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Non-spherical racemic polylactide microarchitectures formation via solvent evaporation method

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ABSTRACT

Usually, amphiphilic or crystalline structure is necessary to prepare nano- or micro-structured materials with various morphologies. Racemic random polylactide (PDLLA) is completely amorphous in nature and was mainly prepared to be of spherical form for the use of sustained drug release. In this paper, we observed novel lath-, sheaf-like morphologies of PDLLA microarchitectures fabricated by the double emulsion-solvent evaporation method in the presence of glycerol or epirubicin. These non-spherical microarchitectures coexisted with amorphous PDLLA microspheres and crystallized poly(vinyl alcohol) (PVA) nanoparticles. On the basis of our experiments, the formation of lath-like microarchitectures is due to glycerol, while the formation of sheaf-like microarchitectures is owing to both glycerol and epirubicin. Therefore, novel assemblies of PDLLA have been developed and the formation mechanism is different from those of well-defined amphiphilic or crystalline polymers. These PDLLA non-spherical microarchitectures might have potential application as non-spherical carriers in drug delivery system.

1. Introduction

Polylactide (PLA), a biocompatible, biodegradable chiral aliphatic polyester, has been widely studied for medical uses as well as the industrial applications as a "green plastic" [1,2]. The stereoregular poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA) are crystalline, while the racemic random polylactide (PDLLA) is completely amorphous in nature, these polymers are simply called PLA all together [3]. PLA spheres as drug carriers were fabricated in ease by means of direct dialysis or emulsion-solvent evaporation [4,5]. The studies of non-spherical microarchitectures of PLA were focused on lactic acid-based amphiphilic block copolymers, PLA stereocomplex and PLLA due to their amphiphilic or crystalline properties [3,6–9]. Fabrication of non-spherical microarchitectures from amorphous PDLLA still remains a great challenge.

Self-assembly of block or graft copolymers with various morphologies to construct interesting materials for their potential applications has attracted increasing attention recently, such as spherical micelles, worm-like micelles, vesicles, flower-like aggregates, and nanotubes [10–17]. Besides that primary structures and the properties of block or graft copolymers dominate the self-assembling processes, recent research has further explored various additional external influences on self-assembly of polymers, including small organic molecules, inorganic acids, artificial chaperones, shear and surface confinement [18–25]. However, amphiphilic or crystalline structure of polymers is necessary to assemble various microarchitectures in general. The above results inspire us to investigate assemblies of biodegradable amorphous polymers such as PDLLA assisted by small guest molecules due to the superiority of amorphous polymers for drug formulations [26].

In drug delivery system, emulsion-solvent evaporation technique is prevalent to prepare polymeric spheres [5,27–29]. Poly(vinyl alcohol) (PVA) is one of the most commonly used surfactants to stabilize the dispersed phase in emulsion. Moreover, PVA can be crystallized from its dilute solution by the flow-induced crystallization [30], which is often neglected for the preparation of polymeric spheres in drug delivery system owing to only emphasis on the residual amount assay on the surface of particles. Additionally, glycerol was added to the double emulsion system instead of the traditional stabilizer for the preparation polymeric

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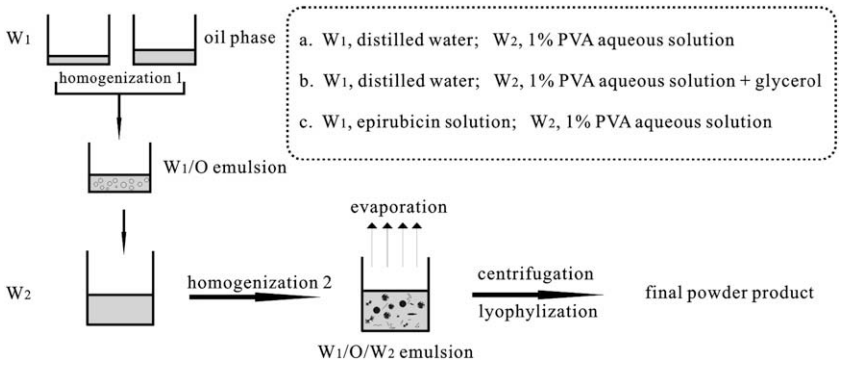


Scheme 1. Schematic illustration for the preparation procedure of PDLLA microarchitectures by double emulsion-solvent evaporation method.

