



## Emulsion-ultrasonic spray method to prepare polylactic acid microspheres

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### ABSTRACT

Polylactic acid (PLA) microspheres are widely used in drug delivery and bone repair, among other applications. Herein, an ultrasonic spray-assisted emulsion method was developed to prepare PLA microspheres. The key factor affecting microsphere formation—duration of ultrasonic spray circulation—was discussed, and the effect of polyvinyl alcohol (PVA) concentration on microsphere morphology was evaluated. Compared with the emulsion solvent evaporation method by mechanical stirring, microsphere preparation time was shortened from 5 h to approximately 1.5 h based on ultrasonic cavitation effect, which converted the emulsion into microatomized droplets and accelerated solvent volatilization. Using this approach, microspheres formed when the PVA concentration was increased to 3 mg/mL, and the particle size decreased with increasing PVA concentration. Moreover, PLA microspheres could construct spaces in favor of cell growth. Altogether, this new method provides technical assistance for the preparation of polymer microspheres for tissue engineering.

### 1. Introduction

Polylactic acid (PLA) is a biocompatible and biodegradable polymer material with excellent mechanical properties and processability [1]. Microspheres are common forms of PLA used for drug delivery and tissue engineering scaffolds. As drug carriers, microspheres provide both a large surface area and volume ratio for spatial and temporal control over drug release [2]. Moreover, microspheres can be used alone or combined with bioactive factors or nanophase ceramics to construct three-dimensional structures as scaffolds for cell culture, which are desirable tools for tissue engineering and biological interfaces [3,4]. Additionally, microsphere-based scaffolds can be directly injected into tissue defect sites, simplifying tissue engineering methodology and reducing pain in patients [5]. Notably, microsphere particle size affects the drug release profile, and the surface properties influence cell proliferation [6,7]. Therefore, controlling microsphere size and surface properties is necessary for their biomedical application.

Various techniques have been used to produce polymer microspheres [8–11]. The emulsion solvent evaporation method is arguably the simplest to employ and can also be promptly optimized [12]. However, traditional mechanical stirring is time-consuming, and the microsphere size distribution is usually difficult to control [11]. Ultrasonic spraying is

a technology for synthesizing material with a low droplet diameter that can enhance the ability of heat and mass transfer and accelerate solvent volatilization [13].

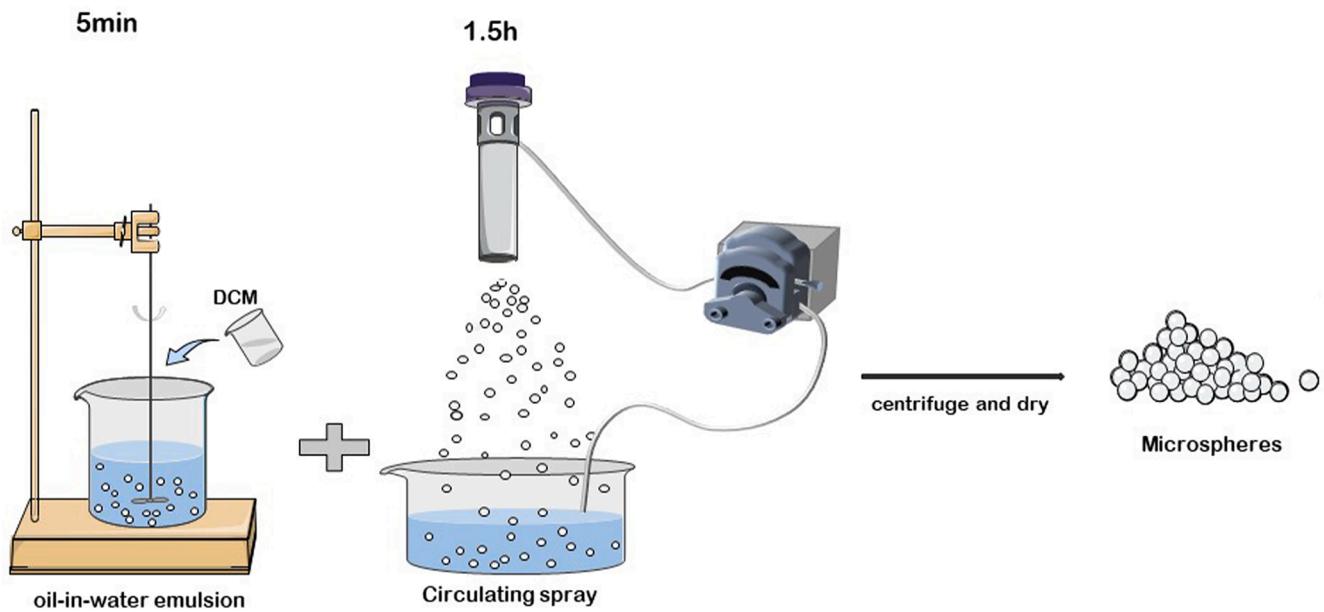
This study developed an ultrasonic spray-assisted emulsion method to overcome the problem of slow solvent evaporation by traditional mechanical stirring. The cycle spraying time was determined to control microsphere formation, and polyvinyl alcohol (PVA) concentration was used to adjust the size of particles obtained by the emulsion–ultrasonic spray method.

