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REVIEW

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ABSTRACT

This review aims to provide a comprehensive overview about various innovative strategies that have been employed by recent researchers to overcome with the shortcomings associated with traditional microspheres. Essentially, optimization strategies from structural aspects have been widely investigated to improve the properties (*e.g.*, enhanced hydrophilicity, reduced initial burst release, *etc.*) of the pristine microspheres. These include bulk alteration, surface modification as well as the formation of sophisticated microsphere design such as core-shell structures. Other than that, various microencapsulation techniques and novel technologies such as spray drying, supercritical fluid technique, membrane, and microfluidics emulsification also have been explored in this review. Additionally, the impact of formulation-related aspects on the drug encapsulation efficiency, particles size and particles size distribution during double emulsification method will also be discussed and reviewed extensively based on the recent literatures reported.

1. Introduction

Lack of rate-controlled release and target specificity are probably the most renowned limitations of drug administration using conventional regimen. In most of the cases, traditional drug intake tends to cause a sharp increase of drug content in plasma, which will then decline below therapeutic limit after a short period of time, making re-administration indispensable in order to achieve the intended pharmaceutical function. In this regards, the controlled release drug delivery system (DDS) which involve therapeutic release kinetic over an extended duration has been advanced rapidly as a better delivery vehicles to overcome the shortcomings associated with traditional administration.[1] Such delivery system enables the

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drug plasma concentration to be maintained within desired therapeutic range and/or allows specific drug delivery to the target region, thus broaden their therapeutic window by offering a more efficacious and safer delivery of pharmaceutical drugs during treatment. This is particularly important for the delivery of therapeutic agents that are rapidly metabolized and removed from the body after administration. While a variety of devices such as nanoparticles, microspheres, dendrimers, liposomes, micelles, *etc*.[2] have been utilized for depot-based delivery system; biodegradable polymeric microsphere is one of the widespread studies one. It is noted that single administration of drug-encapsulated polymer microsphere allow the release of cargos in a continuous and controlled fashion over a long period of time, thus retaining the drug concentration within a target ranges.[3] This can significantly minimize systemic side effect ensued from the repeated administration using traditional regimen, thus increase the patient compliance during drug intake.[4–6] In addition, this carrier also acts as a transient mask to protect unstable and labile active pharmaceutical ingredients (API) (*e.g.*, enzymes, proteins and peptides) from physiological degradation in local tissue surrounding, thus prolong their half-live *in vivo*.[7],[8]

In recent years, polyester-based synthetic polymer such as poly (lactic acid) (PLA) and poly (lactic-co-glycolic acid) (PLGA), which is approved by the U.S. Food and Drug Administration (FDA) and European Medicine Agency (EMA) has received enormous attention among pharmaceutical researchers for the delivery of pharmaceutical agents, such as drugs and other macromolecules such as protein, peptides, DNA and RNA. This is due to its biodegradable and nontoxic characteristics that can be degraded into water soluble oligomers or monomers, which will be further metabolized and eliminated from the body.[9–11] Furthermore, their degradation and drug release kinetics can be accurately regulated over different periods from days to months by changing their molecular weight, chemical compositions (*e.g.*, lactide:glycolide ratio) and crystallinity.[12–15] For instance, PLGA with higher lactide ratio degrades more slowly as the additional $-CH_3$ constitute on the polymer backbone will enhance the hydrophobicity which in turn impose steric hindrance and reduces water infiltration, thus result in a slower cargo release. In the last two decades, several pharmaceutical products based on PLA or PLGA microparticles have been brought to the market[16–19] and some of the commercially available depot formulations are listed in Table 1.

Recently, there are quite a number of literatures reviewing topics related to biodegradable polymeric microspheres.[5],[20–23] Despite this, to date, there are lacks of literatures that have comprehensively and specifically reviewed about the efforts and approaches that have been

Table 1. PLA or PLGA-based microparticles currently available in the market.

Commercial name	Active pharmaceutical ingredient (API)	Polymer	Company	Application
Lupron® Depot	Leuprolide acetate	PLGA	TAP	Prostate cancer, Endometriosis
Nutropin® Depot	Growth hormone	PLGA	Alkermese & Genentech	Pediatric growth hormone deficiency
Zoladex®	Goserelin acetate	PLA	AstraZeneca	Prostate cancer, endometriosis
Decapeptyl®	Triptorelin pamoate	PLGA	Ferring	Prostate cancer
Decapeptyl® SR	Triptorelin pamoate	PLGA	Ipsen-Beaufour	Prostate cancer
Trelstar™ Depot	Triptorelin pamoate	PLGA	Pfizer	Prostate cancer
Somatuline® LA	Lanreotide	PLGA	Ipsen-Beaufour	Acromegaly
Suprecur® MP	Buserelin acetate	PLGA	Aventis	Prostate cancer
Arestin®	Minocycline	PLGA	Orapharma	Periodontal disease
Risperidal® Consta™	Risperidone	PLGA	Johnson & Johnson	Antipsychotic
Bydureon®	Exenatide	PLGA	Amylin Pharmaceutical Inc.	Type 2 diabetes

employed by recent pharmaceutical scientists in improving the therapeutic efficacy of PLA and PLGA microspheres for DDS. Herein, we are going to provide a glimpse of the endeavours that have been taken by recent researchers in relation to this purpose. As in the first section of our review, we will address some of the impediments encountered by conventional PLA or PLGA microsphere to be commercialized into practical excipient in clinical application. Meanwhile, approaches, modus operandi, technologies and some aspects that are worthwhile to notice during microsphere preparation will be highlighted and discussed in the later part of this literature.

2. Limitation of PLA/ PLGA-based microspheres

Despite the promising and peculiar characteristics of PLA/PLGA for biomedical applications, several challenges and technical barriers to the development of an effectual delivery mechanism for drug delivery have indeed emerged. First, the inherent hydrophobicity of PLA/PLGA microspheres as compared to the surrounding extracellular matrix (ECM) has unfavorably elicit their interaction with cells, thus resulted in a poor cell adhesion during *in vitro* and *in vivo* administration.[24] Moreover, accumulation of acidic lactic acid by-products engendering from the chain disruption in wet condition tends to trigger an inflammatory response that might complicate the further use of these microsphere for DDS.[25],[26] This condition becomes even worst for the delivery of highly vulnerable macromolecular therapeutic agents such as protein in which their activities might adversely debilitate under harsh acidic environment. It is also noteworthy to point out that pristine PLA/PLGA surface does not encompass any functional groups for the attachment of bioactive molecules to improve the integrin binding with cell membranes and regulate protein adsorption, thus despairing their feasibility for commercialization.[27–29] Some undesired effects such as high initial burst during the inceptive stage of drug release also tend to transpire for conventional PLA/PLGA microspheres before they can achieve a stable release profile.[30] This bursting effect will significantly reduce the effective lifespan of the drug delivery carrier, thus affecting its effectiveness both therapeutically and economically. Encapsulation of hydrophilic drugs during the synthesis of PLA/PLGA microspheres can be challenged too due to the partitioning of weakly associated drugs from the organic phases to the external water phase during conventional emulsion process.[31] Hence, it is very crucial to tailor-design an effectual delivery system that has all the aforementioned bottleneck to be conquered in order to turn their biological potential into medical reality.

3. State-of-the-art techniques and technologies for drug encapsulation in microspheres

Till now, conventional emulsification techniques as well as sophisticated technologies have been developed for the fabrication of drug-loaded microspheres in order to optimize their properties for drug delivering purposes. These include several types of conventional emulsification method (*e.g.*, O/W, W_1/O/W_2, S/O/W, W/O_1/O_2, and S/O_1/O_2), spray drying, supercritical fluids technique (SCF), Shirasu Porous Glass (SPG) membrane emulsification, microfluidics technology, *etc*. These techniques or technologies have partly competing and complementary characteristics where each of the methods will be felicitous for a particular

delivery formulation, thus require throughout understanding to ensure a well-design of the delivery system.

3.1 Solvent extraction/evaporation emulsification methods

Oil-in-water (O/W) solvent extraction/evaporation emulsification method is one of the simplest and ubiquitous methods used to form microsphere. As shown in Figure 1(a), this methods encompassing four major steps to be taken to form microspheres: (i) dispersion of drug molecules in organic solvent (often methylene chloride (DCM)) containing PLA/PLGA to form the oil phase, (ii) emulsification of oil phase in continuous aqueous phase, usually PVA solution (or methylcellulose (MC), chitosan, sodium dodecyl sulfate (SDS), etc.) to form O/W emulsion, (iii) solvent evaporation as well as solvent extraction from the emulsion droplets into the aqueous phase will transpire, thus leading to the formation of dispersed particles in the water medium, and (iv) harvesting of the microspheres by centrifugation or filtration, and the collected products will be freeze-dried prior to storage.[32]

For drug molecules that are poorly dissolved in DCM, a co-solvent (such as acetone, DMSO and acetonitrile) can be added to the polymer-DCM system in order to facilitate their encapsulation process. The usage of water-miscible co-solvents along with the DCM allowed a rapid mass transfer from the organic solvents into the external aqueous medium, hence resulting in a faster solidification of droplets into polymeric microspheres.[31] On the other hand, microspheres with distinct surface characteristics can be fabricated by selecting a suitable surfactant to be used in outer aqueous medium. For example, microsphere with negatively charged surface can be produced when anionic surfactants such as SDS and dioctyl sodium sulfosuccinate (DSS) is utilized.[33] Based on the processing parameter and operating condition, encapsulation efficiency (EE) and properties of the microspheres can also be tailored. For examples, Reza et al.[34] showed that manipulation of stirring rate from 300 to 1000 rpm can caused a decrease in mean particle size from 89.8 to 38.2 μm in PLGA microsphere. With an increase in PLGA concentration from 5% to 13% (w/v), microparticles size
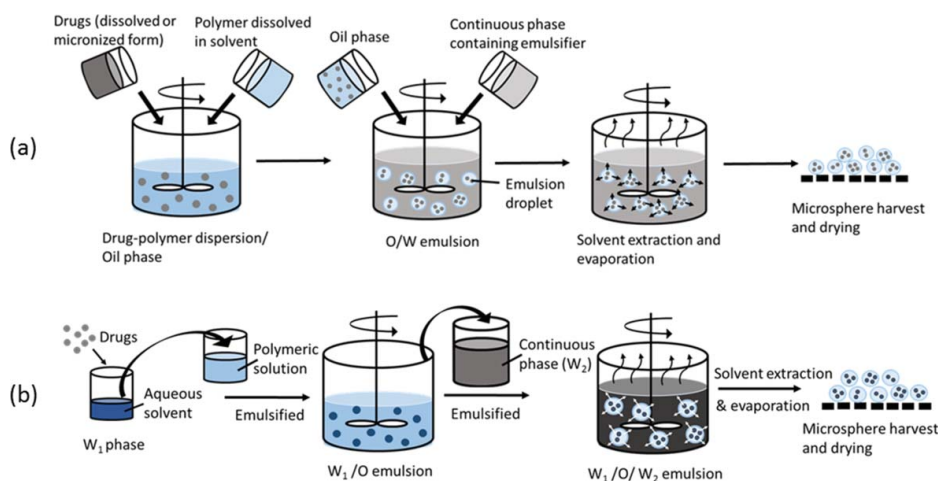

Figure 1. Schematic of the (a) O/W solvent extraction/evaporation technique and (b) W1/O/W2 solvent extraction/evaporation technique for microsphere preparation.

increased from 55.2 to 73.6 μm, due to the more viscous solution that shears differently during O/W process.[35] However, conventional O/W emulsion method is frequently impaired by poor encapsulation for hydrophilic macromolecules, despite high EE have been reported for drugs with hydrophobic nature. Hence, other emulsion-based techniques have been derived from O/W method to overcome the preferential outflow of water-soluble drugs molecules into the continuous phase to improve EE.

