Fabrication of poly(L-lactic acid) porous microspheres via phase inversion emulsion method

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Published in Micro & Nano Letters; Received on 22nd March 2016; Revised on 7th June 2016; Accepted on 27th June 2016

Porous microspheres capable of delivering high payloads of biomolecules with suitable biodegradability and biocompatibility would be valuable in delivery systems to aid tissue regeneration. In this work, a facile and scalable technique was induced to prepare poly(L-lactic acid) (PLLA) porous microspheres by combining an oil-in-oil surfactant-free phase inversion emulsion with thermally induced phase separation method. The morphology and property of the microspheres were investigated by scanning electron microscopy and N_2 adsorption test. Comparing to the conventional biodegradable microspheres, the nanoscale topographic structured PLLA microspheres may find wide applications in biomedical fields.

1. Introduction: In recent years, considerable efforts have been devoted to the design and controlled fabrication of porous biodegradable biopolymeric microspheres motivated by their promising biomedical application prospect, such as controlled drug delivery and tissue engineering [1–3]. The fabrication of microspheres with porous structure has attracted considerable attention due to their attractive characteristics, including large specific surface area, low density, and well-defined porosity. Gas foaming [4, 5], emulsions [6–8], spray drying [9], phase separation [10, 11], and electrospinning [12, 13] have been used to produce porous biodegradable microspheres. Especially, biodegradable microspheres can be effectively obtained using water-in-oil-in-water double-emulsion solvent evaporation method involving porogen. However, this approach is a relatively complex and time-consuming technique requiring multistep procedures to produce double emulsions and an additional processing step to remove the porogen, which limit the practical, large-scale production of porous PLGA microspheres. Furthermore, the surfactants used to stabilise emulsions often remain in the obtained microspheres, which may result in allergy-like reactions and carcinogenicity [8].

Therefore, the convenient and large-scale fabrication of porous biodegradable microspheres is highly desired for the biomedical applications. This Letter describes a facile, scalable technique to produce biodegradable porous microspheres by combining phase inversion emulsion technique with thermally induced phase separation (TIPS) method selecting poly(L-lactic acid) (PLLA) as the raw material. One-step oil-in-oil (O/O) emulsification method without any surfactants and porogens was involved to generate porous PLLA microspheres. The morphologies, size contribution, and specific surface area were especially investigated. Comparing to the conventional PLLA microspheres, this novel technique makes it more practical for the application in biomedical fields.

2. Experimental

2.1. Materials: PLLA with an inherent viscosity of 1.6 dl/g was purchased from Jinan Daigang Biological Co. Glycerol, dioxane, and dichloromethane were from Tianjin Kemiou Chemical Reagent Co.

2.2. Preparation of microspheres: An O/O surfactant-free emulsion technique combining TIPS method was used in this work. PLLA (0.2 g) was first dissolved in 10 ml of dioxane. 30 ml of glycerol was heated to 50°C and then added into the PLLA solution dropwise under stirring. Then the mixture was poured into liquid nitrogen. The solidified mixtures were following blended with ten-folds of ice water to exchange dioxane. The precipitation of PLLA microspheres was washed three times with deionised water to remove the residual dioxane. The microspheres were collected through filtration and lyophilised. For the control group, conventional PLLA microspheres were prepared via a solvent evaporation method using dichloromethane as the solvent. PLLA (0.1 g) was first dissolved in 5 ml dichloromethane and then added into the PVA (1%, 50 ml) solution at room temperature with stirring for 2 h. The stirring was continued for 6 h to evaporate the dichloromethane. Finally, the microspheres were collected after filtration and lyophilisation.

2.3. Characterisations: PLLA microspheres were examined with scanning electron microscopy (SEM) (S-3200N, Hitachi, Japan) after gold coating of 30 s. The size distribution of microspheres was determined and averaged by measuring 100 microspheres from the SEM images using the image analysis software (Nano Measurer 1.2 software). The surface area was measured by N_2 adsorption experiments at liquid nitrogen temperature on a Belsorp-Mini adsorption measuring apparatus (Bel Japan, Japan), after evacuating samples at 25°C for 10 h. Surface area of scaffolds was calculated from Brunauer–Emmett–Teller plot of adsorption/desorption isotherm using adsorption points in the P/P_0 range of 0.1–0.3 (BELSORP-mini analysis software).

3. Results and discussion: Fig. 1 shows the morphology of the PLLA microspheres prepared with different solvents (dioxane and dichloromethane). The size distributions of microspheres were presented in Figs. 1*a*3 and *b*3).