nanocapsules because of some advantages such as high viscosity, polyhydroxyl structure, and nontoxicity [27]. The initial release of insulin from poly(lactic-*co*-glycolic acid) (PLGA) microspheres was able to be controlled strictly by means of adding glycerol in the primary methylene chloride dispersion [28]. Epirubicin is an anthracycline antibiotic and antitumor epimer of doxorubicin which has been used to treat a wide range of cancers (its structure is shown in Scheme 2). At equimolar doses epirubicin is less myelotoxic than doxorubicin and has a lower incidence of cardiotoxicity [31,32]. Previously, PLLA and PLGA spheres were prepared by solvent evaporation technique in the presence of epirubicin [33,34]. Recently, non-spherical microarchitectures of crystalline homopolymers or amphiphilic block copolymers were assembled using emulsion-solvent evaporation method by means of additional external effect, such as PLLA short fibers [9], PDLLA-*block*-monomethoxy poly(ethylene glycol) (PDLLA-*block*-mPEG) cylindrical microparticles [22], isotactic poly([*R*]-3-hydroxybutyric acid)-*block*-mPEG (PHB-*block*-mPEG) sheaf- and star-like microarchitectures [23]. However, the formation of non-spherical microarchitectures of PDLLA induced by glycerol or epirubicin as small guest molecules through emulsion-solvent evaporation method has still not been reported up to date.

Herein, we report some novel PDLLA lath- and sheaf-like non-spherical microarchitectures coexisted with microspheres which were fabricated by the double emulsion-solvent evaporation method in the presence of glycerol or epirubicin. Epirubicin hydrochloride was chosen as a kind of model drug due to sustained drug release as our primary purpose and its similar polyhydroxyl structure with glycerol. The non-spherical microarchitectures originated from PDLLA were demonstrated and the formation mechanisms of non-spherical and spherical microarchitectures were proposed. The novel PDLLA non-spherical microarchitectures have potential application as drug carriers due to the effects of shape on biological systems at the nanoscale [35] and the good biocompatible property of PDLLA.

2. Experimental section

2.1. Materials

PDLLA with molecular weight of M_n 56,000 and 5000 were purchased from the Institute of Medical Instrument of Shandong Province (PR China). Epirubicin hydrochloride was purchased from Zhejiang Hisun Pharmaceutical Company (PR China). Poly(vinyl alcohol) (PVA, degree of polymerization 500, degree of hydrolysis 88%) was kindly supplied by Sinopec Sichuan Vinylon Works (PR China). All other reagents were of analytical grade and used as received.

2.2. Preparation of PDLLA microarchitectures

PDLLA microarchitectures were fabricated by the double emulsion-solvent evaporation method (Scheme 1). The main experimental conditions and corresponding morphologies of polymeric microarchitectures are summarized in Table 1.

2.2.1. PDLLA blank microspheres

Typically, 1 mL of distilled water (W_1) was emulsified with 10 mL of methylene chloride solution of 200 mg of PDLLA containing the complex of sorbitan monooleate (Span-80, 2.5%) and polyoxyethylene sorbitan monooleate (Tween-80, 12.5%) by homogenizer at 10,000 rpm for 30 s (homogenization 1 illustrated in Scheme 1). The prepared water in oil system (W_1/O) was poured immediately into the external aqueous phase (W_2) of 120 mL of 1% PVA aqueous solution, and the whole system was homogenized at 6000 rpm for 5 min (homogenization 2 illustrated in Scheme 1) to form the $W_1/O/W_2$ system. The formed $W_1/O/W_2$ was continued to stir on a magnetic stir plate in the beaker for 18 h under ambient conditions to allow complete methylene chloride evaporation. The produced particles were collected with centrifugation at 20,000 rpm and washed 3 times with distilled water and lyophilized (Scheme 1a). The final powder product was stored at 4 °C.

2.2.2. Preparation of PDLLA microarchitectures in the presence of glycerol or epirubicin

Desired amount of glycerol or epirubicin was added into the external aqueous phase or the inner aqueous phase, respectively.

Table 1
Main experimental conditions and corresponding morphologies of polymeric microarchitectures. Epirubicin is abbreviated to EPI.

PDLLA ($M_n \times 1000$)	Inner aqueous phase	Ratio of glycerol to PVA solution	Types of observed polymeric microarchitectures
56	Distilled water	0	Spheres
56	Distilled water	1:5	Sheaves, laths, spheres
56	Distilled water	1:4	Sheaves, star-like aggregates, spheres
56	Distilled water	1:2	Sheaves, hexapod aggregates, spheres
56	Distilled water	1:1	Sheaves, laths, spheres
56	5 mg mL^{-1} EPI	0	Sheaves, spheres
56	10 mg mL^{-1} EPI	0	Sheaves, spheres
56	20 mg mL^{-1} EPI	0	Sheaves, spheres
5	5 mg mL^{-1} EPI	0	Sheaves, spheres
5	Distilled water	1:5	Nanofilaments, sheaves, hexapod aggregates, spheres
0	Distilled water	1:5	PVA nanoparticles
0	10 mg mL^{-1} EPI	0	PVA nanoparticles

