



A facile strategy for controlling porous PLGA microspheres via o/w emulsion method

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Abstract

PLGA porous microspheres (a porous structure and the characteristics of low-quality density and high pore rate) are widely used in the medical field. Here, we report a preparation of PLGA porous microspheres by a solvent evaporation method, introducing alkanes as porogen. Various morphologies of microspheres can be regulated by controlling parameters such as alkane segment, content, polymer concentration, and temperature. The results show that the phase separation is caused by the incompatibility of the three alkane porogens of 60–90 petroleum ether, n-dodecane, and paraffin liquid. After the hydrophobic alkanes were eluted and pore-formed in situ, the PLGA microspheres could obtain different morphologies. Porous structures range from network porous to hollow. Interfacial tension and polymer phase separation work together on the microsphere morphology. In addition, the shell thickness and surface flatness of the microspheres can be regulated by polymer concentration and temperature, respectively.

Keywords PLGA · Porous microspheres · Alkane porogen · Morphology control

Introduction

Poly(glycolide-co-lactide acid) (PLGA) is an aliphatic copolymer with good biocompatibility and degradability [1]. It was approved by the FDA for use in humans and their success as biodegradable sutures [2]. Porous-structured PLGA microspheres have a high specific surface area and special structure, which have been widely used in tissue engineering, cell culture, soft tissue filling, and drug delivery systems [3–5]. In the cell culture area, not only does the physicochemical properties of the microsphere affect cell growth, but the morphology and porous structure of the microspheres affect cell growth and attachment [6]. Studies have shown that the surface morphology of microspheres and internal pore structure are important factors in controlling the drug release

process [7, 8]. Particularly, porous microspheres can be used as dermal fillers. Porous microspheres are low-density, so they will not deposit in the skin. Moreover, the surface topographic properties can lead to differential adhesion, proliferation, growth, and differentiation of inflammatory cells [9]. The role of the surface morphology and internal structure of microspheres in applications has been attracting strong interest. Therefore, the morphology is precisely controlled during the preparation of the microspheres.

In recent years, great progress has been made in the preparation of porous and hollow microspheres, including the emulsion solvent evaporation method [10], microfluidic method [11], electrospray method [12], and membrane emulsification method [13]. For the latter three methods, they can well control the thickness and size of the monodispersity of the microspheres. However, the equipment for preparing microspheres by these methods is expensive, and the preparation of microspheres takes a long time. The emulsion solvent evaporation method is a common way to prepare microspheres, which is easy to operate and inexpensive [14]. First, various emulsification methods are used to prepare different types of emulsions (w/o, o/w, w/o/w, o/w/o type emulsions), and then the dispersed phase is solidified by solvent evaporation to remove the solvent [15]. Morphology of microspheres and size can be adjusted by the emulsion

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formulation and process parameters. The addition of porogens or surfactants, the type of organic solvent, and the polymer concentration in the inner aqueous phase had a great influence on the morphology of the microspheres [16].

Pore formation by soft and hard template methods is a common method for preparing porous microspheres. Researchers used ammonium bicarbonate as a gas-blowing agent to prepare highly open porous PLGA microspheres [17]. However, with the increase of system pressure, the number of pores on the surface of the microspheres increases, and the assembled structure becomes smaller. The morphology of the microspheres changes and is difficult to control. Liu et al. [18] used n-hexadecane (HD) as a porogen and achieved regulation of pore density by simply changing the HD concentration.

The use of non-solvent alkanes can act as porogens. Because the polymer and the alkane are phase-separated, the alkane can cause pores in situ to obtain porous microspheres, and a series of porous microspheres with different pore sizes can be obtained by adjusting the alkane concentration. Furthermore, this facile method can be applied to various polymers, alkanes, organic solvents, etc. [18]. Herein, we prepared porous PLGA microspheres by emulsion solvent evaporation method and introduced different alkane porogens including petroleum ether, n-dodecane, and paraffin liquid. Systematically studied the effect of microsphere preparation formulation and operating parameters on the porous morphology of microspheres, including emulsion formulation polymer concentration, alkane porogen type, porogen content, and process parameter like temperature. After the microspheres were prepared, the morphology of the microspheres was characterized to explore the influence mechanism of these factors. In this method, the three porogens have different carbon chain lengths and therefore have a different tendency to phase separation from the polymer.

Experiment

Materials

Poly(L-lactide-co-glycolide acid) (PLGA; $\eta = 2.72$, LA:GA = 90:10) is lab homemade, Polyvinyl alcohol (PVA; av mol wt = 30,000 to 70,000), petroleum ether (b.p.60–90 °C), n-dodecane (b.p.216.3 °C), paraffin liquid, ethyl acetate (b.p.180–250 °C), and dichloromethane (DCM) were purchased from Sigma Chemicals (St. Louis, MO). Distilled water was used in the formulation of the microspheres.