Double emulsion-solvent extraction/evaporation techniques have been frequently used to enhance the entrapment efficiency of hydrophilic macromolecules, such as proteins and peptides. Water-in-oil-in-water ($W_1/O/W_2$) technique is the most common double emulsion used to encapsulate water-soluble drugs. As depicted in Figure 1(b), the hydrophilic drug solution to be encapsulated is first emulsified in the organic polymer solution to form the primary water-in-oil emulsion (W_1/O). This W_1/O is subsequently added into a large volume of emulsifier-containing aqueous phase to form double emulsion of $W_1/O/W_2$. In this emulsion system, the organic layer will serve as a barrier to hinder the diffusion of hydrophilic drug into the external water medium. Eventually, solidified particles will formed upon extraction and evaporation of organic solvent.[36],[37] Notably, there are several preliminary parameters and variables that need to be adjusted to optimize the characteristics (including size, size distribution and EE) of the microspheres prepared using this method. These including polymer concentration, drug concentration, volume ratio of the W_1/O, type of stabilizer used, amount of the external aqueous medium, agitation rate, *etc*. Primarily, the manipulation of the W_1/O volume ratio in $W_1/O/W_2$ method is very crucial for the encapsulation of both hydrophilic and hydrophobic drugs. For example, as the W_1 volume approaches the volume of the organic phase, the stability of the emulsion droplets during second emulsification will be severely afflicted. This will result in microspheres with poor drug EE and high initial burst, thus affecting their application when implied *in vitro*.[38] Others variables and parameters will be detailed and discussed thoroughly in Section 4 for better understanding and control of the microspheres properties, specifically for the encapsulation of hydrophilic drugs.

Sometimes, when $W_1/O/W_2$ double emulsion methods are abortive in encapsulating a particular hydrophilic drugs, S/O/W emulsion approach can be used instead. In this method, drug particles in the form of solid state can be dispersed in the organic polymer solution to form inner S/O suspension[39],[40] and the rest of the procedure is similar as reported for the previous discussed method to fabricate microsphere. This approach is especially useful for certain cases where the compounds to be encapsulated are sensitive and have the tendency to loss their activity at the W_1/O interface. Furthermore, this emulsion technique also reduce the possibility of drug leakage into the outer aqueous medium, since solid-state drugs molecules are required to obviate dissolution step before able to diffuse out from organic droplets into outer water phase. By using S/O/W method, EE of amoxicillin reached 61% in PLGA microparticles, while its encapsulation merely showed 35% when $W_1/O/W_2$ approach was employed.[41],[42] In this method, it should be bear in mind that the encapsulating agents are required to be in fine micrometer size to allow a complete entrapment of the drug crystals in the microsphere. If comparatively bigger drug crystals are used in the primary S/O suspension, the scanty amount of encapsulated crystals will penetrate through the polymer shell and cause initial burst release. To alleviate this problem, microsphere prepared using S/O/W method can be extracoated by incorporating organic polymer/chloroform solution into the pre-microsphere suspension, or by

increasing the viscosity of the polymer solution used in the emulsification process. Due to the low but different solubility of certain API in the organic solvent, some of them might dissolve in the solution of the S/O/W emulsion. Hence, it is not suggested to store the drugs suspensions in the organic phases to avoid crystal growth.[20]

As reported by massive amount of previous literatures, solvent extraction/evaporation emulsification involving aqueous external phase (*i.e.*, $W_1/O/W_2$ and $S/O/W_2$) tend to associated with the partitioning of drug from the internal phase into the continuous phase during the encapsulation of hydrophilic and amphiphilic drugs. Regarding to this problem, some attempt to employ nonaqueous based emulsification approaches (such as $W/O_1/O_2$ and $S/O_1/O_2$ methods) to prepare microsphere. For $W/O_1/O_2$-based approach, microsphere can be prepared by emulsifying the primary W_1/O emulsion in a large volume of nonmiscible organic phase encompassing suitable stabilizer (*e.g.*, lecithin or cotton oil). Nafea *et al.*[43] showed that the encapsulation of alendronate sodium (a bone protective agent) in PLGA microsphere reached an EE of nearly 100% using this method as compared to $W_1/O/W_2$ that only attained 1%. Similar approaches also have been applied to compare the encapsulation of betamethasone phosphate disodium, a type of corticosteroid drug into the PLGA microspheres. It was revealed that the $W/O_1/O_2$ using light white oil containing lecithin as external continuous phase yield significantly higher EE (~78%) compared to $W_1/O/W_2$ method ($\leq$ 15%).[44] On the other hand, alternative organic emulsification approach, which involve similar initial concept and methodology of S/O/W have been explored to entrap some hydrophilic drugs that failed to be encapsulated well in $W_1/O/W_2$ and S/O/W. Several drugs such as pamidronate disodium,[45] ganciclovir,[46] and ciprofloxacin[47] have been encapsulated using this approach and relatively high EE were reported. For organic emulsification performed in oil continuous phase, it is noteworthy that the fabricated microsphere is required to be washed using petroleum ether or hexane instead of aqueous solution during harvesting step in order to remove residual solvent and emulsifier attached to the microparticles. Lyophilization can be then carried out to ensure the further removal of these solvent impurities to a safe level.[20]

3.2 Techniques and technologies for the preparation of monodisperse microparticles

Microsphere size and their size distribution are said to be the prime factors for the design of a drug delivery carrier, as these features will potentially influence the product injectability, biodistribution, microsphere degradation as well as their drug release kinetics and therapeutic efficacy.[48],[49] Furthermore, the *in vivo* fate and longevity of the microspheres also largely rely upon the size distribution of the fabricated microparticles. Typically, small microparticles (with particles diameters less than 10 μm) are likely to be phagocytosed by immune cells while microparticles larger than 200 nm might as well elicit inflammatory response. Based on the conventional emulsification approaches using homogenizer to create turbulence for microsphere formation, the controlling of mean particles size in a desired range is very hard to attain and batch-to-batch variation in both size and morphology are commonly observed. This will lead to the diversity of the drug release profiles as a result of broader polydispersity. Recently, advance microengineering technologies have been developed for the preparation of monodisperse microspheres. One of the widely studied techniques is the so-called membrane emulsification. This technique consists of a SPG membrane that serves as a screening tool to control colloidal droplets in the emulsion, where the

principle of the drug encapsulation is similar to the aforementioned emulsification method. In SPG emulsification process, the emulsion droplets will be forced to pass through the pore of the membrane and detach from the membrane surface to form smaller and uniform-size oil droplets.[50] Noted that the O/W or W_1/O/W_2 system will be first prepared in a separate premix reservoir to form coarse colloidal system prior to be membrane emulsified (Fig. 2). Narrow particle size distribution with tunable particle diameter can be easily manufactured by altering the pore sizes with SPG membrane. As a result, it is convenient to precisely control the drug release behavior from the drug carriers according to the desired cargo release that are prerequisite for a particular DDS.[51],[52] One important issue related to membrane emulsification is handling the cleaning procedure due to membrane fouling. To ensure the membrane is reusable for subsequent emulsification reaction, a series of cleaning protocol (usually including the ultrasonic treatment with appropriate detergent solution, organic solvent and chemical treatment) are crucial not only to remove the emulsification foulants but also to avoid microbial growth.[53],[54]

Monodisperse microspheres with tunable size and shape can also be produced by microfluidics technologies based on single or double emulsion techniques.[55] Generally, the microfluidic devices can be divided into three types: (a) co-flow capillary, (b) flow-focusing capillary, and (c) combination of co-flow and flow-focusing capillary (Fig. 3). In co-flow capillary device, the organic phase containing polymer, and drug is pumped through a syringe with a constant flow rates into the central channel of the device while the aqueous continuous phase will be directed into the side channels in the same direction. Monodisperse droplets will be periodically formed at the tip of the combined channels, therefore promoting the microspheres with narrow size distribution.[56] On the other hand, the size of the microparticles is dependent on the polymer concentration and relative flow rate of both aqueous and organic phase in the system. For example, PLGA microspheres with average diameters of 11
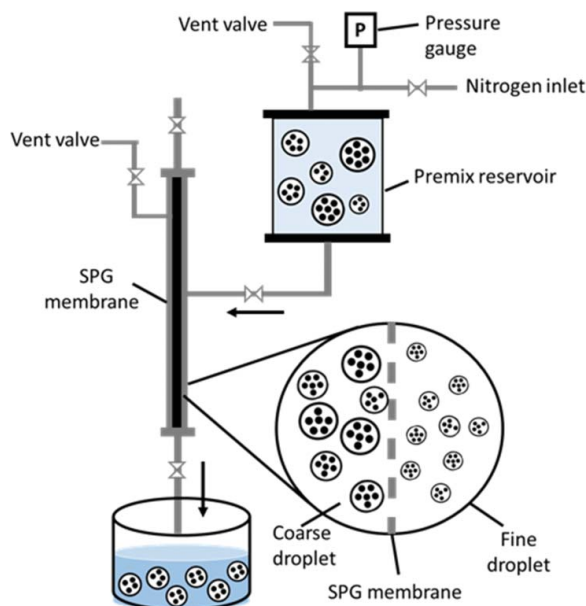

Figure 2. Schematic representation of SPG membrane emulsification process.
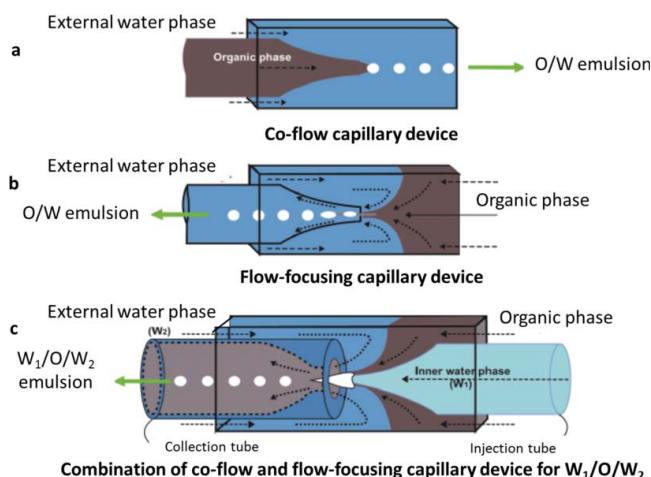
Figure 3. Schematic representation of microfluidics devices for the preparation of monodisperse micro-spheres. (a) Co-flow capillary device, (b) flow-focusing capillary device and (c) combination of co-flow and flow-focusing device for the preparation of microparticles with W1/O/W2 emulsion approach.31.

and 41 mm can be obtained when different flow rates (outer water phase: 4.0–20.0 mL hr^{-1}, organic phase: 0.5–2.0 mL hr^{-1}) are utilized.[57] When PLGA concentration increases from 0.5% to 5%, an increase in the particles volume from 0.52 to 9.2 pl were observed. This is because at higher polymer concentration, larger particles size will be obtained as the large amount of polymer molecules in the system will slower down the solvent diffusion process, thus causing aggregation to form bigger particles.[58] Microsphere with defined size (ranging from 10 to 50 μm) and low polydispersity were successfully prepared by Xu and colleagues using microfluidics device and their correlation with drugs release behavior were studied. Due to the more homogenous distribution of the drugs molecules in the PLGA microspheres prepared by microfluidic method, lower initial burst and overall release kinetics were observed when compared to the polydisperse microparticles prepared using traditional emulsion method. This is very promising for the fabrication of monodisperse drug carriers to be delivered in a sustain manner with less variation than microparticles prepared via con-ventional emulsification technique. In flow-focusing capillary approach, the continuous water phase consisting emulsifiers will be introduced from the two sides of the channels while the drug-containing dispersed phase will be fed to the central channel, both flow in an opposite direction to be focused into microfluidics mixing chamber. The inner oil phase will be hydrodynamically flow-focused by the outer water phase through the narrow orifice. Due to the constriction orifice geometry and shear force imposed by the aqueous phase, a highly periodic dripping or jetting of the organic phase will be formed to yield monodisperse emul-sion droplets with smaller size than the orifice diameter. This feature allows the usage of big-ger orifice in order to minimize the possibility of clogging in the orifice.[59]

Microfluidics device combining both co-flow and flow-focusing component also have been used to fabricate double emulsion-based microspheres. In this approach, there are three phases flowing in different capillary, *i.e.*, inner water phase (W$_1$) in the injection tube, organic (O) and external continuous phase (W$_2$) in the square capillary. Briefly, W$_1$ phase containing dissolved drug molecules is injected through a narrow tube to form fine aqueous

droplets into the organic phase, which is then processed in collecting tube via flow-focused approach to form monodisperse $W_1/O/W_2$ emulsion droplets.[60],[61] Utada *et al.*[62] employed this approach to prepare double emulsion with a core-shell geometry. It was reported that the droplet size and the shell thickness can be tuned by adjusting the relative flow rates and the microfluidics orifice geometry. Similarly, monodisperse PLGA-alginate core-shell microparticles were also successfully prepared using similar concept.[63] Unlike single microfluidics devices that are commonly constrain with low production scale, scaling up the production using this device can be performed by concomitantly employing multiple microfluidics devices in parallel.[31]

It is known that the conventional emulsification based on mechanical stirring is the simplest and cheapest method that can be offered to synthesis PLA or PLGA microspheres. Nevertheless, as mentioned previously, routine agitation using homogenizer would result in the particles with nonuniform size, mainly due to the heterogeneous emulsification caused by the rotor rotation that predominantly confined to the central plane of the reaction flask. In other words, the dispersion of the emulsion can be greatly varied relying on the distance from the stirring rotor. To overcome this problem, Liu *et al.*[64] employed an economically practical technique, *i.e.*, glass beads as co-adjuvant to enhance the homogenous distribution of the mechanical dispersion produced by rotor. The size distribution of PLGA microspheres fabricated using this novel preparative approach were significantly narrower compared to routine mechanical stirring, which indicating the potent of glass beads as dispersion aids in assisting a more homogenous dispersion during emulsification process (Fig. 4).