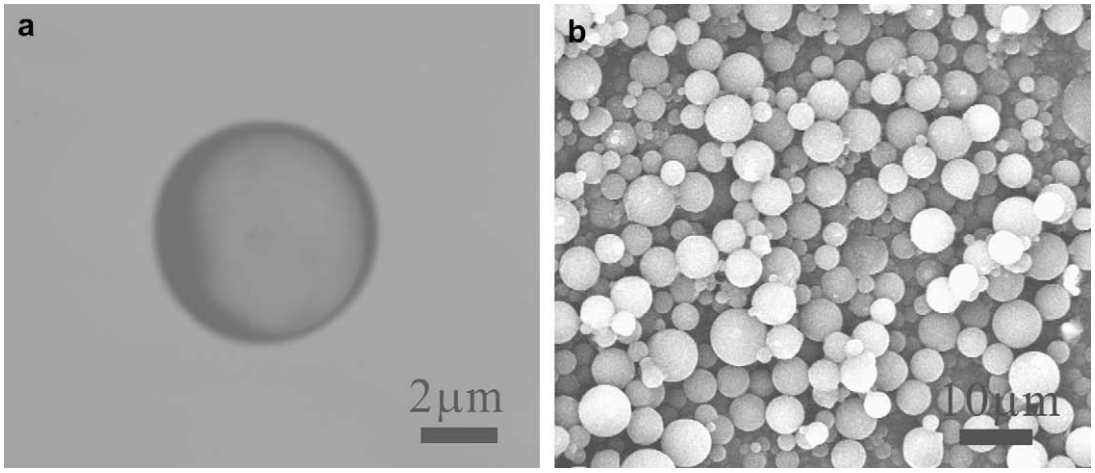


Fig. 1. TEM (a) and SEM images (b) of PDLLA blank microspheres fabricated by the double emulsion-solvent evaporation method.

Other parameters were the same as the above blank experiment (Scheme 1b and c).

2.3. PVA nanoparticles crystallized in the presence of glycerol or epirubicin

The procedures for these control experiments were the same as above-mentioned. Only PDLLA was not added into the oil phase and desired amount of glycerol or epirubicin was added into the external or inner aqueous phase, respectively. The experimental conditions and corresponding results are shown in Table 1.

2.4. Transmission electron microscopy (TEM)

For TEM (Hitachi H600) and high-resolution TEM (HRTEM, TECNAI F-30), a drop of diluted aqueous suspension of microparticles was deposited on copper grids and air dried prior to observation. Copper grids were coated with a thin film of Formvar (for H600) or carbon film (for F30), respectively.

2.5. Scanning electron microscopy (SEM)

For field emission scanning electron microscopy (SEM, LEO-1530), a drop of the sample suspension was placed on a silicon wafer. After drying, the sample was coated with gold by using Ion Sputter (JEOL, JFC-1100). Coating was provided at 20 mA for 25 s. Observation with SEM was performed at 20 kV.

2.6. Confocal laser scanning microscopy (CLSM)

For confocal laser scanning microscopy (CLSM, Leica TCS SP2 SE), a drop of the sample suspension was placed on a glass slide. After drying, epirubicin in the PDLLA microarchitectures was excited with a 488 nm argon laser and emission lines were collected after passage through a DD 488/543 filter in a spectral window ranging from 515 nm to 600 nm.

2.7. X-ray diffraction analysis (XRD)

An X-ray powder diffractometer (Philips PANalytical X'Pert) equipped with Cu Kα radiation ($\lambda = 1.54178$ Å) over the 2θ range of 5–60° was used to characterize the structure of the raw materials of PVA.

2.8. FTIR spectroscopy

IR spectra were recorded on a Nicolet Avatar 360 FTIR spectrometer in the transmission mode. Samples were analyzed as powders by preparing KBr pellets.

3. Results and discussion

3.1. PDLLA blank microspheres

Fig. 1 shows the typical TEM and SEM images of PDLLA blank microspheres. We observed that spheres are the sole form in the blank experiment. The size of these microspheres is about 2–5 μm predominantly and the distribution of size is not uniform. The results indicate that we fabricated PDLLA blank microspheres which are in accordance with previous literatures [5,29].

3.2. Effect of glycerol on PDLLA microarchitectures

Fig. 2 displays the typical TEM and SEM images of the various morphologies of PDLLA microarchitectures when different amounts of glycerol were added in the external aqueous phase. Fig. 2a shows the TEM image of the product when the ratio of glycerol to PVA aqueous solution was 1:5, which displays the coexistence of lath-, sheaf-like microarchitectures and microspheres, even more lath- or sheaf-like microarchitectures than microspheres in the same grid. The lath-like aggregates are approximately 100 nm in width and 400–1500 nm in length. However, we did not observe lath- or sheaf-like non-spherical microarchitectures except microspheres using SEM (Fig. 2b). By increasing the ratio of glycerol to PVA aqueous solution to 1:4, the lath-like assemblies of PDLLA are still dominant and most of them tend to aggregate to star-like microarchitectures (Fig. 2c). Besides the lath- and star-like morphologies, the sheaf-like is also observed in the product (Fig. 2c inset). By further increasing the ratio of glycerol to PVA to 1:2, we observed more sheaf-like morphologies of PDLLA (Fig. 2d) and regular hexapod aggregates (Fig. 2d inset).