Microsphere formulation

PLGA microspheres were prepared by an oil-in-water (o/w) prepared using emulsion–solvent evaporation/extraction

method. Preparation microspheres: The polymer is dissolved in an organic solvent and the porogen is either dispersed in this organic phase. The oil phase consisted of 1.5 g PLGA and 50 mL DCM, 1.5 g Petroleum ether. The oil phase was added to a three-necked flask containing 250 mL of 0.2 wt% PVA aqueous solution, decompress filter with 1 h/0.6 L circulating water, and mechanically stirred at 600 rpm for 4 h to form an o/w emulsion system. The obtained emulsion was stirred magnetically. The plate was stirred for about 7 h to completely evaporate the solvent and solidify the droplets into microspheres. The microspheres were collected by suction filtration under decompress filter, washed three times with distilled water, placed in a vacuum oven for 5 h, and the residual DCM was dried by suction. Obtaining different batches of microspheres by changing the parameters to be explored. When n-dodecane or paraffin liquid is used as the porogen, the microspheres collected by suction filtration need to be eluted with 15 wt% Ethyl acetate: Petroleum ether. The microspheres batch abbreviations are shown in Fig. 1. PVA is a typical surfactant, and DCM is a common solvent used to match the general framework of most previous studies [19].

¹ AA refers to the number after the percentage multiplied by 10 (the percentage is the mass of PLGA to the volume of DCM). BB refers to an acronym for alkane, like petroleum ether means PE. CC refers to the number after the percentage multiplied by 10 (the percentage is the mass of alkane to the volume of DCM).

Characterization of microspheres

Characterization examined the morphology of PLGA microspheres using scanning electron microscopy (SEM, INSPECT, F0825-1900, FEI, America). Mount the microspheres to the metal stubs using double-sided tape. Then, the samples were coated with a thin layer of gold (100–150 Å). Samples were directly observed with SEM in high vacuum mode using an acceleration voltage of 5 kV or 20 kV. The internal morphology of microparticles was observed after grinding in liquid nitrogen. Before the samples were observed with a polarized optical microscope (POM, Model XPN-203, Shanghai Changfang Optical Instrument, China),

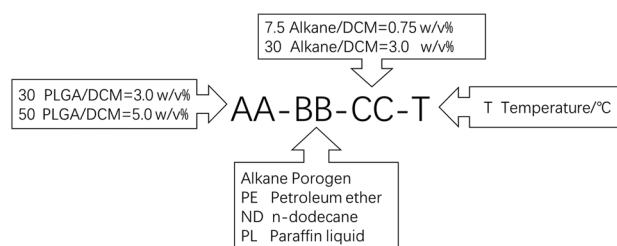


Fig. 1 Abbreviation codes for the batch names¹

dried microspheres were first redispersed in distilled water and placed on glass slides. The size distribution and surface morphology of PLGA microspheres was determined using SEM images. The internal morphology of microspheres was observed with SEM and POM images.

Micrographs were digitally taken and analyzed using Image-Pro software (National Institutes of Health, USA, Java 1.6.0–20) to calculate individual microsphere size and their mean value. All microsphere sizes were statistically averaged ($n > 100$) and results are reported as mean \pm SD (standard deviation).

Interfacial tension

The interfacial tension (IFT) between polymer and dichloromethane solution containing alkane porogen and PVA solution was measured at 25 °C by contact angle meter (CAM, JC2000D1, POWEREACH®, China), and the measurement method was the suspension drop method. Firstly, a certain amount of PLGA was dissolved in an appropriate amount of dichloromethane, and then alkane porogen was added. The mixture was sealed and stirred to make the mixture evenly mixed. The 0.2 wt% PVA aqueous solution was added to the sample tank, and then the 100 μ l micro-sampler containing alkane porogen PLGA-dichloromethane solution was vertically fixed. The sample platform was raised, and the needle of the sampler was immersed in the aqueous solution. Then push down the injector to drop the polymer alkane-dichloromethane solution into the sample pool. Photograph the droplet before dropping and input the parameters to get the IFT [20].

Results and discussion

Characterization of the emulsion IFT

The IFT is an important factor affecting the shape and size of the microspheres [21]. Table 1 shows the IFT between the aqueous and oil phases. Different oil phases have different IFTs with the same water phase (0.2 wt% PVA in H₂O). It can be seen that the IFT between different pure alkanes and the water phase are quite different, and the IFT between the liquid paraffin and the water phase is the largest, which is 32.54 mN·m⁻¹. The $\gamma_{o2/w}$ is similar to $\gamma_{o1/w}$ (oil phase 1 refers to PLGA in DCM, oil phase 2 refers to PLGA in DCM and alkane), probably because the alkane content is less than that of PLGA in DCM. Thus IFT of adding alkane is close to the original IFT.