3.3 Technologies for microspheres preparation

Spray-drying method is a single-step, continuous particles production process that has been widely scrutinized for protein encapsulation to enhance the stability of the biomacromolecules. This approach is feasible for any emulsion system as discussed previously, such as dispersion of aqueous drug solutions (W/O) or micronized drug particles (S/O) in the polymeric organic phase. Principally, the dissolved or dispersed drugs in the organic phase will be first sprayed through a nozzle as ultra-fine droplets into a drying chamber (usually consists of stream of hot air). Due to their high surface to volume ratio, a relatively fast
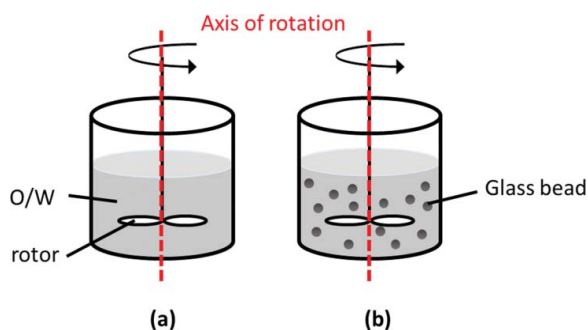

Figure 4. (a) Routine mechanical stirring which involves rotation of rotor around a fixed axis on a single plane, where dispersion of droplets can be varied depending on the solution and the distance from the rotor, (b) glass beads-facilitated mechanical stirring approach that uses random distributed glass beads together with mechanical rotor to assist homogenous dispersion.

solvent evaporation will favor the efficient and rapid drying of the droplets, and dried solid microparticles will be formed.[12],[16] Besides, spray-drying technique is also capable of producing drug-loaded microspheres with high EE, even for the encapsulation of hydrophilic and amphiphilic drugs and the product quality is highly dependent on the spray-drying condition and formulation. This is due to the absence of outer solvent phase that will lead to the drug loss during microsphere preparation. For instances, high EE (84.2–99.5%) of vancomycin was achieved when drug-loaded PLGA microspheres was spray-dried using the process condition: inlet air-temperature: 80–85 °C, outlet-air temperature: 68–70 °C with spray rate of 10 min/ml.[65] Furthermore, spray-drying technique was employed to encapsulate triamcinolone, a corticosteroid drug into PLGA microspheres and 90–98% of EE was demonstrated by da Silva-Junior et al.[66] Chaw et al.[44] have shown that betamethasone phosphate-loaded PLGA microspheres prepared using spray-drying technique possess EE of more than 90%, while only 15% was achieved for microparticles prepared using conventional $W_1/O/W_2$ method. Indeed, the most appealing advantage of spray-drying method is that the final drying step required for conventional emulsion technique is becoming nonobligatory, and associated with their more beneficial economical view compared to freeze drying due to their lower energy consumption involved to dry sample. Additionally, this technique also can overcome the problematic issue associated with solvent contamination in conventional emulsion method. Despite the remarkable advantages presented by the spray-drying approach with respect to other fabrication method, their viscosity limitation, thermal instability, polydispersity and low yield for small batch production (due to the probable loss of product in the wall of the drying chamber) have become the drawbacks for microsphere preparation.[67],[68] Modification of conventional spray-drying using atomizer which is capable of producing uniform droplets (e.g., monodisperse droplet generators) can be useful to produce microparticles with homogenous size.[69]

The use of techniques exploiting the properties of supercritical fluids (SCF) is also becoming one of the propitious alternatives for the fabrication of microspheres. Generally, a SCF can be achieved when a substance is subjected to temperature and pressure higher than its critical point. SCF possesses both the low viscosity properties of gas and dissolving ability of a liquid where the temperature and pressure alteration around the vicinity of the critical point can lead to tremendous changes in their solubility. It also has high penetrating power which make them potent for extraction purposes. Ascribed to the low environmental impact, ease of accessibility, low cost, low critical point (P = 7.38 MPa, T = 304.25 K) and low residual solvents, supercritical CO_2 is frequently used as extracting phase to fabricate microspheres. Basically, there are several SCF approaches that have been employed to form PLA or PLGA microspheres, i.e., rapid expansion of supercritical solutions (RESS), supercritical emulsion extraction (SEE), and supercritical assisted injection in liquid antisolvent (SAILA). RESS method involves the rapid depressurization of emulsion through a nozzle to cause rapid nucleation of microparticles. For the past decades, RESS has become an attractive fabrication method to form drug carriers due to the absence of organic solvents use. Unfortunately, this method displays deficiency of low solubility that will affect their affordable process yields, thus restricting RESS to compound with low polarity properties.[70–72] In addition, control of particles size, particles size distribution, and morphology can be problematic during the particle growth processes, condensation and coagulation that occur in the downstream region. Thus, modification of RESS using a

liquid solvent or a solution, namely rapid expansion of a supercritical solution into a liquid solvent (RESOLV) has been proposed to overcome the shortcomings associated with RESS. This method could prevent aggregation phenomenon by the presence of a polymer or another protecting agent in the receiving solution.[73]

On the other hand, SEE method, which often processes with classic O/W or $W_1/O/W_2$ emulsion with the involvement of supercritical CO_2 as organic extracting agent has emerged as green and robust route to prepare microspheres of high drugs EE with better size and distribution control.[74],[75] In this method, countercurrent-packed tower has been used to ensure rapid solvent extraction and shorter emulsion processing time. As described by Porta et al.,[76] the mechanism of solvent extraction by supercritical CO_2 from an O/W emulsion and a $W_1/O/W_2$ emulsion during the production of PLGA microspheres can be eventuated in two routes. First pathway involves the diffusion of organic solvent into aqueous medium, which then followed by the succeeding solvent extraction by supercritical CO_2 from the water phase. For the second route, supercritical CO_2 can extract the organic solvent directly upon their contact inside the emulsion droplet. Due to the enhanced mass transfer rate imposed by SCF, particles aggregation can be greatly circumvented as a result of rapid hardening of the polymeric particles. Therefore, the drugs to be encapsulated are less likely to be loss during microsphere formation stage, i.e., higher EE relative to traditional emulsion method. SAILA approach on the other hand is a continuous process that useful in the field of micronization, where microspheres with controlled particle size distribution can be fabricated. This method applied SCF to solubilize the organic solute and forming expanded liquid solution, where the expanded ternary system will be sprayed directly into aqueous medium filled with surfactants. As the polymeric solute is not water-soluble in which the organic solvent will be miscible in, the water phase will serve as liquid antisolvent that leads to the solute precipitation to form microspheres.[77] Campardelli et al.[78] recently applied SAILA technique to prepare PLA and PLGA microspheres, and the effect of processing parameters (i.e., gas to liquid ratio (GLR), temperature and polymer concentration) on the microparticles morphology and size distribution were studied. Unlike RESS, smooth, spherical, non-aggregated microspheres with narrow particles size distribution were obtained. It was also shown that a reduction of particle mean diameter has been observed when a lower temperature and reduced GLR ratio were employed, which is concomitant with the reduced surface tension of the expanded liquid that allow a more efficient mixing between the solvent and the antisolvent phase. Similarly, reduction of polymer concentration in the expanded liquid mixture can also decrease the particle mean diameters, which is related to the ease of diffusion from solvent to antisolvent during low solute concentration that can reduce particle aggregation.

4. Encapsulation of hydrophilic drugs during $W_1/O/W_2$ emulsification

Encapsulation of hydrophilic drugs via $W_1/O/W_2$ double emulsion method is often found to be sophisticated as it involves lots of formulation variables and operation condition that may affect their morphologies and EE. Thus, it is very crucial to acquire an in-depth understanding about the impact of processing parameters on the properties of the microsphere for ease of subsequent planning guidelines, so that some predetermined criteria can be integrated during microsphere fabrication. Many have presumed that the solidification stage during double emulsification can dictate the EE of drugs, since drug partitioning into the

external aqueous phase will be greatly hampered when the emulsifier droplet have solidified. Meaning, formulation variables that can facilitate the hardening of the microparticles are anticipated to improve the EE of hydrophilic drugs.[79–81] Other than that, some other designed parameters that can create an environmental condition to prevent drugs outflow into the continuous phase are also found to be useful for attaining better EE. Based on these conceptions, we are summarizing the recent literature regarding to the impact of various formulation variables to the microsphere properties and provide a glimpse of the compiled aspects that should be taken into consideration during microspheres preparation for better drug entrapment.

4.1 Effect of oil phase's viscosity

It is intelligible that the polymer molecular weight and concentration used in the fabrication formulation are interrelated to the resulting viscosity of the organic phase, where this aspect will play an important role for the subsequent emulsification as the diffusion behavior of drugs will be greatly governed by the viscosity of the organic barrier. Organic phase with high viscosity, which can be either engendered from polymers with relatively high molecular weight or high concentration, can positively affect the EE of drugs and lead to a bigger particles size. A sensible reason upon this outcome can be elucidated by the bigger emulsified droplets obtained due to the more viscous solution that shear differently during emulsification to form a bigger particles.[22] Moreover, increasing polymer concentration will reduce the porosity on the microsphere surface, which is ascribed to the thicker polymer film at the interface between organic and outer aqueous phase at high organic viscosity that can effectively prevent the pores formation and subsequently minimizing drugs loss.[82],[83] Chaisri et al.[84] demonstrated the increasing of polymer concentration from 10% to 20% (w/v) had resulted in about fourfold increase in EE of gentamicin sulfate (GS) due to their concurrent increase of oil phase's viscosity at higher concentration. Additionally, it is also explicated that high polymer concentration will facilitate the solidification of polymer droplets, thus avoiding the diffusion of drug into the external phase.[31] Nevertheless, it should be noted that the increment of polymer concentration will reach a critical point where a further upraising of concentration will only result in lower EE. For example, the EE of dextran increases proportionally when the polymer concentration increases from 3% to 9% (w/v), however, for an increase to 15% (w/v), a decrease in drug EE is observed instead.[85] This is because the solution has become viscous enough to encapsulate the maximum amount of drug at the critical concentration and further increment in polymer concentration will only aggravate the drug loading.

4.2 Influence of volume of W_1 and the theoretical drug loading

It is also important to underline that the volume of W_1 and the theoretical drug loading used in $W_1/O/W_2$ emulsion process are crucial in affecting the drugs EE. According to some previous researches, it can be surmised that the drugs EE and mean particles size are inversely correlated to the volume of W_1 used. With an increase in W_1 volume, a surfeit amount of water used to solubilize drugs molecules tend to produce large holes in the microparticles during polymer solidification and evaporation stage. Once the organic solvent evaporates, contraction of the inner emulsified droplets will transpire and subsequently drive the intake

of water, thus creating a phase barrier between polymer-rich phase and water-rich phase. Upon microsphere drying, water vacates from the microspheres to form interconnecting pores, thus leading to a faster efflux of entrapped drugs molecules from the microspheres and resulted in lower entrapment efficiency.[86],[87]

The precise formulation of theoretical drug loading during emulsification stage should be well-designed for the synthesis of drug-loaded microspheres in order to control the drugs entrapment efficiency as well as to avoid wastage of precious therapeutic drug during fabrication process. Flores et al.[88] conducted studies on the effect of theoretical GS loading on the practical drug loading in PLGA microspheres. Further increasing theoretical drug loading from 5% to 45% will bring adverse outcome for their EE, which drops drastically from 55% to 30%. Similarly, Gaignaux et al.[87] demonstrate the decline trend in clonidine EE from 6% to 1.13% when theoretical clonidine loading progressively increase from 1% to 17% (w/v). It was proclaimed that increasing clonidine concentration in the primary W_1/O emulsion will gradually increase their concentration gradient that will lead to the loss of drug molecules into the outer W_2 medium. Ito et al.[85] also explained the positive correlation of dextran concentration with inner water phase viscosity that may facilitate the leakage of inner aqueous phase into the external water medium.