### 2. Experimental section

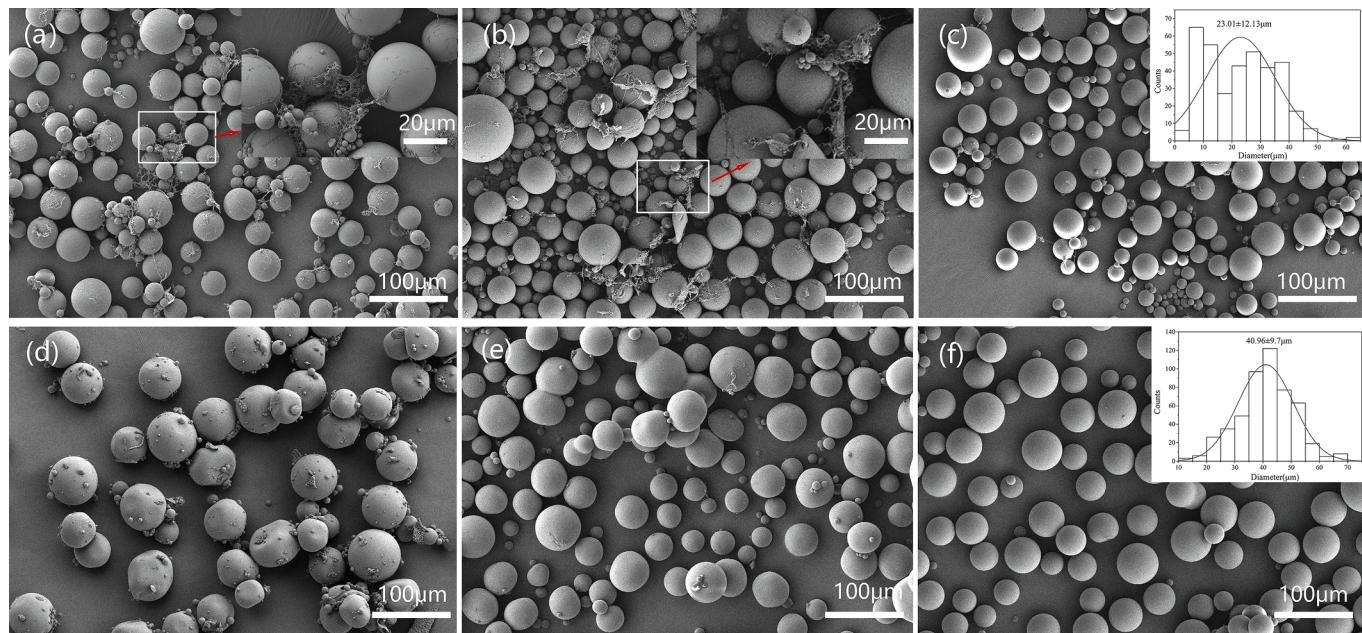
Fig. 1 schematically illustrates PLA microsphere preparation using the emulsion-ultrasonic spray method. Aqueous PVA and dichloromethane PLA solutions were mixed and mechanically stirred for 5 min at 1000 r/min. PLA concentration (30 mg/mL), oil-in-water ratio (10:1), and spray flow rate ( $1.83 \times 10^{-5} \text{ m}^3/\text{s}$ ) were determined. The mixture was then subjected to circular ultrasonic spraying to remove dichloromethane. The precipitate was washed four times with deionized water and freeze-dried for 24 h to obtain the microspheres.