Microsphere morphologies

Since the size of the polymer microspheres is one of the most important indicators to ensure safe use. When the diameter of microspheres is less than 20 μ m, there is a risk of phagocytosis by macrophages, and the durability of fillers cannot be guaranteed [22]. To exclude the influence of the stirring speed on the particle size of the microspheres, the same stirring speed was controlled at 600 rpm in this experiment. Several batches of microspheres were prepared by changing the o/w emulsion formulation parameters, and the data particle size distribution map was obtained by scanning SEM images and analyzing the size with Image-Pro

Table 1 The IFT between the water phase and different oil phases

Water phase	Oil phase			$\gamma_{O/W}/\text{mN}\cdot\text{m}^{-1}$	
	Alkane Porogen	C_{PLGA} (w/v %)	C_{alkane} (w/v %)		
0.2 wt% PVA in H ₂ O	—	3.0	—	11.09	
	Petroleum ether	—	—	—	21.74 ^a
		—	3.0	1.0	11.43
		—	3.0	2.0	12.67
		—	3.0	3.0	12.94
		—	3.0	6.0	13.09
		—	3.0	12.0	13.96
	N-dodecane	—	—	—	10.26 ^a
		—	3.0	3.0	11.13
		—	3.0	6.0	13.54
	Paraffin liquid	—	3.0	12.0	12.45
		—	—	—	32.54 ^a
		—	3.0	1.5	13.51
		—	3.0	3.0	14.08
		—	3.0	12.0	11.52

^aOil phase conditions for three IFTs for pure alkane porogen, DCM as solvent under other oil phase conditions

software. The relevant parameters during the preparation of these microspheres are listed in Table 2.

Figure 2 shows the morphology and particle size distribution of microspheres prepared with different porogens and concentrations. For petroleum ether, it can be seen from Fig. 2A–D, and the corresponding average particle size that with the increase of petroleum ether content, the particle size of the microspheres increases (Table 2). In addition, with the increase of petroleum ether content, the IFT also increases (Table 1). We speculate that the increase in the size of the microspheres is related to the increase in the IFT between the organic and aqueous phases. As can be seen in Fig. 3, the increase in the IFT of the water–oil phase containing petroleum ether is conducive to the formation of larger droplets during the emulsification process, thereby forming larger-sized microspheres. This observation is supported by the work of Ito et al. [23]. With the increase of the porogen petroleum ether, the solubility of PLGA in droplets becomes worse. Therefore, PLGA precipitation occurs earlier, and the particle size increases when the droplet solidifies.

For the microspheres with n-dodecane and liquid paraffin as porogens, with the increase of porogen content, the particle size of the microspheres increases (Fig. 2E–J). Meanwhile, the IFT first increases and then decreases (Fig. 3). When the alkane concentration is low, the particle size of the microspheres increases with the increase of the IFT between o/w (Table 1). It is probably because the alkanes act as porogens to occupy the inner volume of the microspheres, which increases the size of the microspheres. When the concentration of n-dodecane increases to 6 w/v% and the concentration of liquid paraffin increases to 3 w/v%, the particle size of microspheres increases slightly with the decrease of IFT

(Table 1). Here, the IFT is not the decisive factor to control the particle size. Similarly, like the petroleum ether mentioned above, when the contents of n-dodecane and liquid paraffin are increased, the polymer will be phase-separated and precipitated in advance, and the particle size of the microspheres will also increase. However, the difference is that petroleum ether has a lower boiling point and tends to leave droplets along with DCM. While both n-dodecane and liquid paraffin have boiling points higher than 200 °C and have greater space-occupying capacity in droplets. Furthermore, it can be seen from Fig. 2(I, K) that the microspheres have two morphologies: porous surface and smooth surface.

Study the surface morphology of microspheres

According to the classification of the International Union of Pure and Applied Chemistry [24], the microspheres in Fig. 4 are ultraporous materials (greater than 200 nm). It can be seen from Fig. 4 that when the petroleum ether content increases, the pore size on the surface of the microspheres gradually increases, which is similar to the previous results [25]. It is worth noting that in the porous microspheres, with the increase of the IFT between the oil–water phases containing the alkanes (Table 1), the surface pore size of the microspheres tends to increase (Fig. 4A2–D2, E, F, I, J). This may be because when the IFT increases, the tendency of incompatibility between the polymer and the porogen increases. The change in IFT results from the change in composition, and the change in composition leads to the ease of phase separation. As stated above, since the early precipitation of the polymer leads to a large particle size of the microspheres, the pore size is naturally large (ignoring

Table 2 Parameters for preparing microspheres and mean size of dry microspheres

Sample names	Alkane Porogen	C _{PLGA} (w/v %)	C _{alkane} (w/v %)	Temperature (°C)	Mean Size (μm)
30-PE-7.5–25	Petroleum Ether	3.0	0.75	25	52.07 ± 19.36
30-PE-20–25		3.0	2.0	25	55.62 ± 24.41
30-PE-30–25		3.0	3.0	25	87.32 ± 17.95
30-PE-60–25		3.0	6.0	25	177.62 ± 57.68
30-PE-30–5		3.0	3.0	5	76.18 ± 24.44
30-PE-30–35	n-dodecane	3.0	3.0	35	91.38 ± 28.82
30-ND-10–25		3.0	1.0	25	47.90 ± 18.99
30-ND-30–25		3.0	3.0	25	60.78 ± 11.84
30-ND-60–25		3.0	6.0	25	83.71 ± 26.71
30-ND-120–25	Paraffin Liquid	3.0	12.0	25	86.32 ± 24.62
10-PL-150–25		1.0	15.0	25	—
20-PL-90–25		2.0	9.0	25	153.67 ± 33.18
20-PL-150–25		2.0	15.0	25	87.86 ± 16.72
30-PL-7.5–25		3.0	0.75	25	80.43 ± 21.61
30-PL-30–25	3.0	3.0	25	93.52 ± 30.85	
30-PL-120–25	3.0	12.0	25	104.69 ± 26.91	