4.3 Influence of the volume W_2 and the surfactant concentration in W_2

Other fabrication variables including volume of W_2 and the surfactant concentration in W_2 used in double emulsification approach should also be considered wisely in order to obtain optimal entrapment efficiency and appropriate particles size for their delivery purpose. Commonly, increasing W_2 volume in the formulation tends to increase the mean diameter of microparticles ascribed to the less effective agitation and shearing force in disintegrating the droplets.[89] On the other hand, the large aqueous volume that is accessible for organic solvent as a result of increasing volume of W_2 allows a faster polymer coalescence to form bigger particles, thus restricting the drug efflux and enabling more internalization of drugs to be entrapped in microspheres. As evidenced, Srivastava and Sinha[90] reported an increase in EE of stavudine in PLGA microsphere from 12.44% to 65.53% when the volume of continuous phase used in the emulsion system increases from 10 to 100 ml. Often, excessive concentration of surfactants (usually PVA) in W_2 tends to lead to a smaller microsphere with low entrapment efficiency. At high surfactant concentration, increase in the outer phase viscosity will lead to a higher shear forces and consequently formation of smaller droplets. Plus, hardening of polymer is greatly hindered attributed to the reduced surface tension as a result of high surfactant concentration, which tends to induce more leakage of drugs out from the inner droplet to cause poor drugs entrapment.[85],[87]

4.4 Solubility of drug in external aqueous phase

Alternatively, drug partitioning during microencapsulation process can also be prevented by restricting the diffusivity of drugs within the organic phase, which can be accomplished based on two ideas. One of the strategies is to depress the drugs solubility in the outer aqueous phase with the intention to enervate their diffusion movement during emulsification process. Whereas, the second idea involves the designation of an appropriate osmotic surrounding in both dispersed and outer continuous phases, where a higher osmotic

concentration in external phase will effectively prevent the influx of outer aqueous into the primary emulsion, thus ensuring better inner emulsion stability. For instance, in the case of ionic drugs encapsulation, some attempts to curb the aforementioned drawback by incorporating inorganic salts or osmogens (such as NaCl, NaBr, NaClO$_4$, and Na$_2$SO$_4$) into the external aqueous medium, where the function of impregnated salts can be elucidated using the two concepts explained above. It is conceivable that the salts addition at high concentration will decrease the solubility of drug in the external aqueous due to the 'salting-out' of drugs from aqueous salts solution. Meaning, there is no excess water molecules to allow the solvation of drugs molecules in external medium. Besides, the formation of less soluble new salts between protonated drugs and the uncommon anion (from the initial salts added) also pose difficulty for drug partitioning during microencapsulation process.[91] In terms of osmosis explanation, salts inclusion into the aqueous medium will create a hyperosmotic environment that can hinder the drugs diffusion into the outer medium.[92] This is attributed to the formation of osmotic pressure gradient across the two phases that can prevent the intake of water into internal emulsion to cause destabilization and subsequently preventing drugs output. These combined effects therefore lead to a better drug internalization around the microsphere, thus resulting improved drugs EE using double emulsion process. For example, Dorati et al.[82] has successfully increased the EE of GS in PLGA-PEG microsphere from 72.1% to 97.5% when 5% w/v NaCl is added to the external phase. Similarly, the effective increment of EE from 31% to 100% was also observed for the loading of quinidine sulfate when 0.5 M of NaSCN salts is dispersed in the external continuous phase.[93]

Apart from inorganic salts, Ca^{2+} ions also has been added as counter ions for alendronate to reduce their solubility in aqueous solution. Cohen-Sela et al.[94] showed that incorporating Ca^{2+} using the formulation of 1:2 of alendronate to Ca^{2+} ratio had remarkably improved their EE from 4.5% to 83.4%. This is due to the ability of Ca^{2+} to couple with the phosphate moiety of alendronate, forming hydrophobic bis-alendronate calcium salts that can effectively prevent the drugs partition into the outer phases. Attempt to reduce the solubility of GS in aqueous by means of incorporating sparingly soluble solvent such as ethanol has also been elucidated by Cohen-Sela et al. Nonetheless, rather low entrapment efficiency was observed instead, probably due to the increase in DCM solubility in external water medium leading to a rapid extraction of solvent, thus resulting in the formation of microparticles with porous-like morphology, making leaching of drugs molecules highly probable.

Other than that, the concentration of solvents used to prepare outer aqueous medium also play a pivotal role in affecting the drugs EE, since the primary W$_1$/O emulsion emulsified in different concentration of outer aqueous medium will lead to a different osmotic pressure between two phases. Again, Chaisri et al.[84] studied the impact of buffer concentration (i.e., PBS) used to prepare aqueous PVA solution on the resulting GS loading. By using 0.3 M of PVA/PBS buffer instead of mere aqueous PVA solution, GS EE increased substantially from 5% to 18%, with not much difference in their particles size. It is surmised that emulsification of primary emulsion in a mere PVA solution (dissolved in aqueous water only) will built a higher osmotic pressure in the GS/PBS droplets compared to external PVA phase. This will cause the intake of water into the inner droplets and unfavorably affecting their stability and therefore their EE. Thus, using a buffer solution at appropriate concentration to adjust their osmotic flow seems to be a wise approach to avoid drug loss.

4.5 Stability of inner emulsion (W$_1$/O)

The inner stability of the primary emulsion is said to be the key aspect in controlling the encapsulation of hydrophilic drugs in double emulsion approach. One of the tactics to achieve inner emulsion stability is by incorporating stabilizer such as PVA, PEG, chitosan, and Span 40 into the oil phase. For example, addition of Span 40 into the primary emulsion has successfully increased the iodo-2'-deoxyuridine (IUdR) loading from 4% to 36.5%.[34] However, employment of inapt candidate as surfactant for a particular primary emulsion will unfavorably affect the drug loading into the microsphere. It is showed that neither pluronic F68 nor benzalkonium chloride is effective in optimizing the GS loading in PLGA microspheres and there are lack of explanations regarding this observation.[84] Incorporation of lecithin as surfactant on the other hand might further increase the solubility of hydrophilic drugs into the aqueous phase, especially when their concentration surpass their critical micelle concentration. For instance, the EE of 5-FU will decrease from 79% to 35% when the concentration of lecithin added in external phase increases from 0.05% to 2%. Hence, it is very crucial to select an appropriate surfactant for a particular W$_1$/O emulsion system in order to avoid further aggravation to the drug loading.

5. Structural optimization of microspheres

5.1 Bulk modification of PLA/PLGA

Numerous bulk modification approaches involving physical blending or development of various kinds of lactide based-copolymer with distinct block structure have been explored in order to synthesis precursor/starting material to form microsphere matrix. The properties of the microsphere, including surface characteristics (hydrophobicity and functionalities), degradation rate and thus their drug release kinetic can be tailor-design by utilizing appropriate bulk modifier to modify PLA/PLGA. Similar to the pristine PLA/PLGA microsphere, various type of preparation techniques and technologies have been successfully employed to fabricate bulk-modified polyester microsphere, including solvent extraction/evaporation emulsification, membrane emulsification, spray drying, and microfluidics method (Table 2). Various consideration including the type of fabrication technique employed, formulation parameters (type of polymer, polymer concentration, emulsifier concentration, volume of W$_1$, *etc.*), nature of drug to be encapsulated and operating condition as discussed in the previous section will considerably affecting their microencapsulation properties.

One of the most common bulk modification is the incorporation of hydrophilic polyethylene glycol (PEG) into the bulk polyester architecture. In recent years, various forms of amphiphilic block copolymers or blended constitutions of polyesters based on PEG have been developed and employed to encapsulate therapeutics agents in the form of microsphere.[95–104] Basically, PEG is a nonionic biomacromolecules which possess terminus –OH groups in their structure that can be used to furnish polyester with hydrophilicity. Meanwhile, acidic microclimate issue attributed to the degraded by-products formed during drug delivering can be alleviated concurrently when PEG is incorporated into the polyester formulation.[26],[105] Indeed, some studies have presumed that the hydrophilic PEG segments of the PEGylated polyester will enable a better transport that permits a faster clearance of degraded products away from local, thus mitigating the local acidic surrounding upon microsphere degradation.[98] Along with their improvement in hydrophilicity, accelerated

Table 2. Compositional varieties of bulk-modified PLA/PLGA microsphere prepared using different fabrication methods.

Polymeric material	Organic solvent	Drug	Stabilizer	Fabrication technique	Other remarks	References
PEG-PLGA	DCM	Insulin	PVA (in W_2)	W_1/O/W_2 emulsion	—	[95]
PEG-PLGA	DCM	CsA	PVA (in W_2)	Microfluidic method (O/W emulsion)	Flow rate of O phase: 0.3–1.2 ml/hr, flow rate of W phase: 15–90 ml/hr	[97]
PEG-PLGA	DCM	rhBMP-2	PVA (in W_2)	W_1/O/W_2 emulsion	Solvent extraction/ evaporation was performed in ice	[98]
PEG-PLGA, PLGA-PEG-PLGA, PEG/PLGA blend	DCM	BSA	PVA (in W_2)	W_1/O/W_2 emulsion	—	[99]
PLA-PEG-PLA	DCM/acetone	Paclitaxel	PVA (in W_2)	O/W emulsion	—	[100]
PLA–PEG–PLA	DCM	—	—	RESS method	First spraying of polymer solution (dissolved in DCM) into supercritical CO_2 with spray rate of 1.0 ml/min, followed by second spraying of their mixture into ambient chamber.	[101]
PLGA-PEG-PLGA	DCM-acetone	Protein	PVA (in W_2)	S/O/W emulsion	—	[102]
PEG/PLGA blend	DCM	—	—	Spray drying	Spray rate 600 l/hr inlet temp: 40 °C, outlet temp: 30—32 °C	[104]
PEG/PLGA blend	DCM	OVA	Span 60 (in W), Poly(vinyl pyrrolidone) (in O_2)	W/O_1/O_2 emulsion	Methanol as continuous phase solvent (O_2 phase)	[103]
PCL-PLA	DCM	Clodronate	PVA (in W_2)	W_1/O/W_2 emulsion	—	[112]
PCL-PLGA	DCM	BSA	PVA (in W_2)	W_1/O/W_2 emulsion	—	[113]
PCL/PLA blend	DCM-acetone	Colchicine	Pluronic F68 (in O), PEG/PVA (in W)	O/W emulsion	—	[114]
Cellulose-g-PLA	DCM	—	PVA (in W_1 and W_2)	W_1/O/W_2 emulsion	—	[115]
PVMMA/PLGA blend	Ethylformiate (PLGA), Acetone (PVMMA)	BSA	—	Spray drying	Spray rate: 3 ml/min, inlet temp: 42 °C, outlet temp: 72 °C, pressurized air flow of 500 l/hr	[116]

drug release profile is commonly observed due to its rapid swelling properties that will increase the degradation rate of the microspheres. Therefore, PEG can serve as a versatile modifier to control the rate of cargo release by varying the amount PEG insertion in the microsphere formulation.[96],[98],[106]

Moreover, inclusion of PEG into polyester formulation either in the form of copolymers or blended products is suitable for the delivery of proteins or peptides as it conquers the protein instability issue that transpire in conventional microspheres devices. By speeding up the release of proteins or peptides prior to microsphere degradation, these labile macromolecules can be successfully delivered and released without confronting any harsh acidic condition that will affect their biological performances.[107] Furthermore, these amphiphilic precursors can devitalize the contact of proteins with the oil–water interface or with the hydrophobic matrix, thus minimize the aggregation of loaded protein during microencapsulation.[95][108] However, PEG-based polyester microsphere device is not suitable for the encapsulation of small drug molecules such as clonidine as they can possibly leach out through the hydrophilic PEG segments during emulsification process.[81],[87]