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**Fig. 1.** Schematic illustration of poly(lactic acid) microspheres prepared by emulsion-ultrasonic spray method.

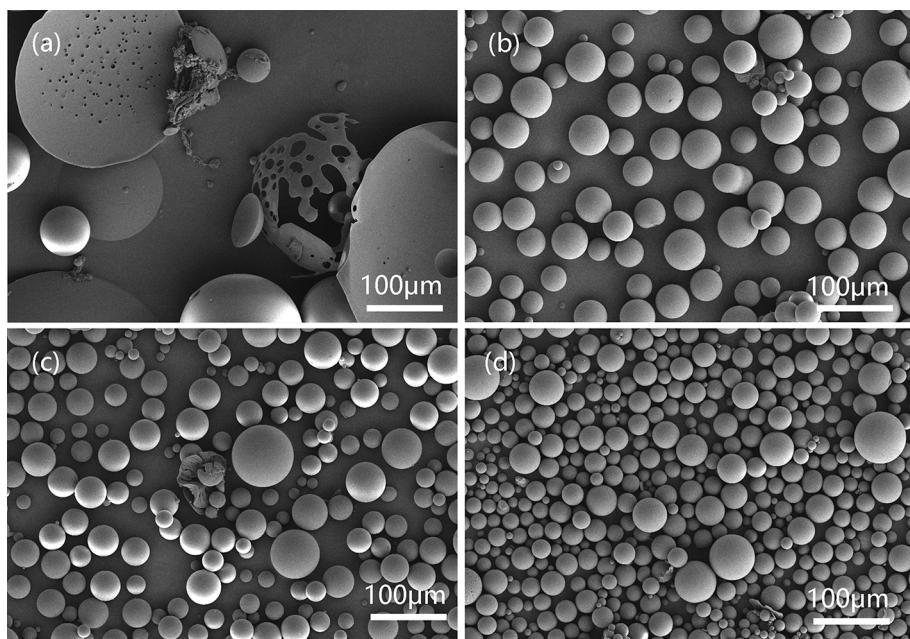


**Fig. 2.** Field-emission scanning electron microscopy images of poly(lactic acid) microspheres obtained by the emulsion method with mechanical stirring (a–c: 1.0, 3.0, and 5.0 h respectively) and ultrasonic spray (d–f: 0.5, 1.0, and 1.5 h, respectively).

### 3. Results and discussion

PLA microspheres were successfully produced by the introduction of an ultrasonic spray. Fig. 2 shows FESEM micrographs of microspheres obtained by two different methods (PVA = 3 mg/mL). The preparation methods and reaction time had noticeable effects on sphere formation rate and morphology. PLA microspheres obtained by mechanical stirring for 0.5 h showed many fibrous substances in the

mixed solution (Fig. S1). However, the solution was solidified into microspheres with a few wrinkles on their surfaces after circulating spraying for 0.5 h (Fig. 2d). After 1 h, the samples prepared by mechanical stirring (Fig. 2a) still contained unreacted fibrous substances with aggregated spheres. Ultrasonic spray accelerated microsphere solidification (Fig. 2e), and partial adhesion near the microspheres was observed, but no fibrous substance remained. After mechanical stirring for 3 h, the reaction was incomplete with obvious fibrous substance