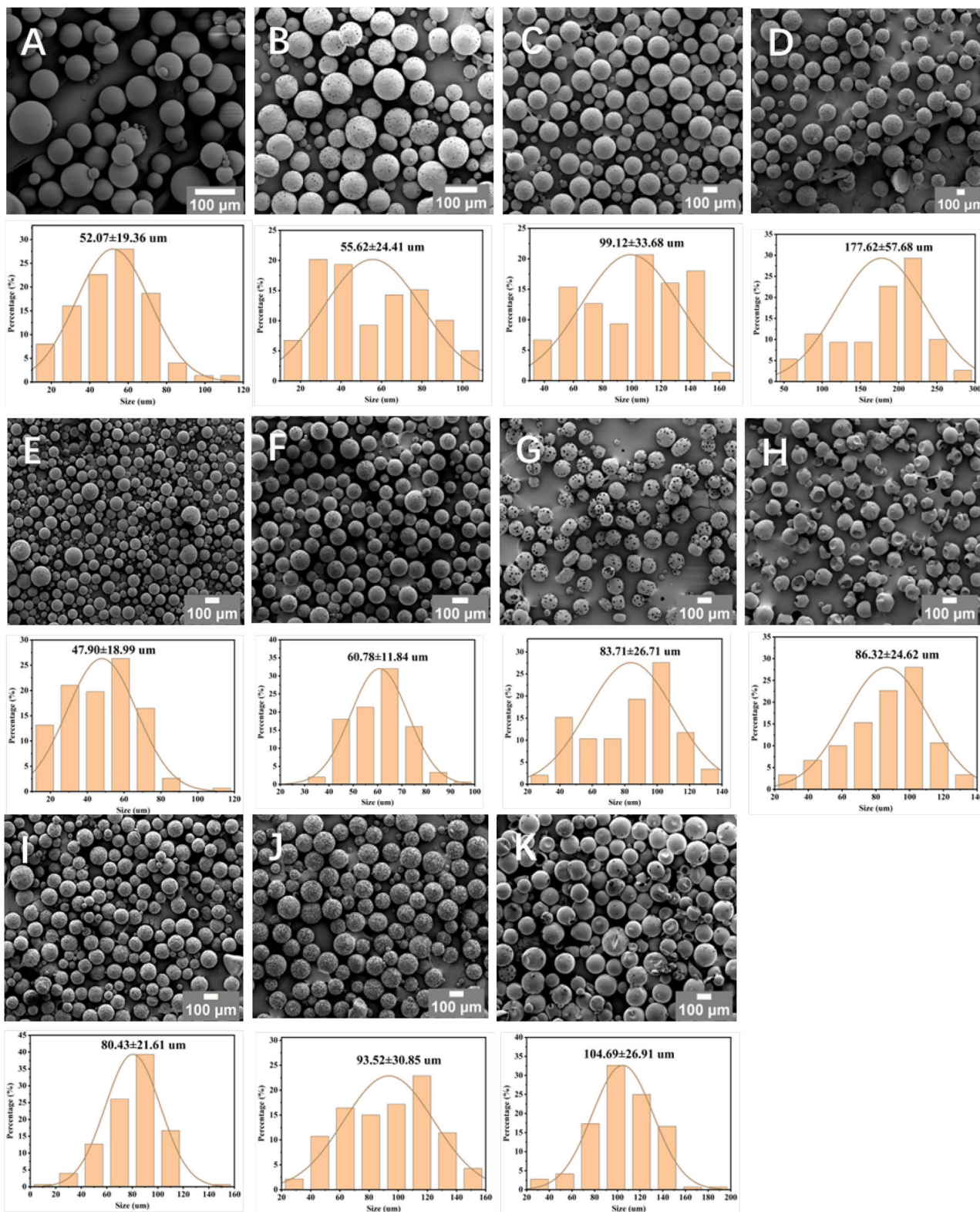
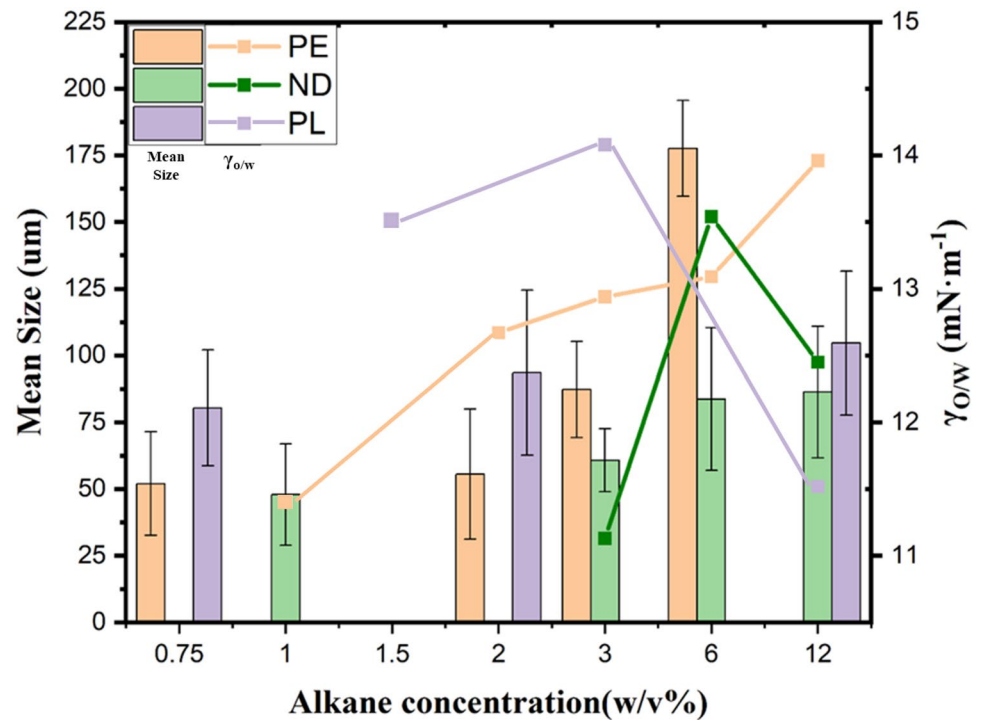