In general, there are two types of PEG based copolymers that are commonly employed to form microsphere, i.e., A-B diblock copolymer and A-B-A/ B-A-B triblock copolymer. Usually, these copolymers can be synthesized by ring-opening polymerization (ROP) of lactide in the presence of PEG (or its end group derivatives) as initiator and stannous octoate (Sn(Oct)$_2$) as catalyst (Fig. 5).[102] Noted that A represents the hydrophilic PEG portion and B symbolizes the hydrophobic PLA or PLGA segment. Different conformation of copolymers will result in different properties of microspheres. According to Buske et al.,[99] the formation of microsphere produced from A-B diblock monomer depicts a surface with a rough and spongy-like structure, where the hydrophilic PEG segments will orient toward the internal and surrounding aqueous phase during precipitation stage. In the case of hydrophilic drugs such as bovine serum albumin (BSA), they will adhere to the hydrophilic PEG segments which will be predominantly located at the microsphere surface to facilitate drug release. Notwithstanding, a biphasic release profile with a large initial burst is normally observed in diblock-based microsphere due to the high solubility of hydrophilic PEG segments in water that can enhance the diffusivity of drugs and water molecules across the microsphere. On the other hand, when B-A-B triblock monomer is used as microsphere precursor, a slow continuous release of BSA with no initial burst is observed instead. This is due to their rapid hydrate
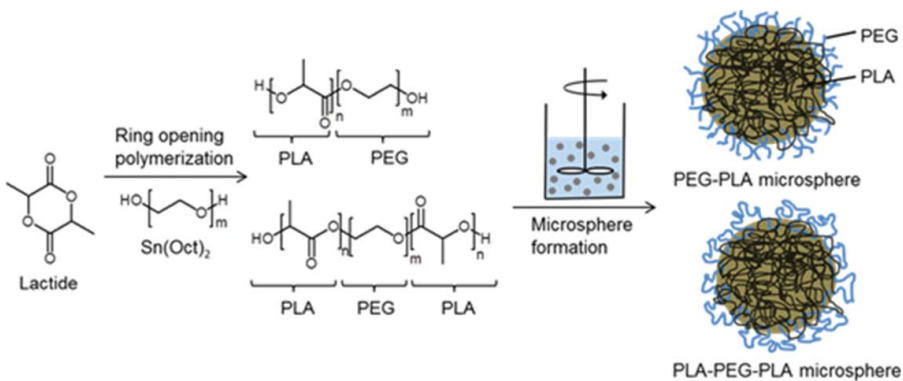

Figure 5. Synthesis of PEGylated diblock and triblock copolymers via ROP and its employment to form microsphere.

properties that will form hydro-gel structure which enable the drugs to be release continuously through their swollen matrix by diffusion-degradation mechanism.[99],[109]

It is noticed that the molecular weight of both hydrophilic and hydrophobic segments plays a prime role in determining the drugs EE. For B-A-B-triblock copolymers structure, high EE can be achieved when a short segment of hydrophilic PEG and a long segment of hydrophobic polyester are used for microencapsulation of water-soluble drugs. For instance, a smaller size of PEG segment can lower the water infiltration level into the microsphere which subsequently aid in minimizing protein leaching during microsphere encapsulation. By considering all the aforementioned statements, the optimal formulation of protein encapsulation as demonstrated by Tran et al.[102] can be attained when PLGA-PEG copolymers with 40 kDa of PLGA segment and 4 kDa of PEG segment are used. This is ascribed to the best hydrophilic-hydrophobic balance in this formulation leading to about 77% of EE, with little initial burst and sustained release profile up to 36 days.

In order to make their way for commercialization, it is very crucial to make sure that the polyester delivery vehicles are able to persist longer in human systemic circulation to ensure a successful liberation of drug molecules at the target sites for therapeutic purposes. Unfortunately, polyester based microspheres which are nonstealth to the mononuclear phagocytic system (MPS) are susceptible to rapid clearance from our bloodstream due to the opsonization of microspheres which will direct their removal to macrophages via phagocytosis process. For this purpose, hydrophilization of polyester using PEG can be used to address this limitation, where the formation of PEGylated precursor or surface modification of polyester microsphere via PEGylation can be used to avoid opsonization process (surface modification of microsphere using PEGylation will be discussed in Section 5.2.2). Despite numerous scientific results that proved the aptitude of PEG in escaping the phagocytic system, the mechanism behind their role in enhancing the plasma half-life of the microsphere still remains unclear. Huang et al.[26] deciphered that the hydrophilic dynamic cloud created by the PEG barriers on the microsphere surface can reduce their nonspecific interaction with liver Kupper cell. On the other hand, some have presumed that the Van der Waal interactions as well as the steric repulsion produced by the corona-core microsphere will prevent their attachment with opsonin protein, thus significantly reduce their affinity for macrophages. Additionally, the flexible free-rotating PEG chains will form a hydrophilic stealth corona surrounding the microspheres, which can help them to mask and evade from the human defense system and thus increase their residence time when implied in vivo.[110]

Other than PEG, poly (caprolactone) (PCL) also has been used as bulk modifier for PLA/PLGA to fabricate microspheres. Both PLA/PLGA and PCL are categorized under bio-based polymer which is equipped with both biocompatibility and biodegradability values, however, had quite a different property in term of their rate of degradation. Typically, PCL exhibited a slower degradation behavior compared to PLA/PLGA due to their highly hydrophobic backbone and crystallinity structure than the later polymer.[111] On the other hand, PLA/PLGA contains higher ester group content in their backbone structure, thus making them feasible to degrade with relatively faster rate compared to PCL. Therefore, in the case of long-term delivery application, copolymerization or blending of PLA/PLGA with PCL seems to be useful in compromising the fast degradation rate of PLA/PLGA microsphere, where their degradation rate can be controlled by varying the copolymeric/blended ratio in the formulation. In other words, a series of copolylactides/blended polyesters with half-life of degradation ranging from several weeks to more than two years can be obtained by adjusting the component

ratio of PLA/PLGA to PCL. Zhou et al.[112] attempted to regulate the release of clodronate from the microsphere by altering different ratio of PLA and PCL in their formulation. It was found that the inclusion of PCL in the formulation will reduce the amount of pores on the resulting microsphere, which can significantly affect the release of drug during *in vitro* studies. Another interesting research based on a four-arm star-shaped PLGA-b-PCL microsphere was also studied by Dong et al.[113] to modulate the release rate of BSA from microspheres. One of the primary features of this star-shaped architecture is that they possess relatively short polymer chain, high molecular weight with more –OH moieties than linear polymer. This cause star-shaped microsphere to display a faster degradation rate compared to linear polymer with similar molecular weight engendering from their more hydrophilic character. Nevertheless, high molar content of PCL in the formulation will adversely affect the EE of BSA (due to their hydrophobicity), thus their PLGA: PCL composition must be well controlled for the optimization of drug-encapsulated microsphere design. Notably, degradation will begin at PLGA segment ascribed to their amorphous and less hydrophobicity relative to PCL partitions. In overall, the star-shaped microsphere based on PLGA-b-PCL displayed a good released profile with a nearly constant release from day 20 to 110, which is due to the slow diffusion of drug from PCL segments that are balanced and attenuated by the fast drug release from the PLGA segments. Since both PCL and PLA/PLGA are highly hydrophobic compared to the surrounding ECM, microsphere prepared using this polymers should be extra-coated using a layer of hydrophilic polymer (*e.g.*, chitosan, PEG) to improve its biological properties.[114]

Grafting modification of PLA/PLGA with cellulose using ring-opening copolymerization also has been devoted to the fabrication of promising precursor for microsphere with favorable features such as enhanced wettability and biocompatibility since the presence of abundant –OH groups originated from cellulose structure is beneficial for cell adhesion and proliferation. According to the research conducted by Yang et al.,[115] cell cultivation study in HepG-2 cells showed that the amount of cell adhered on the cellulose-g-PLA microspheres (with 6.7 and 11.5 molar substitution of PLA) were significantly higher with some pseudopod present on the surface compared to neat PLA microsphere. This finding significantly substantial the usefulness of cellulose backbone in overcoming the limitation of pure PLA microspheres, provided that the ratio of cellulose and PLA used in the fabrication of microsphere is well-controlled and designed.

As discussed earlier, grafting ligands to the surface of the PLA/PLGA microspheres might not be an easy task to accomplish due to their limited availability of functional groups for further functionalization. In this matter, incorporation of poly (methyl vinyl ester)-co-(maleic anhydride) (PVMMA) to the bulk polyester structure to form microsphere seems to be useful especially for lectin coupling which requires quite an amount of –COOH density for covalent immobilization. PVMMA, which is a copolymer of methyl vinyl ether and maleic anhydride (MA), can feasibly undergo hydrolysis by cleaving the ester bond in the MA backbone to generate free acid groups. By utilizing the –COOH groups present on the surface of microsphere, coupling of lectin onto the microsphere surface can be optimized by providing functional site for the bonding of amino groups from the lectin backbone. Related research regarding the modification of PLGA using PVMMA and its function in augmenting the lectin conjugation on the PLGA microsphere was elucidated by León-Rodriguez et al.[116]

5.2 Surface modification of microspheres

Apart from bulk modification, attempts to augment the efficacy of microsphere performance have also been made from the view of surface modification, where their surface hydrophilicity and chemical functionalities can be introduced to improve their cytocompatibility by virtue of the surface modifying species or techniques employed. This is indeed crucial as the assorted mechanisms of biomaterial and host interaction require specific surface characteristics in order to prevent any detrimental effects when implied *in vivo*. For the subsequent conjugation of bioactive molecules, surface modification of microsphere will play an important role as an intermediate step, in which the particular techniques or surface modifier must introduce or possess special functional groups such as –NH$_2$, –COOH, –OH, *etc.* to the microsphere surface.

5.2.1 Chemical treatment

Chemical treatment, which basically includes simple surface hydrolysis (with an alkali) or aminolysis is a simple, inexpensive and accessible method that can be utilized to endow PLA/PLGA microsphere surface with hydrophilicity. In general, base treatment is a postsurface treating process that can be performed by immersing as-prepared microsphere in base solution for predetermined interval in order to induce chemical functionality via hydrolysis (Fig. 6a). In alkaline-catalyzed treatment, the cleavage of backbone ester linkages as a result of hydrolysis will create free –COOH and –OH groups on the microspheres surface, where NaOH is mostly used as catalyst.[115],[117] Basically, the mechanism of this treatment can be elucidated as a two-steps process: (i) Attack of nucleophile (OH$^-$ ions) at the electron deficient carbonyl carbon to form tetrahedral intermediate, (ii) Restoration of carbonyl group to form free –COOH and –OH groups with negatively charged surfaces (Fig. 6b).[27] On the other hand, surface aminolysis route using diamine as reagent (*e.g.*, 1,6-hexanediamine) also
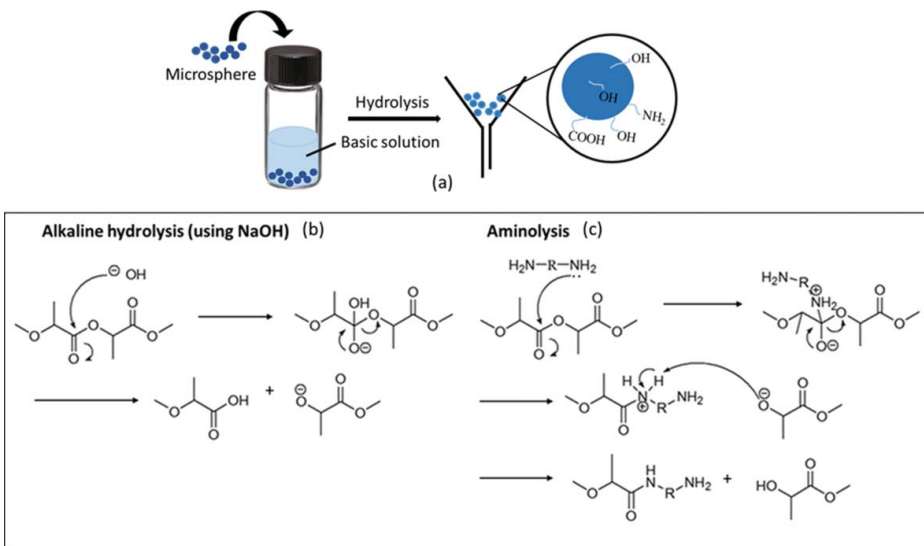

Figure 6. (a) Schematic illustration of base hydrolysis, (b) and (c): mechanism reaction for alkaline hydrolysis and aminolysis.

can be used to create aminated functionalities. During aminolysis reaction, one of the $-NH_2$ group will covalently interacts with the $-COO-$ group to form $-CONH-$, keeping the other amino group free and unreacted (Fig. 6c). Since the massive amount of researches devoted to the fabrication of hydrophilic surface is related to their enhancement of biocompatibility aspect for biomedical purposes, the impact of surface-hydrolyzed microspheres in terms of cell toxicity and proliferation has been evaluated to test their feasibility for this purpose. In this case, Sabee et al.[25] demonstrated that the PLA microspheres treated with 0.5 M NaOH have displayed the highest cell viability in day 3 with no toxicity effect on rabbit fibroblast cells during in vitro studies, which significantly corroborates the potential of alkaline-treated PLA microspheres as drug carrier.

