handling. In contrast, perfluoro-15-crown-5-ether (boiling temp. 146 °C) emulsions are very stable and exhibit a reversible droplet to bubble transition upon exposure to ultrasound triggering drug release and providing ultrasound contrast (Rapoport et al., 2011). The highly fluorinated perfluorocrown ether provides a single magnetic resonance ^{19}F peak acting as a strong MRI contrast agent, however, the contrast it provides is dependent on the oxygenation/vascularity of the tissue of interest.

Conclusion

Nanoemulsions have attracted significant interest in past decades for various applications due to their unique structures and properties. They can be easily produced at large scale using industrial methods including high-pressure homogenization and ultrasonication. By virtue of their small size and easy-to-disperse components with different hydrophobicity (e.g. hydrophobic drugs in the dispersed oil phase and hydrophilic proteins in the continuous aqueous phase), they have great potential in applications including food, cosmetics and pharmaceutics. For example, nanoemulsions have been formulated to deliver hydrophobic drugs, and have been used as adjuvants for vaccines, demonstrating their clinical impacts. Because of these clinical successes, nanoemulsions have been further developed for emerging high-end applications such as immunotherapy, targeted therapy by incorporating multiple functions, for example, encapsulating drugs or imaging probes in the oil droplet, and decorating the nanoemulsion surface with targeting ligand or antibodies for targeted delivery and immunotherapy. Although many of these preclinical studies are in very early stage, more systematic studies and fundamental understanding of the complex interactions between nanoemulsions and biological systems from cells to tissues and organs will accelerate the translation of their real clinical applications.

Declaration of interest statement

All authors declare no other competing interests.

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