**Fig. 3.** Field-emission scanning electron microscopy images of samples prepared with different concentrations of polyvinyl alcohol (a-d: 1, 3, 5, 7 mg/mL, respectively) synthesized by emulsion-ultrasonic spray method.

residue (Fig. 2b, c). Moreover, the microspheres did not solidify completely until 5 h, reaching a size of  $23.01 \pm 12.13 \mu\text{m}$ . When spray time reached 1.5 h (Fig. 2f), the solidification effect of the microspheres was obvious, revealing regular morphology and mean diameters of  $40.96 \pm 9.7 \mu\text{m}$ . Therefore, microspheres prepared by ultrasonic spray presented a shorter preparation time and narrower size distribution than the microspheres prepared by mechanical stirring. Differential scanning calorimeter (Fig. S3) and FT-IR (Fig. S4) analyses showed that the microspheres prepared by ultrasonic spraying were consistent with those obtained by conventional mechanical agitation.

The effect of PVA was further explored based on the determination of the ultrasonic spray preparation time. At a PVA concentration of 1 mg/mL (Fig. 3a), the obtained products consisted mostly of unreacted flocules and sherds with only minimal microsphere formation, which could be due to the small size of the spray droplets and large specific surface area. Thus, a stable emulsion could not be formed with low PVA concentration, making sphere formation difficult. Numerous regularly-shaped microspheres were formed with increasing PVA concentrations (Fig. 3b-d). Nonetheless, the mean diameter decreased slightly from  $40.96 \pm 9.7$ – $21.16 \pm 9.5 \mu\text{m}$  with 3–7 mg/mL PVA, respectively (Fig. S2). This may be due to the higher emulsifier concentration and smaller interfacial surface tension between the water and oil phases, resulting in smaller droplets [14].

As a bone repair material, low toxicity and good biocompatibility are essential for PLA microspheres. The CCK-8 result (Fig. S5) shows that PLA microspheres synthesized by the emulsion-ultrasonic spray method have great biocompatibility. Fig. 4(a-f) shows two kinds of cells differentiate in the plane direction. Compared with the control plate, cells on the surface of the microsphere scaffold adhered and

showed a larger spreading area after culturing for 24 h. Although PLA is hydrophobic, the protrusions formed on the surface of microspheres promoted cell differentiation [15]. Nile Red labeled microspheres were excited under blue and ultraviolet light, respectively (Fig. 4g-i). The stained cells and microspheres emitted bright lights of different colors simultaneously. Thereafter, we found that cells drilled into the microsphere scaffold after 1 day, and the morphology of cells between microspheres was observed by fluorescence microscope inside the microsphere scaffold. After 7 and 14 days (Fig. 4j-o), rMSCs adhered to microspheres and stretched into the space among the structures, showing a behavior similar to that seen in three-dimensional growth. ALP assessment (Fig. S6) showed that rMSCs differentiated into osteoblasts, and the positive staining area of ALP activity of the PLA samples was greater after 14 days than 7 days. Overall, rMSCs can proliferate and differentiate when seeded in PLA three-dimensional scaffolds.

#### 4. Conclusion

In this study, PLA microspheres were successfully synthesized using an emulsion-ultrasonic spray method. The preparation time was reduced from 5 h to approximately 1.5 h, and the size of the microspheres could be adjusted by changing the PVA concentration. The uniformity of the microspheres was also improved. Cell culture showed that PLA microspheres could provide a three-dimensional environment to support cell growth. In our future study, we will prepare composite microspheres, regulate their surface characteristics, and explore their effects on biological properties. Collectively, the method described herein can provide technical assistance in preparing polymer microspheres for tissue engineering.