Fig. 2 SEM images and size distributions ($n > 100$) of PLGA microspheres illustrate the sizes obtained in various experiments. SEM images of **A–D** PLGA porous microspheres fabricated with 0.75 w/v%, 2.0 w/v%, 3.0 w/v%, and 6.0 w/v% petroleum ether respectively. **E–H** 1.0

w/v%, 3.0 w/v%, 6.0 w/v%, and 12.0 w/v % n-dodecane respectively, **I–K** 0.75 w/v%, 3.0 w/v%, and 12.0 w/v% paraffin liquid respectively, **A–K** 3.0 w/v% PLGA. Scale bar = 100 μm

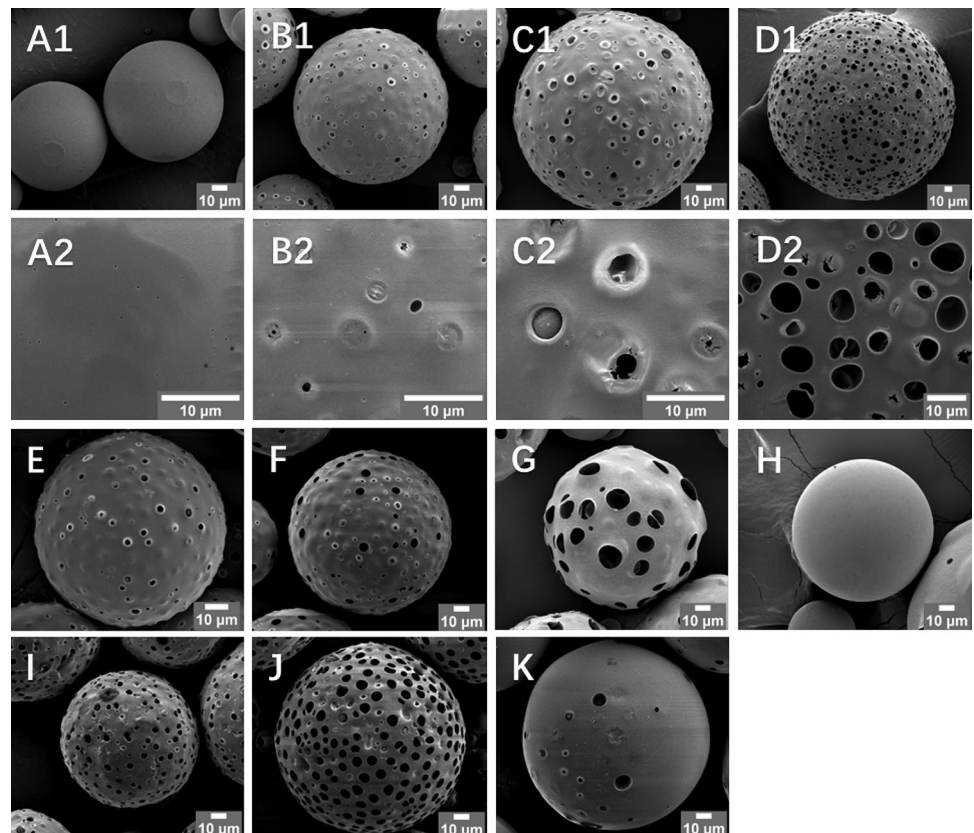
Fig. 3 Microsphere particle size ($n > 100 \pm SD$) and IFT between o/w phase at different alkane concentrations at 25 °C



the shrinkage after curing). On the contrary, in the late precipitation, the density of the microsphere shell is large, and the pore size is naturally small.