Notably, hydrophilization via chemical treatment also present as an indispensable step for the subsequent deposition or covalent attachment of other biomolecular species for the synthesis of truly biomimetic-based materials. For example, negatively-charged surface generated from alkaline hydrolysis facilitate the deposition of apatite coating onto the microspheres surface.[118] Subsequent coverage of chitosan coating onto the surface of microsphere can also be enhanced through surface hydrolysis.[119] Grafting of arginine-glycine-aspartic acid (RGD)-containing peptides onto the surface of microspheres can also be made possible through the hydrophilic functional groups introduced during surface hydrolysis treatment.[120] Yuan et al.[121] also demonstrated increased cell adhesion on surface of poly(L-lysine)(PLL)-adsorbed PLGA microsphere mediated through basic alkaline hydrolysis.

Despite the seemingly easy process that can render microsphere surface with hydrophilicity, alkaline-catalyzed hydrolysis have been receiving drawback due to its aggressiveness that might affect the bulk properties of microspheres.[122] Hence, the duration of hydrolysis process should be kept as short as possible to prevent bulk disintegration that might cause drug leakage.

5.2.2 Surface coating

Surface coating is one of the mostly exploited methods used to improve the cytocompatibility of pristine PLA/PLGA microsphere for DDS. Conceptually, this modifying approach involves the adsorption or conjugation of modifying agent onto the surface of the microsphere, thus introducing hydrophilic element to avoid biological irritation. Basically, surface coating of microsphere can be performed either during in situ microsphere synthesis or in a postsynthesis process (ex situ coating). In most of the cases, the employment of hydrophilic coating during microspheres preparation is able to enhance the EE of drug, which is attributable to their role as stabilizer that will prevent the drug loss to external phase during emulsification process.[123] Postcoating on the other hand is usually carried out by incubating the as-prepared microsphere in the coating agent solution to permit their association on the microsphere surface. Sometimes, surface activation reaction involving chemical treatment (i.e., alkaline or aminolysis) are applied to microsphere surface prior to ex situ coating in order to optimize the extent of surface modification. For ex situ surface coating of drug carriers that are weakly associated without covalent immobilization, entrapment of surface modifying agent by grafting-coating protocol,[124] or cross-linking[125] have been sought to overcome the desorption or instability issue ascribed to their noncovalent interaction. Herein, miscellaneous coating materials that have been reported will be detailed in this

Table 3. Representative surface coating of selected polyester-based microspheres and its brief coating description.

Coating agent	Type of microsphere	Coating description	References
PEG	PLA	*In situ* surface coating during emulsification (using PEG as external emulsifier) - Oil phase containing taxol and polymer solution was dispersed in aqueous phase containing 2.5% of PEG as emulsifier to form O/W emulsion.	[126]
PLL-g-PEG	PLGA	Postcoating of microsphere surface - Equal volume of PLGA microsphere dispersion (dispersed in HEPES buffer solution) and PLL-g-PEG solution was mixed and incubated at room condition for 15 min.	[129]
Chitosan chloride	PLA	Postcoating of microsphere surface - Microspheres were immersed in 1% (w/v) chitosan solution and incubated at room condition for 4 hr.	[134]
Chitosan	PLA	*In situ* surface coating during emulsification (using chitosan as external emulsifier) - W_1/O phase containing 5-FU solution dispersed in organic phase was emulsified in 1% chitosan aqueous solution to form W_1/O/W_2 emulsion.	[132]
Chitosan	PLGA	Postcoating of microsphere surface by covalent conjugation - Hydrolysis of PLGA in 4 mol/L NaOH for 8 hr to increase the –COOH content. - Activation of –COOH groups of microsphere by dispersing them in EDC/NHS solution for 1h. - 12% of chitosan solution was added to allow direct coupling onto the microsphere surface.	[119]
Chitosan	PLA	Postcoating of microsphere surface - Microspheres were suspended and stirred in 3% chitosan solution, followed by injection of 1N NaOH solution to form spherical gel.	[133]
Alginate	PCL-PLA	*In situ* surface coating during emulsification (using alginate as external emulsifier) - Oil phase containing cisplatin and polymer solution was emulsified in aqueous solution containing 2.5% of alginate to form O/W emulsion.	[139]
Palmitic acid-avidin conjugate	PLGA	*In situ* surface coating during emulsification (using avidin conjugate as co-stabilizer) - W_1/O phase (fluorescent-BSA solution + organic medium) was emulsified in external phase (containing 5% PVA and avidin conjugate) to form W_1/O/W_2 emulsion.	[140]
SF	PLGA	Postcoating of microsphere surface - Suspension of microspheres in 1% (w/v) SF solution was incubated for 2 hr followed by cross-linking using 2% (w/v) glutaraldehyde.	[125]
SF	PLGA	Postcoating of microsphere via layer-by-layer deposition - Microspheres were immersed in 0.1% (w/v) silk fibroin solution and shaken for 2 min, then followed by bath ultrasonication for 1 min. - Repeat the step for three times to generate three SF coating layers on the microsphere surface.	[30]
HA nanoparticle	PLA	*In situ* surface coating via Pickering emulsification - Aqueous dispersion of HA nanoparticles (pH: 6.5) was added to PLA solution, shaken (HA nanoparticle as stabilizer) and followed by solvent evaporation.	[151]

HEPES: (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); NHS: N-hydroxysuccinimide; EDC: 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide.

section, where most of them are bio-based in nature (some of the selected surface coating procedure of microsphere will be displayed in Table 3).

Polyethylene glycol (PEG) is one of the most common hydrophilic polymers used to alter the surface properties of microsphere. The widespread employment of PEG as an ideal

surface modifier in various drug delivery carriers such as nanoparticles and microspheres is ascribed to their shelf stability as well as their capability to regulate the drug release over a long period of time.[126] Das *et al.* prepared PEG-coated PLA-PCL microspheres using solvent evaporation fabrication method, where PEG was utilized as outer phase emulsifier. In their research, it was shown that the PEG displayed a dual role as (a) protective colloid to hamper the aggregation of emulsion droplet, thus improving the stability and payload of the encapsulated drugs, (b) surface modifier to reduce the initial burst rate while allowing a sustained drug release over a longer period of time. Besides their satisfactory properties such as biodegradability and biocompatibility, the main reason for the interest of modifying PLA/PLGA microspheres with PEG is to enhance their circulation time during drug delivering process. As mentioned in the previous section, it is supposed that the hydrophobic feature of the polyester microcarriers will make them to be assumed as foreign by human body and will be taken up rapidly via phagocytosis. Similar to PEG copolymer or blended constitution precursor mentioned before, PEG will thus usefully serve as a hydrophilic protective coating that shield and camouflage the microspheres from macrophages, thus allowing them to persist longer in the body.[120],[127],[128] Such microcarrier design is very desirable for DDS as it is not only eliminating the indiscriminate interaction with the macrophage and allowing the liberation of encapsulated drug with an optimal rate, but also with their nontoxic degraded products which can be easily removed from the body. Nonetheless, nonionic PEG coating is susceptible to desorption when in contact with water. Thus, some attempt to copolymerize PEG with charged-polymer such as poly (lysine) prior to PEGylation coating with the intention to abrogate and suppress protein adsorption.[129] For instance, Müller *et al.*[130] reported the use of PLL-g-PEG coating on the surface of PLGA microsphere as protein repellent. Their results suggested that the PLL-g-PEG coated PLGA microsphere displayed a declination of protein adsorption by two orders of magnitude, as compared to unmodified microsphere. PLL-g-PEG coated PLGA microdevices also reported to be useful for ligand-specific phagocytosis, where integrin ligand can end-capped onto the adsorbed PEG on the surface of the biopolymer devices to improve immune response.

Other than PEG, polyelectrolyte, which can be either polycationic (*e.g.*, chitosan, poly (lysine) and protamine) or polyanionic polymers (*e.g.*, alginate) can also be electrostatically decorated onto the microsphere surface to tune their surface characteristics besides introducing peculiar specificity for a particular delivering purpose. Chitosan, due to its biodegradability, biocompatibility, nontoxicity and bioadhesion properties, has recently emerged as one of the most famous auxiliary agent in pharmaceutical applications. Apart from that, chitosan's unique cationic character ascribed from its primary amino moieties also make it promising as a coating agent for polyester-based microspheres to improve their applications. In drug delivery application, chitosan coating has been applied in polyester devices for different purposes. First, chitosan-coated surface offer binding site for the succeeding immobilization of bioactive molecules using their free amino groups exposed from their surface. For instance, Fischer *et al.*[131] showed a successful conjugation of NHS-PEG-biotin on the surface of the chitosan-coated PLGA microparticles, as evidenced by their accessibility for succeeding binding with streptavidin. Also, chitosan may be utilized as a coating material for the delayed release of encapsulated drugs. This is due to the lining and coverage of chitosan on the micropores of the microsphere that lead to a control or modulation over the release of encapsulated drugs.[119],[132],[133] Likewise, injectable chitosan-covered HA/PLGA microparticles have also been proposed for the controlled release of GS and teriparatide in the

treatment of osteoporosis. It is revealed that the diffusion coefficient of chitosan-coated devices is one order of magnitude smaller than uncoated samples, which corroborate the feasibility of chitosan coating as an additional barrier in delaying the liberation of drug by diffusion. Besides, the initial drug burst of the HA/PLGA microspheres will be greatly reduced when the coating of chitosan is added. These remarkably showed the potent of chitosan coating in sustaining the delivery of drug, thus eliminating the necessities of daily dose injection and maintaining drug levels within a desirable range. Additionally, surface-engineered polyester microspheres using cationic chitosan also hold great promise as adjuvants in vaccine delivery application due to the deposition of positively charged coating that permit the adsorption of antigen (*e.g.*, recombinant HBsAg) onto the surface of microsphere based adjuvant by means of electrostatic interactions.[134],[135] Similarly, protamine, which is a FDA-approved compound, also serves as a useful coating for the association of antigen and nucleic acid on the surface of the controlled-release system as demonstrated by Gómez *et al.*[136]

Alginate coating is also another possible approach that has been used to modify the microspheres surface from hydrophobic into hydrophilic. Alginate is an anionic polysaccharide that capable of forming strong gels with Ca^{2+} ions, thus leading to microspheres with great strength and flexibility. Moreover, the hydrocolloid-like behavior of alginate coating can be useful in the design of controlled release system.[137],[138] Liu *et al.*[123] studied the impact of alginate coating on the surface of tetracycline filled-PLGA microsphere for their local delivery application to periodontal pocket. The authors proclaim that the endeavor of alginate coating not only can improve the EE of drug but also could prolong the release of drug and increase the drug's release quantities. It is noticed that the high drug released profile was probably due to the easy hydration exhibited by alginate-coated microspheres that aid in the migration of encapsulated drugs out of the microcarrier to the external releasing medium, thus improving the efficacy of tetracycline delivery to the periodontal pocket. While there are quite a number of coating agents that can be used for surface modification, selection of the most appropriate candidate for the maximization of the surface properties for a particular delivery application becomes paramount. Therefore, with the aim of scrutinizing the most suitable polyelectrolyte coating for microspheres in the target delivery of antiproferative agent (*i.e.*, cisplatin), Chandy *et al.* prepared PLA-PCL microspheres by using PEG, chitosan and alginate as coating agents. It appeared that alginate and PEG coating resulted in a higher microsphere yield, cisplatin recovery and content with more drug release to the site for better therapeutics function relative to chitosan-coated system. The outer semi-permeable layer of alginate or PEG also auspicious to reduce the degradation and improve the biocompatibility of the PLA-PCL microspheres.[139]

Surface modification of microsphere using fatty acid conjugated with avidin also shown satisfactory for targeting drug delivery application. In the case of encapsulation of biotinylated compounds, the amphiphilic feature of fatty acid-avidin can serve as co-stabilizer to augment the microsphere formation yield and EE, notably, when simultaneous encapsulation and surface modification are performed during their emulsification stage (*in situ* coating).[140]

Furthermore, endeavor in ameliorating the surface properties of microsphere using protein-based biomacromolecules such as collagen, gelatin, silk, *etc.* has also been burgeon within these recent years. Collagen is one of the most abundant natural ECM proteins that found in the wide range of applications in biomedical fields for their natural turnover characteristic and low immunogenicity, which is the key contribution for its biodegradable and biocompatible features when implied *in vivo*. Particularly, the employment of collagen as

modifying species to form surface-coated microspheres seem to be a pragmatic approach for DDS and tissue engineering ascribed to their triple helix ligand kind-of structure which can specifically interact and attach with cell surface receptors, thus effectively facilitate the cell adhesion and proliferation. Furthermore, their innate ability that can mimic and improve the compatibility of the pristine PLA/PLGA microspheres with the biological surrounding also make them as a feasible coating agent to improve the surface characteristic of the drug-loaded microdevices.[141],[142] This is evidenced by Hong et al.[142] when the improvement of cell proliferation rate of collagen-coated microsphere was observed in chondrocyte cultures. Similar to other coating agent such as PEG and polyelectrolyte, initial burst release and drug release rate of PLA or PLGA microspheres can significantly reduce and prolong respectively upon surface modification with collagen coating.[143] On the other hand, pretreatment of microsphere surface via aminolysis and glutaraldehyde can also be performed to stabilize the collagen coating around the microspheres surface, by utilizing the concept of grafting-coating protocol. The formation of –CHO groups on the microsphere surfaces as a result of aminolysis and glutaraldehyde treatment allow the covalent grafting of collagen molecules using their amino moieties, where the free collagen molecules will then intertwine and interact with the grafted collagen to form a much stable and thicker collagen-rich layer around the microspheres surface (Fig. 7).