It was found that the microspheres were prepared with dioxane shows a more complicated structure and also exhibits a good size distribution. The average diameter is about 38 μm and there is no obvious aggregation within visual range. The outer surface of the microspheres shows a coarse nano-topography and numerous nano-scale coral textures (100–500 nm per unit). Note that there are many larger microholes with diameters of 1–2 μm in the interior of the microspheres. The complex micronano structure leads to a considerable specific surface area (45 m^2/g). On the contrary, the

doi: 10.1049/mnl.2016.0115

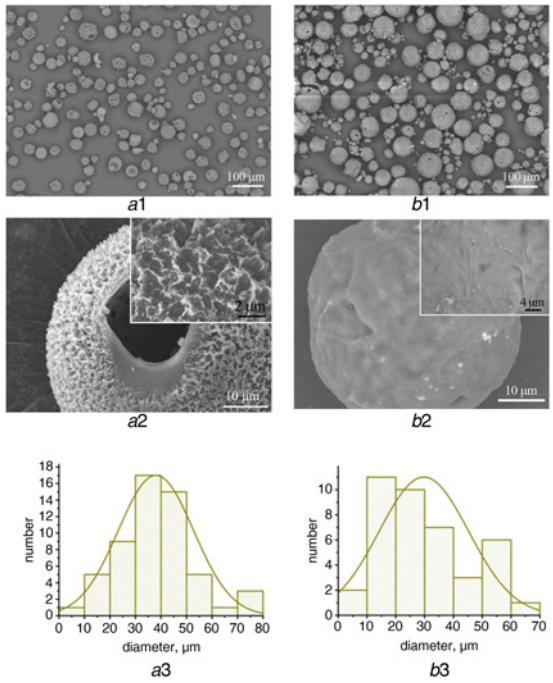

Fig. 1 *SEM photographs of PLLA microspheres prepared with different organic solvents*
a1–a3 Dioxane
b1–b3 Dichloromethane

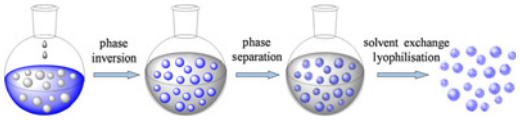

Fig. 2 *Schematic procedure to fabricate porous PLLA microspheres*

microspheres prepared with dichloromethane showed a solid internal structure and smooth surfaces, as shown in Figs. 1*b*1–*b*3. Although the microspheres prepared with dichloromethane exhibit a smaller average size (about 25 μm), the microspheres showed an uneven size distribution and the specific surface area is merely 1.2 m^2/g. In tissue engineering, since cells inevitably respond to micro, even nanoscale of materials, the nanosurface textures and high specific surface area of the microspheres is advantageous for the cell shape, orientation, growth, and differentiation.

An O/O surfactant-free emulsion technique was used to prepare porous PLLA microspheres in this work. A likely scenario of the formation of microsphere with nanotextures is as follows (as shown in Figs. 2 and 3): during the initial addition of glycerol into the PLLA/dioxane solution under stirring, the glycerol was dispersed as liquid drops (dispersed phase) in the PLLA solution. As more glycerol was added, there was a phase inversion process in which glycerol became a continuous phase and PLLA/dioxane solution became a dispersed phase. As shown in Fig. 3, under stirring,

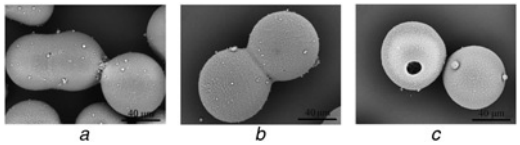

Fig. 3 *Formation process of PLLA microspheres during phase inversion*
a Distortion of PLLA microspheres under shear force
b Splitting of PLLA microspheres under shear force
c Formation of smaller PLLA microspheres

the shear force acts on the PLLA/dioxane globules and breaks up the droplets, resulting in smaller microspheres (from Figs. 3*a–c*). As the continuous phase, glycerol has a freezing point of about 20°C and has high viscosity at room temperature. The high viscosity of glycerol as continuous phase is favourable to stabilise PLLA microspheres during the initial step. As the temperature decreases to below the freezing point of glycerol, glycerol is solidified and further stabilises PLLA microspheres. At the same time, thermally induced phase separation occurs between PLLA and dioxane, and PLLA porous architecture is generated throughout the entire microsphere. As we can see in the SEM images, there are many larger microholes in the interior of the microspheres than the outer surface. This can be explained as follows. The surface of dispersed PLLA/dioxane microdroplets is first solidified due to the rapid cooling in liquid nitrogen, leading to the nanotopography on the surface of microspheres. The PLLA macromolecules inside the microdroplets continue to solidify and phase separate during the cooling process, resulting in a relatively larger porous inside. Gelation is the critical step and controls the porous morphology of the microspheres. Low gelation temperature leads to the formation of the porous structure.