Pores for polymer phase separation are not entirely due to thermodynamic driving forces, but also to solvent-selective dissolution effects [26]. Phase separation occurs earlier, the

Fig. 4 SEM images of porous morphology on PLGA microspheres were obtained by different alkane parameters. SEM images of **A1–D1** PLGA porous microspheres fabricated with 0.75 w/v%, 2.0 w/v%, 3.0 w/v%, and 6.0 w/v % petroleum ether respectively. **E–H** 1.0 w/v%, 3.0 w/v%, 6.0 w/v%, and 12.0 w/v % n-dodecane respectively, **I–K** 0.75 w/v%, 3.0 w/v%, and 12.0 w/v% paraffin liquid respectively, **A–K** 3.0 w/v% PLGA. **A2, B2, C2, and D2** are the magnified images of **A1, B1, C1, and D1** respectively. The mean pore size of microspheres: **A** 0.21 μm , **B1** 2.43 μm , **C1** 4.32 μm , **D1** 5.39 μm , **E** 2.16 μm , **G** 11.06 μm , **H** 3.25 μm , **I** 5.56 μm . The scale bar is 10 μm



solvent changes from good solvent to poor solvent or even non-solvent, the polymer precipitates and migrates, and the non-solvent phase also fuses. The hydrophilic phase of the polymer is easy to form a hollow structure, and the hydrophilic phase of the porogen is difficult to form a hollow structure. The longer the alkane carbon chain and the higher the content, the less hydrophilic it is [27]. Therefore, n-dodecane and liquid paraffin have a smooth surface and few pores at high concentration (Fig. 4H, K).

Evaluate the main mechanisms leading to spherical particle shape

To explore the morphological formation mechanism of the microspheres, the internal and external morphologies of the microspheres in Fig. 5 were analyzed. The POM diagram shows that the transparent chamber in the microspheres is the internal pore after the porogen elution. The SEM images showed that Fig. 5A, E were porous network structures. Figure 5C, I show the hollow structure. Correspondingly, it can be inferred from the corresponding POM that Fig. 5E is a porous network structure, and Fig. 5G is a cavity convex porous structure. When petroleum ether is a porogen, it is a porous network of microspheres. When the n-dodecane concentration is 6 w/v%, it is a cavity convex porous microsphere (B), and when it is 12 w/v%, it is a hollow microsphere (C). With the increase of the liquid paraffin concentration, 3.0 w/v% and below are network porous microspheres (D, E, F), 12.0 w/v%

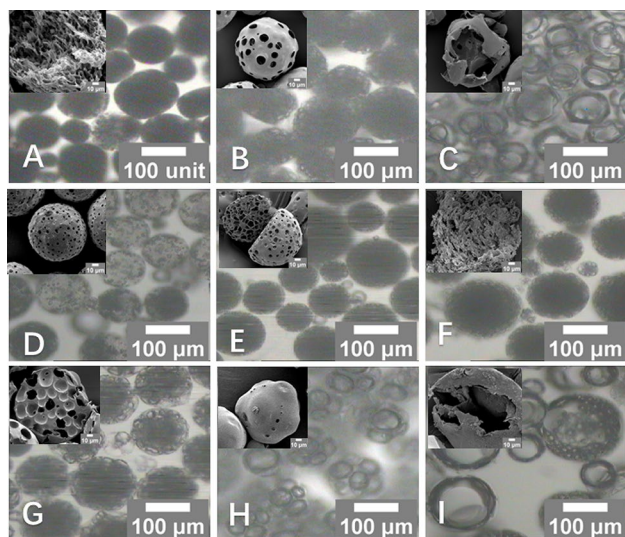


Fig. 5 POM image and SEM image of microspheres morphology by different parameters. POM images of **A** PLGA microspheres fabricated with 3.0 w/v% petroleum ether, **B–C** 6.0 w/v%, 12.0 w/v% n-dodecane respectively, **D–I** 0.75 w/v%, 1.5 w/v%, 3.0 w/v%, 6.0 w/v%, 9.0 w/v%, and 12.0 w/v% paraffin liquid respectively, **A–G** and **I** 2.0 w/v% PLGA, **H** 2.0 w/v% PLGA. The scale bar of small graphics is 10 μm, the bigger one is 100 μm

are hollow microspheres (I), and the intervening cavity is convex. Formed microspheres (G, H). At the same alkane concentration (3.0 w/v%: A and F; 6 w/v%: C and G), the chamber with more alkane carbon number is larger. This may be due to the increase in carbon number and viscosity, and the dynamic change of alkanes in emulsion droplets tends to aggregate rather than disperse.