Silk fibroin (SF) also has considerable potential to be used as a coating agent for microsphere carriers. SF is one of the strongest and toughest natural fibers (produced by silkworms or spiders) that proved to be both biocompatible and biodegradable.[144] Due to its robust mechanical properties, SF coating can be used to maintain the shape of the microspheres and hindered them from aggregation and decomposition. Moreover, this coating can slow down microsphere degradation and delay the release of encapsulated therapeutic agents by conferring a diffusion barrier around the drug-loaded devices, which is very auspicious for depot delivery application. The drawback of plain PLA or PLGA microsphere that have a large initial burst release can also be remarkably overcome through the coating of silk layer around them. For instance, Wang et al.[30] showed the role of silk coating in sustaining the drug release, where only about 20% of the encapsulated Horseradish peroxidase were liberated from the PLGA microspheres over 30 days of observation, with almost no initial drug
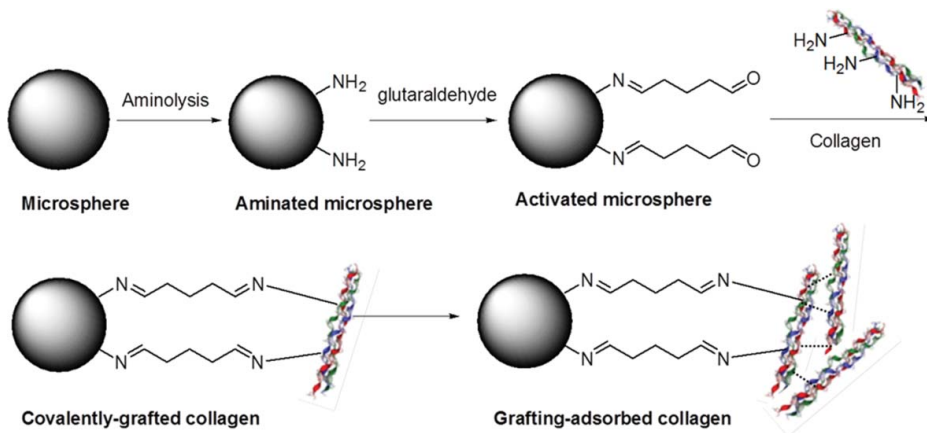

Figure 7. Overview for the steps of collagen grafting-coating process around the microsphere surface.

burst on the first 5 days. Notably, the extent of degradation and the rate of drug release can be tailor-made by varying the number of SF films deposited around the microsphere surface using layer-by-layer assembly technique. By using this kind of assemble coating protocol, drugs not only can be embodied into the microsphere but also can be sandwiched within the multilayers of silk coating, thus making them very potent for the sequential drugs release from one drug carrier device.[145]

PLA/ PLGA and its derivatives based microspheres modified with apatite have also been widely reported to overcome the limitation related to pristine microsphere. This is because the association of apatite with these biodegradable microspheres will engender an alkaline environment which will buffer the acidic by-products generated during polyester degradation.[146] Thus, mineral-coated microspheres appeared to be auspicious for bone tissue engineering as well as bone DDSs compared to their solidary form as their assemble form not only possess better control over their degradation kinetics but also concurrently displayed less inflammatory effect.[118],[147],[148] In recent years, one of the most common biomineralization method used to coat apatite onto the microsphere surface is biomimetic process, where the key of this method is employing simulated body fluid (SBF) as coating carriers. In this approach, partial hydrolysis of polyester will transpire to produce negatively charged surface, thus making the nucleation of apatite possible. For example, Kang et al.[148] synthesized PLGA microsphere and coated apatite onto the surface by incubating them in SBF. It is revealed that the cell adhesion and bone formation on apatite-coated PLGA microspheres were significantly higher than the controlled PLGA microspheres. On the other hand, HA coating of microsphere surface using alternative approach have also been explored. According to a research conducted by Xu and Czernuszka,[149] the PLGA microspheres bearing a negatively charged surface were coated with HA using dual constant composition method. Noted that sodium dodecyl sulfate (SDS) was employed as surfactant instead of PVA during microsphere preparation in order to render the microsphere surface anionic. SDS, which serves as a strong chelating group for Ca^{2+} ions discard the necessity of any pretreatment to induce surface negativity. This permits a more rapid nucleation of HA during subsequent coating process, thus resulting in a low processing time. These HA-coated microspheres showed a sustained release profile for at least one month with little initial burst release, which is higher compared to HA-coated lysosomes. Another way to coat HA onto the surface of PLGA microspheres was also proposed by Zhe et al.[150] using a two steps process, i.e., (i) Affixing/absorption of Ca^{2+} ions onto the microspheres surface, (ii) Bonding of phosphate ions with absorbed Ca^{2+} ions to form HA coating (Fig. 8). This method confers discernible advantage in term of duration of coating compared to classic biomimetic process, as the latter is rather time-consuming during the precipitation of HA (~3–7 days). HA-coated microsphere also can be synthesized via Pickering emulsion
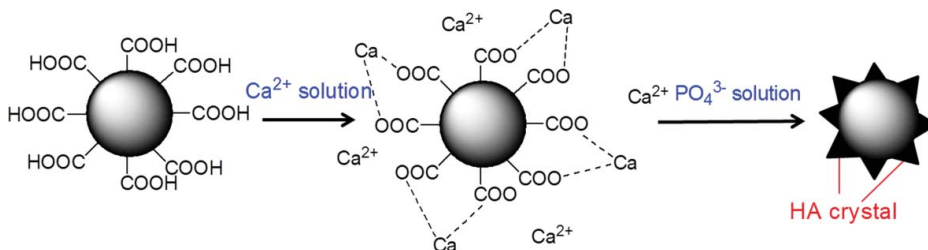
Figure 8. Coating protocol of HA on the surface of microsphere as proposed by Zhe et al.

approach as reported by Fujii *et al.*,[151] where HA nanoparticles will be used as an emulsifier. Due to the good absorbability of HA nanoparticles for cell-adhesive protein which possess integrin binding character, it was reported that HA coating will aid in promoting the cell adhesion and spreading in PLA microspheres. The increase in surface roughness after HA nanoparticles coating also substantially improves their interfacial interaction with cell, thus providing more opportunity for cell adhesion.

5.2.3 Irradiation or plasma-induced grafting

Surface modification by means of irradiation is a prevalently used method to induce hydrophilicity and compatibility in bulk PLA/PLGA, though, it still remain scarce for PLA/PLGA based-microsphere to be modified using this approach and their usage in DDS awaiting further elucidation. Indeed, most of the microspheres dealing with irradiation are studied in the sense of sterilization impact, not as much as in a way of modification.[152–155] Generally, radiation sources which might be γ-rays, X-rays, and electron beam, can initiate the covalent grafting of hydrophilic monomer onto the microsphere surfaces without the use of any initiator or catalysts. Upon irradiation, radicals produced will interact with hydrophilic monomer to initiate grafting, where reactive intermediates formed will engender to the rearrangement or formation of new bonds between them, thus leading to a hydrophilic grafting on the surface of the microspheres.[156] Surface modification of microsphere based on radiation approach seems to be advantageous not only for their role as an initiator for the grafting process but also with their associate plus point for the concurrent sterilization effect that possibly happened.[25]

Besides, graft copolymerizing hydrophilic monomers using plasma treatment also has been used to introduce chemical functionalities to the microspheres. Unlike chemical treatment which might compromise the surface topology and obliterate the potential surface pattern of the microspheres, plasma treatment seems to be promising in improving the surface characteristics of the polymer without altering their bulk properties.[157] Gas plasma such as O_2, CO_2, N_2, NH_3, and H_2 is usually used as a gas source in plasma-induced polymerization to initially create useful reactive sites such as –COOH, –OH, and –NH_2 on polymer surface, thus offering a reaction platform for subsequent grafting with other hydrophilic monomers. While the aim of introducing these specific functional groups onto the surface of the biodegradable polyester is mostly used in improving their surface wettability, hydrophilicity and thus their cell-material interaction, utilization of a biomolecules-derived monomer encompassing natural cell recognition site (*e.g.*, collagen and gelatin) can be useful to improve their biological properties. Baki *et al.*[158] studied the surface modification of PLGA microspheres with gelatin methacrylate (gel-MA) and further investigated their interaction with human mesenchymal stem cells. Several modification approaches, including plasma treatment, adsorption and entrapment method were compared by the authors to probe their viability in introducing gel-MA into the PLGA microspheres. The study showed that oxygen plasma treatment produce the highest density of gel-MA deposition around the microsphere surfaces, with relatively higher amount of cell growing on the surface of PLGA microspheres compared to the adsorption method. The effectiveness of gel-MA deposition using oxygen plasma treatment is due to the unstable peroxide groups that form reactive radical species that prone to attack the electron-rich vinyl groups from the gel-MA monomer, thus initiate the entire grafting process. Additionally, some of the peroxides moieties also transmute into –OH and –COOH groups that make easy for the anchorage of gel-MA molecules onto the PLGA microspheres via covalent bonding. This biopolymer-derived monomer to be used

for grafting is indeed potential for further immobilization of biomacromolecules with specific tissue functionalities.

5.3 Core-shell systems

The synthesis of microspheres with exceptional architecture design also has enticed considerable attention as a way to improve the performance of microcarriers. In this regard, novel integrated system known as core-shell systems has been introduced as an effort to attenuate some limitation related to polyester microspheres. Briefly, core-shell microsphere can be described as a drug-entrapped polymeric core which is encased with single or multiple layers of other polymer that constitute the shell. Double-walled microsphere (DWMS) is one of the common core-shell constitutions classified under this category. The architectural advantage of core-shell system continue to gain recognition as an effective carrier for drug delivering due to the following perceive advantages compared to monolithic carrier: (a) The outer shell or the corona of the microsphere serves as an effective diffusion barrier that prevents the diffusion of hydrophilic drugs located at the inner core surface, thus alleviating the initial burst release phenomenon during drug release study,[159] (b) modulation of drugs release rate can be attained ascribed to the role of external shell as rate-limiting barrier for drug release, where their rate of drug release can be tailor-made based on the mass ratio, the thickness or the types of the polymeric materials used, (c) for highly water-soluble drugs, core-shell microsphere exhibited higher EE relative to monolithic microspheres,[160] and (d) sequential DDSs can also be designed by selectively incorporate drugs into different spatial locations of microsphere, *i.e.*, the core or shell phase, thus expanding the efficacy of delivery device.[161] Recently, core-shell microspheres have been used to encapsulate GS,[160] 5-fluorouracil (5-FU),[162] BSA,[163–165] doxorubicin (DOX),[166] etanidazole,[167] exenatide,[168] and cyclosporine (CyA).[169]

Generally, fabrication of core-shell microsphere can be broadly fall into two categories, *i.e.*, two-step and single-step method. For two step core-shell fabrication method, the polymeric core is first prepared via conventional emulsification (*e.g.*, O/W, $W_1/O/W_2$, S/O/W, *etc.*) to form monolithic polymer microsphere. Subsequently, core-shell microsphere is formed by dispersing the as-prepared polymer microsphere (core) in another polymeric solution followed by re-emulsifying them in outer extracting solvent to solidify the shell layer.[170] On the other hand, there are two possibility of core-shell fabrication using single step method. First type involving the emulsification of drug-suspended/dispersed primary polymer solution (W/O or S/O) in secondary polymer solution (O) followed by solvent extraction and evaporation in external aqueous medium (W). This can be known as $W_1/O_1/O_2/W_2$ or $S/O_1/O_2/W$ emulsification method. Second type involve slight modification of double emulsification where secondary polymer will be either dispersed in internal organic phase or in external continuous phase. Besides, technology such as microfluidics device also have been used to form core-shell microsphere. Table 4 summarize the fabrication of various type of core-shell microspheres using different approaches.