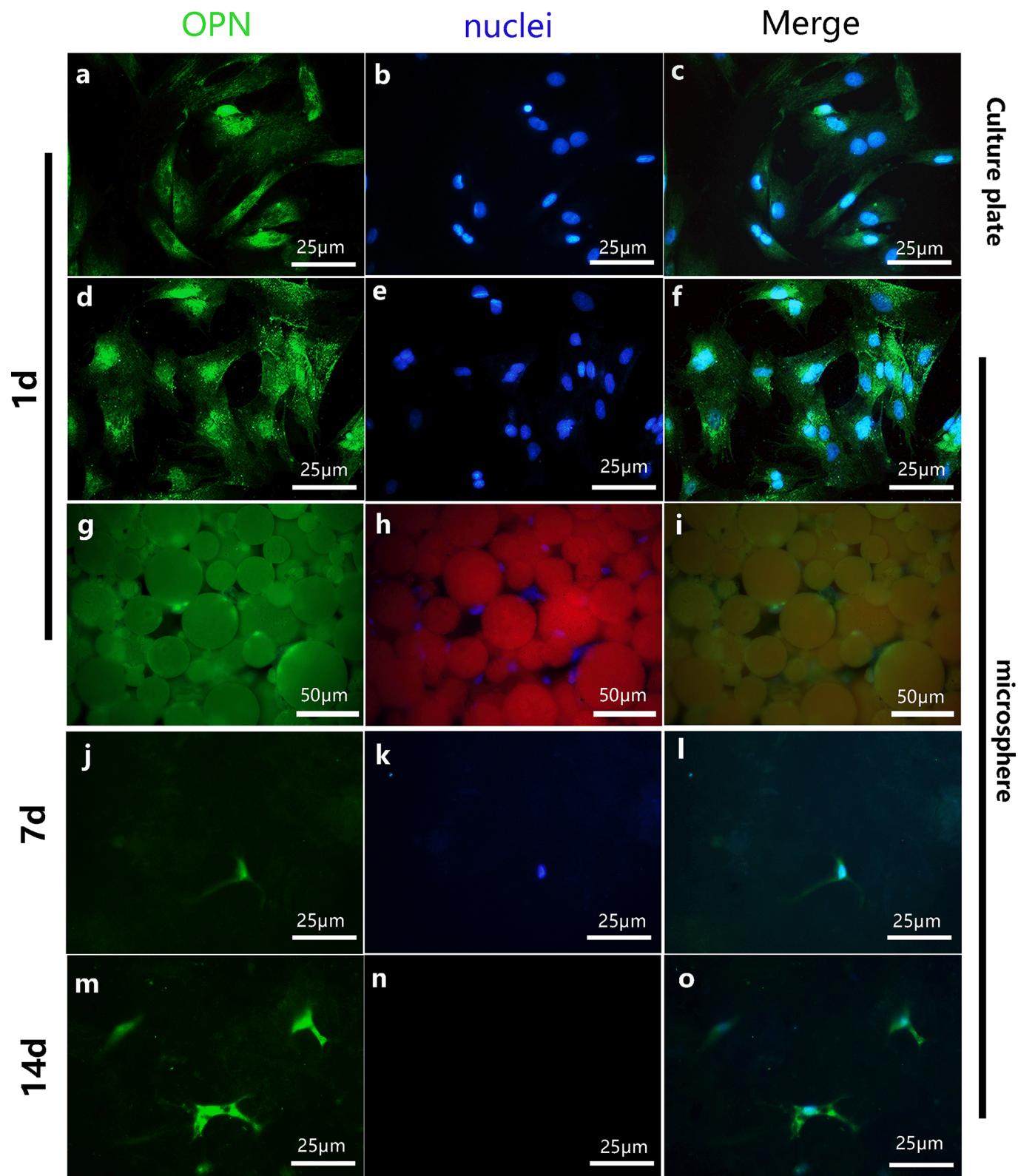


Fig. 4. Cell adhesion on culture plate and microsphere samples after culturing for 1 (a-i), 7 (j-l) and 14 days (m, o), respectively.

## CRediT authorship contribution statement

**Xiaoting Yuan:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Si Lin:** Resources. **Kang Zhao:** Resources. **Yingchao Han:** Supervision, Funding acquisition, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.matlet.2021.131461>.

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