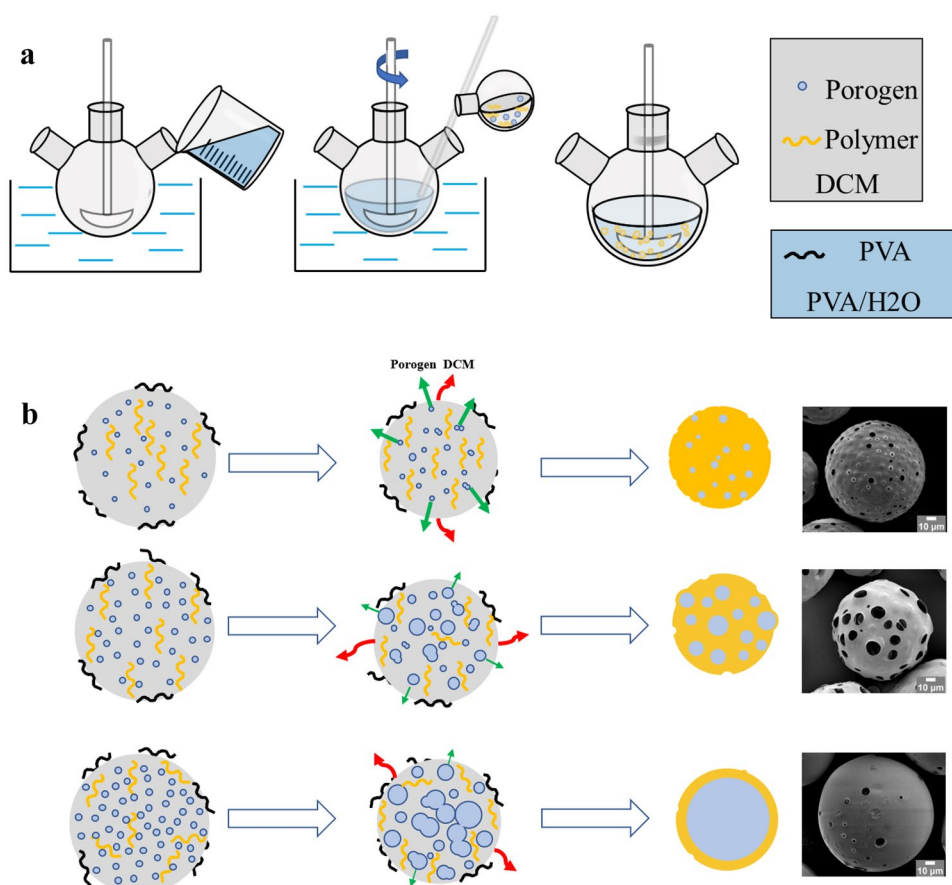
In the microsphere preparation of Fig. 6a, a large amount of water phase ($o/w \geq 1/4$ v/v) dilutes the oil phase, and mechanical stirring provides shear force to separate the oil phase into small emulsion droplets. It acts as a surfactant to stabilize emulsion droplets at the water–oil interface containing PLGA, a hydrophobic alkane porogen, and the solvent DCM. DCM has a low boiling point and can be drawn off with stirring under reduced pressure. At this stage, alkanes may also leave the emulsion droplets along with the DCM. With the volatilization of the solvent DCM, the concentration of PLGA increases, the viscosity increases [28]. Then the movement of molecular chains weakens, which is frozen in PLGA. It followed that the emulsion droplets are solidified. The shape of the microspheres is maintained until the DCM is completely volatilized in the system, and the original alkane porogen remains in the system.

Figure 6b depicts possible pathways for the formation mechanism of the hollow microspheres proposed in the current study [18, 29]. According to the type and content of porogens, in-situ porogens can be divided into three types: porous network structure, cavity protruding porous structure and hollow structure. Petroleum ether is volatile, and has a high probability of migrating from emulsion droplets to the surface phase. Because it has low viscosity and boiling point, it is easy to disperse. When n-dodecane and liquid paraffin were used as porogens, with the increase of the content, the porous network structure, the cavity-protruding porous structure and the hollow structure have successively appeared. With the increase of pore-forming agent content, the trend of pore-forming agent fusion increases. For this reason, the pore size of in-situ pore-forming chamber increases, even forming a hollow structure. When the porogen content is less than 6 w/v%, the microspheres are porous network structures, and the internal and external pore sizes are similar. Although the internal pore size cannot be uniformly controlled, the pore size range can be adjusted by alkane content.

Effect of the polymer concentration in the oil phase

It can be seen from Fig. 5I that the microspheres are single-chamber hollow microspheres when the liquid paraffin is used as the porogen concentration of 12 w/v%. In Fig. 7, the microspheres with a polymer concentration of 1 w/v% are almost all concave and do not maintain a spherical state.

Fig. 6 **a** Schematic diagrams of the process to prepare polymeric microspheres; **b** The mechanism to control the morphology of polymeric microspheres



When the concentration is increased to 2 w/v%, the concave spheres are significantly reduced, and the microspheres basically keep the microspheres three-dimensional. However, there is a slight degree of depression, and at a polymer concentration of 3 w/v%, the microspheres are spherical.