In the case of DWMS, the fabrication of this sophisticated morphology relied upon the immiscibility between the core and shell material, where phase separation between distinct polymers will be formed to produce wall-like structure. For example, DWMS based on PLA and PLGA is usually prepared using two distinct solvents, *i.e.*, dichloromethane (DCM) and ethyl acetate (EA). Basically, PLA is soluble in DCM but not in EA, whereas PLGA is miscible in

Table 4. Representative literatures of core-shell microsphere prepared using various method.

Core	Shell	Drug	Preparation method	Schematic route/ representation	References
PLLA	PLGA	GS	Single step: $S/O_1/O_2/W$ emulsion	- Emulsification of GS in PLLA solution to form S/O_1 suspension. - Emulsification of S/O_1 in PLGA solution to form $S/O_1/O_2$. - Emulsification of $S/O_1/O_2$ in MC solution (W) allow solvent extraction and evaporation to form core-shell microsphere.	[160]
PLGA	PLLA	5-FU	Single step: $W_1/O_1/O_2/ W_2$ emulsion	- Emulsification of aqueous 5-FU in PLGA solution to form W_1/O_1 emulsion. - Emulsification of W_1/O_1 in PLLA solution to form $W/O_1/O_2$ emulsion. - Emulsification of $W_1/O_1/O_2$ in aqueous PVA solution (W_2) allow solvent extraction and evaporation to form core-shell microsphere.	[162]
POE	PLGA	BSA (Shell) CyA (Core)	Single step: $W_1/O/W_2$ emulsion	- Emulsification of BSA in aqueous PVA solution to form W_1. - Mixing of PLGA, POE and CyA in DCM to form O. - Emulsification of W_1 and O phase to form W_1/O emulsion. - Emulsification of W_1/O in external aqueous PVA solution allow solvent extraction and evaporation to form core-shell microsphere.	[169]
PLGA	GC	CHA (shell) bFGF (core)	Single step: $W_1/O/W_2$ emulsion	- Emulsification of bFGF solution in PLGA solution to form W_1/O. - Mixing of CHA and GC in aqueous PVA solution to form W_2. - Emulsification of W_1/O in W_2 allow solvent extraction and evaporation to form core-shell microsphere.	[172]
PLGA	Chitosan	GDNF	Two-step process: $W_1/O/W_2$ emulsion, *in situ* crosslinking in W/O emulsion	Preparation of GDNF-loaded PLGA microsphere via $W_1/O/W_2$ emulsion - Emulsification of GDNF solution and heparin in PLGA solution to form W_1/O. - Emulsification of W_1/O in aqueous solution containing Tween 80 allow solvent extraction and evaporation to form PLGA microsphere. Preparation of PLGA-Chitosan core-shell microsphere via W/O emulsion - Re-suspending microsphere in chitosan solution (W). - Emulsification of W in liquid paraffin (O) containing Span 80 to form W/O emulsion. - Addition of STPP solution into W/O emulsion to cross-link and solidify the core-shell microsphere.	[170]
Dextran	PLGA-PLA	rIL-2	Two-step: Freezing-induced phase separation, $S/O_1/Oh_2/W$ emulsion	Formation of rIL-2-loaded dextran particles - Co-aqueous solution of rIL-2, PEG, and dextran was vortexed, frozen overnight and lyophilized to powder. - Re-suspending lyophilized powder in DCM and centrifuged to remove PEG. Formation of dextran-(PLGA-PLA) core-shell microsphere - Emulsification of dextran particles in co-solution containing PLGA and PLA to form S/O_1 emulsion. - Further emulsification of S/O_1 in hydrophilic oil phase (Oh) (containing ethylene glycol, glycerol and PVA) to form $S/O_1/Oh_2$ emulsion.	[173]

Table 4. (*Continued*)

Core	Shell	Drug	Preparation method	Schematic route/ representation	References
PLGA	Alginate	Rifampicin	Microfluidic method to form O_1/ W/O_2 emulsion followed by alginate cross-linking	- Emulsification of $S/O_1/Oh_2$ in aqueous continuous phase (W) containing NaCl to allow solvent extraction and evaporation to form core-shell microsphere. - PLGA solution containing rifampicin (O_1) was injected through narrow tube to form fine organic droplet in W phase (aqueous PVA solution containing alginate) to form O_1/W. - O_1/W is then processed to O_2 phase (toluene containing Span 80) to form $O_1/W/O_2$ emulsion. - Cross-linking of $O_1/W/O_2$ droplet in $CaCl_2$ solution in collecting beaker followed by solvent evaporation to form core-shell microsphere.	[63]

STPP: sodium tripolyphosphate

both of these solvents. During emulsification process, the addition of PLA solution (PLA dissolved in DCM) containing dispersed drugs into the PLGA solution (PLGA dissolved in EA) will create PLA droplets in the emulsion system, where the PLGA will remain dissolved in EA solution. As DCM begins to evaporate, solidification will first occur at the PLA-PLGA interface, where PLGA is still dissolving in the EA solution. Upon the subsequent addition of this suspension into the water medium, evaporation of EA will lead to the succeeding precipitation of PLGA to form the outer shell layer, thus completing the double-walled structure.[165]

Usually, the most widely studied research regarding the synthesis of DWMS is based on PLA and/ or PLGA, where the selection of either of them as core or shell is highly dependent on the prerequisite delivery application. Generally, PLA possess a slower degradation rate than PLGA due to their more hydrophobic moieties compared to the latter one. Therefore, for a sustained delivery application, PLGA with different lactide:glycolide ratio will be utilized instead of PLA, where different mass ratio of PLGA will be used to construct the core-shell structure for the microsphere. For instance, Zheng synthesized a double-walled PLGA microsphere for the delivery of a hydrophilic 5-FU using emulsion method. The building block of the DWMS composed of PLGA 75/25 as the core and the outer shell that is made up of PLGA 80/20, both in mass ratio of 2:1. It is revealed that about 86.5% of EE has been obtained when a DWMS is used to encapsulate 5-FU, which is significantly higher than the single-walled microsphere reported by some previous literatures.[132],[171] Moreover, their initial burst release is as low as 4.2% where a prolonged release of drugs has been observed over 70 days. This finding remarkably substantiate the usefulness of the outer shell layer to suppress the burst effect arising from the deposition of drug on the microsphere surface during microsphere fabrication. On the other hand, Tan and Ye[160] also design a DWMS based on PLA and PLGA for the encapsulation of hydrophilic GS to treat osteomyelitis. Similarly, the uses of this core-shell microcarrier profoundly increase the GS EE from 42.01% (for single-walled PLGA microsphere) to 75.68% (with PLA to PLGA mass ratio of 1:1) and the sustained drug release of about 40% was observed for 30 days. This is because the drug encased inside the core matrix will only be released when degradation of microsphere or the influx of water occurs. Only a small amount of drugs reside on the surface will be liberated in the first few days during drugs release study. Therefore potential local inflammation caused by

excessive bursting in monolithic system can be effectively prevented by implying the core-corona structure to encapsulate drugs. DWMS based on PLGA and poly (orthoester) (POE) has also been used for the dual delivery of drugs with different water solubility due to the different degradation rate exhibited by the core and shell materials. Attributable to the slow degradation of PLGA shell and the rapid erosion properties of POE core, a hollow structure will be formed after 1 week during *in vitro* study. From here, the controlled release of two drugs model at different rate can be attained. Shi *et al.*[169] encapsulated both BSA and CyA using double-walled PLGA/POE microsphere. It is proclaimed that BSA which has a higher affinity to PLGA will preferentially stay at PLGA phase during emulsification while the more hydrophobic CyA tends to reside on POE core. The BSA-CyA encapsulated-DWMS allows a more sustain and rapid liberation of CyA compared to mere CyA-loaded DWMS ascribed to the more porous morphology exhibited by PLGA shell. It is also worthwhile to point out the role of outer PLGA shell as a carrier for BSA and as a reservoir for CyA in this core-shell microdevice. As revealed from the literature, most of the BSA is released from PLGA shell during the first 5 days, where only about 14% of CyA will be liberated from the microsphere. For the subsequent 25 days, accelerated CyA released was observed (more than 80% of CyA was released) due to the generation of more pores during the earlier leaving of BSA from PLGA shell, which have less biphasic CyA release profile compared to mere CyA-loaded POE/PLGA microsphere. This distinct combination fascinatingly suggests a potent delivery device for the controlled release of therapeutic drugs.

Composite core-shell microsphere based on PLGA and glycol-chitosan (GC) has also been studied for the sequential release of chlorhexidine acetate (CHA) and basic fibroblast growth factor (bFGF) for wound healing application. By using modified $W_1/O/W_2$ emulsion method, bFGF-CHA-loaded PLGA-GC microspheres were successfully prepared by dissolving bFGF in oil phase while dissolving CHA in outer aqueous phase containing GC. Sequential release of cargos can be achieved due to the diffusion barrier that are imposed differently for each drugs in the microsphere. CHA entrapped in the outer GC shell will be liberated rapidly from the drug carrier due to their fast diffusion from GC, while the two polymeric barriers (both GC and PLGA) imposed to bFGF will result in their sustained release, where their release rate can be adjusted by altering the concentration of GC in the formulation.[172] While core-shell system emerged as promising approach to improve the efficacy of monolithic microsphere, there are still lacks of researches regarding the incorporation of cell-adhesive group onto the microsphere surface via surface modification. This is indeed crucial for core-shell system that lack of functional binding site on the surface of shell layer (*e.g.*, PLA-PLGA DWMS) for subsequent binding with surrounding cell. However, unlike monolithic microsphere that can be easily modified via various approaches, further modification might disturb the core-shell configuration ascribed to their softer outer shell layer. The control of organic solvent residue to a safe level could be another limitation due to the core-shell structure that could restrain the effective solvent evaporation during microsphere fabrication.[174]

6. Conclusion

Despite numerous advantageous properties of PLA/ PLGA microspheres for drug delivery application, several limitations such as low hydrophilicity, poor drug encapsulation, biphasic drug release behavior, polydispersity, *etc.* have restricted their widespread employment in various biomedical applications. Herein, recent strategies and advances in developing

effectual drug-loaded delivery vehicles based on PLA/PLGA microspheres are summarized in this review. Among them, synthesizing or modifying the bulk precursor by means of bulk modification is the most straightforward approach used to design and impart the desired properties to the polyester constitution prior to the formation of microsphere. From this approach, modifying agents which usually possess favorable characteristics such as hydrophilicity, enhanced cytocompatibility or free functional groups can be introduced into the bulk polyester architecture by either blending or copolymerization process. Or else, surface modification can also be performed on the microsphere to augment the interaction between the carrier and the biological environment. Sometimes, the drug release kinetic can also be tailored and regulated depending on the modifying agent used. Due to the low drug EE, uncertain particle size and particle size distribution for microsphere prepared using conventional solvent extraction/evaporation emulsification method, development of new techniques, technologies and novel approaches to improve the current existing method have been developed and investigated. Technologies such as membrane and microfluidics emulsification which are useful in forming inner emulsion droplets with controllable size have been demonstrated as an effective methods to prepare monodisperse microspheres. Spray-drying and supercritical fluid approach which involve water-free emulsion and rapid extraction process respectively also shown to be promising in fabricating drug-loaded microspheres with high EE. Moreover, formulation parameters and operating condition also play crucial roles in determining the drug loading and microparticles size during emulsification process, especially for the microencapsulation of water-soluble macromolecules. As the partitioning of hydrophilic drugs into the aqueous continuous phase will adversely affect their EE in the microspheres, aspects that can prevent this phenomenon (such as the organic phase viscosity, solubility of drugs in W_2, theoretical drug loading, surfactants concentration and the stability of W_1/O) should be taken into consideration during the microencapsulation process. With improvements in microsphere design and efficacy, microsphere-based controlled delivery is expected to contribute significantly to biomedical and pharmaceutical applications.

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