4. Conclusions: A facile, scalable technique was used to produce biodegradable porous microspheres by combining phase inversion emulsion with TIPS. The microsphere consists of nanoscale topographic outer surface and internal microholes. Our method involves a surfactant-free emulsion process and overcomes the concern of residual surfactant retaining in the microspheres. The porous PLLA microspheres may act as ideal microcarriers for tissue engineering and drug delivery.

5. Acknowledgments: This research was supported by the Natural Science Foundation of Hebei Province (E2013201230), the Research Fund for the Doctoral Program of Higher Education of China (20131301120006), the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.

6 References

[1] Paik D.H., Choi S.W.: 'Entrapment of protein using electrosprayed poly(D,L-lactide-Co-glycolide) microspheres with a porous structure for sustained release', *Macromol. Rapid Commun.*, 2014, **35**, (11), pp. 1033–1038
[2] Xu Q.X., Leong J.Y., Chua Q.Y., *ET AL.*: 'Combined modality doxorubicin-based chemotherapy and chitosan-mediated P53 gene therapy using double-walled microspheres for treatment of human hepatocellular carcinoma', *Biomaterials*, 2013, **34**, (21), pp. 5149–5162
[3] Chung H.J., Park T.G.: 'Surface engineered and drug releasing pre-fabricated scaffolds for tissue engineering', *Adv. Drug Deliv. Rev.*, 2007, **59**, (4–5), pp. 249–262
[4] Zhao C., Chen A.Z., Wang S.B., *ET AL.*: 'Preparation of Mtx-Plla porous microspheres by a liquid Co2/organic solvent removing process', *J. Control. Release*, 2013, **172**, (1), pp. E20–E20
[5] Chen A.Z., Dang T.T., Wang S.B., *ET AL.*: 'The in vitro and in vivo anti-tumor effects of mtx-Fe3o4-Plla-Peg-Plla microspheres prepared by suspension-enhanced dispersion by supercritical Co2', *Sci. Chin.-Life Sci.*, 2014, **57**, (7), pp. 698–709
[6] Chen A.Z., Zhao C., Wang S.B., *ET AL.*: 'Generation of porous poly-L-lactide microspheres by emulsion-combined precipitation with a compressed Co2 antisolvent process', *J. Mater. Chem. B*, 2013, **1**, (23), pp. 2967–2975
[7] Hu Y., Zou S.W., Chen W.K., *ET AL.*: 'Mineralization and drug release of hydroxyapatiteipoly(L-Lactic Acid) nanocomposite scaffolds prepared by pickering emulsion templating', *Colloids Surf. B Biointerfaces*, 2014, **122**, pp. 559–565
[8] Hu Y., Zou S., Yang Y., *ET AL.*: 'Facile fabrication of macroporous Plga microspheres via double-pickering emulsion templates', *Macromol. Chem. Phys.*, 2015, **216**, (7), pp. 714–720
[9] Xu J.Q., Jiao Y.P., Shao X.H., *ET AL.*: 'Controlled dual release of hydrophobic and hydrophilic drugs from electrospun poly (L-Lactic acid) fiber mats loaded with chitosan microspheres', *Mater. Lett.*, 2011, **65**, (17-18), pp. 2800–2803

[10] Blaker J.J., Knowles J.C., Day R.M.: 'Novel fabrication techniques to produce microspheres by thermally induced phase separation for tissue engineering and drug delivery', *Acta Biomater.*, 2008, **4**, (2), pp. 264–272
[11] Go D.P., Nicolin Tirtaatmadja D.J.E.H., Gras S.L., *ET AL.*: 'A simple, scalable process for the production of porous polymer microspheres by ink-jetting combined with thermally induced phase separation', *Part. Part. Syst. Charact.*, 2014, **31**, (6), pp. 685–698
[12] Qiu K., He C., Feng W., *ET AL.*: 'Doxorubicin-loaded electrospun poly (L-lactic acid)/mesoporous silica nanoparticles composite nanofibers for potential postsurgical cancer treatment', *J. Mater. Chem. B*, 2013, **1**, (36), pp. 4601–4611
[13] Bock N., Woodruff M.A., Hutmacher D.W., *ET AL.*: 'Electrospraying, a reproducible method for production of polymeric microspheres for biomedical applications', *Polymers*, 2011, **3**, (1), pp. 131–149

doi: 10.1049/mnl.2016.0115