The surface smoothness is reduced and a small number of pores appear. We speculate that as the polymer concentration increases, the shell of a single microsphere becomes thicker. Therefore, the large wall thickness can provide mechanical support for a larger shell, and tends to be more

Fig. 7 SEM images of microspheres prepared with liquid paraffin as porogen at different polymer concentrations were 1 w/v%, 2 w/v%, and 3 w/v%. **A2**, **B2**, **C2**, and **D2** are the magnified images of **A1**, **B1**, **C1**, and **D1** respectively. The yellow marks in Fig. 7A2–C2 are wall thickness displays. The respective wall thicknesses of the three types of balls: **A2**: 0.426 μm, **B2**: 1.357 μm; **C2**: 2.644 μm. The scale bar is 10 μm, the bigger is 100 μm

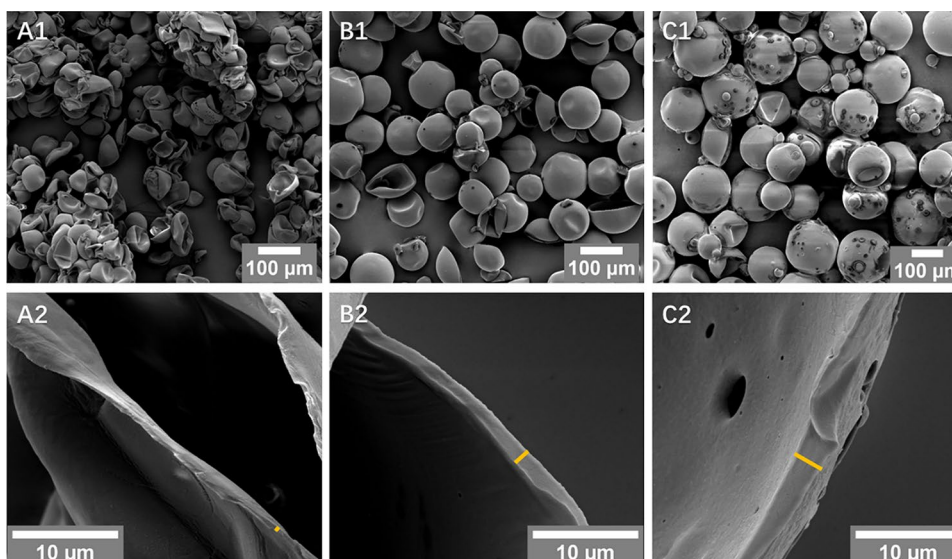
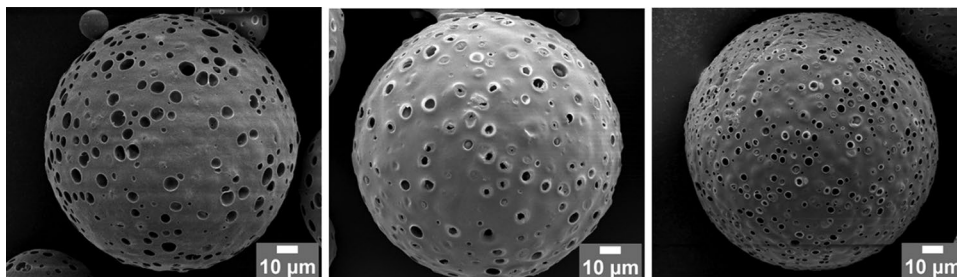


Fig. 8 SEM images of microspheres prepared by petroleum ether at different temperatures at 5 °C, 25 °C, and 35 °C. The scale bar is 10 μm



stable spherical. It can be seen that the spherical state of the microspheres requires a suitable polymer concentration, which is more suitable at 3 w/v% or more. When the content of porogen is 12wt %, it can be speculated that PLGA concentration continues to increase, the shell of microspheres is thickened and not easy to sink. In addition, SEM confirmed the existence of porosity on the surface of the microspheres, and the pore size affects the smoothness of the surface of the microspheres.

Effect of the temperature

It can be seen from Fig. 8 that when the temperature is low, the number of pores on the surface of the microspheres is less and the pore size is larger. On the other hand, the particle size of the microspheres decreases with the increase of temperature. It is speculated that the slow volatilization of petroleum ether porogen at low temperatures leads to a long stay on the surface of the microspheres. The surface of the microspheres leaves a concave hole that is in-situ porogenized by petroleum ether. In line with previous studies [30], with the increase of temperature, the volatilization rate of petroleum ether increases, and a flat hole is left on the surface of the microspheres. In addition, the increase of temperature will make petroleum ether choose more apertures to volatilize, leaving small and dense holes on the surface. The number and morphology of pores on the surface of microspheres were achieved by adjusting the temperature.

Conclusion

In this study, we demonstrated control of polymer morphology by changing parameters and porogen. The internal structure of polymer microspheres can be controlled from porous to hollow by adjusting the microsphere formulation parameters, and the number and size of pores on the surface can also be controlled. Notably, the particle size and pore size on the particle shells can be easily adjusted by changing the porogen type and content. In addition, the thickness of the hollow microspheres can be controlled by adjusting the polymer concentration, and the surface morphology of the microspheres can also be adjusted by changing the temperature.

This general approach can be extended to generate porous polymer particles for a wide range of hydrophobic polymers, alkanes, and organic solvents. These porous polymer particles with tunable structures can potentially be used for soft tissue fillers from the perspective of new applications. And biological studies of evaluating the number of substances related to collagen regeneration in follow-up studies.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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