The viscosity of the system is further investigated by Ubbelohde viscosity technology. When the ratios of glycerol to PVA aqueous solution changed from 1:5 to 1:1, the flow times ranged from 348.8 s to 1255.6 s at 25 °C. The viscosity of the system increases with increasing the amount of glycerol. Careful examination of sheaf-like microarchitectures shown in TEM images suggests that these microarchitectures were in fact assembled from well-aligned individual nanofilaments. Sheaf-like microarchitectures spontaneously

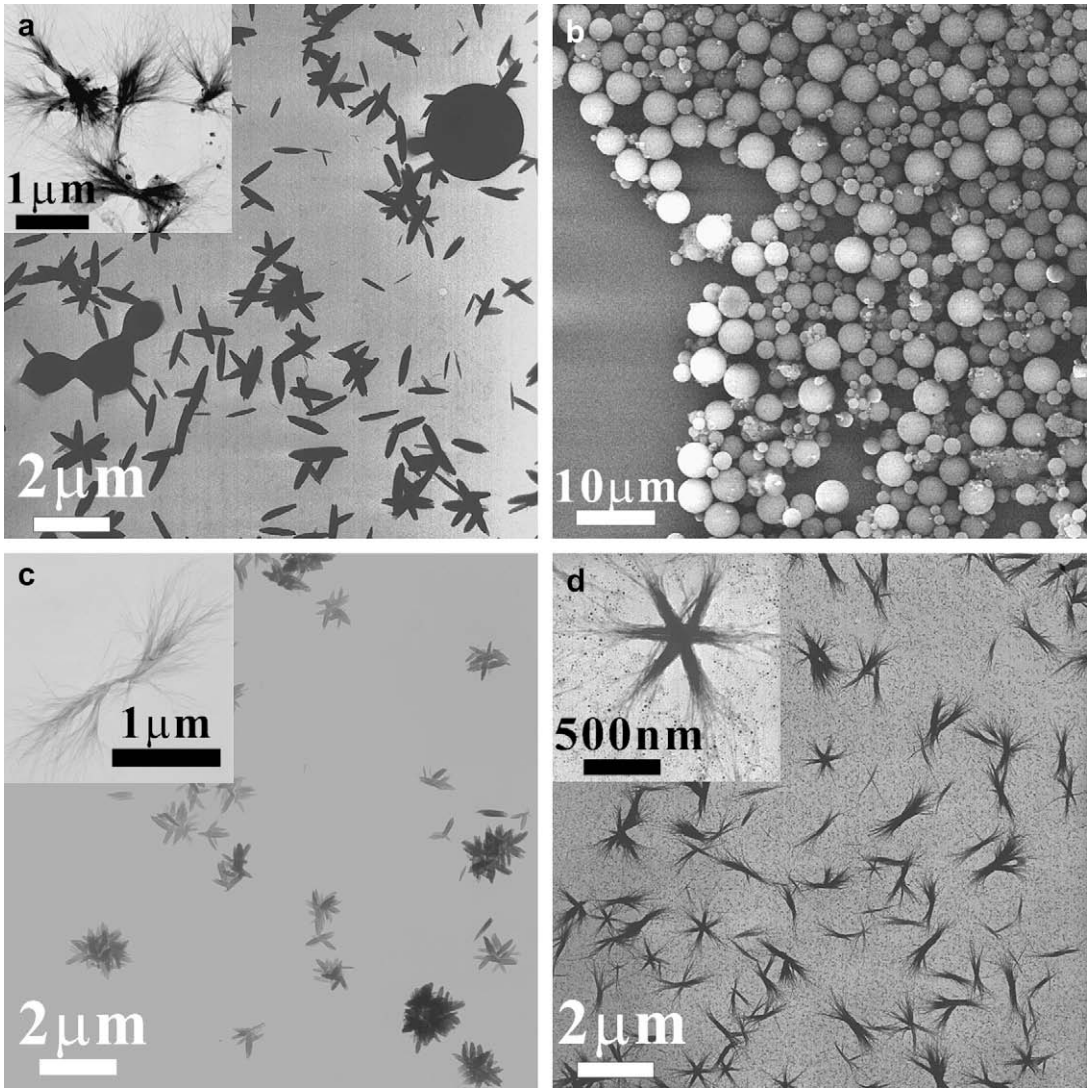


Fig. 2. TEM (a) (c) (d) and SEM (b) images of PDLLA microarchitectures fabricated by the double emulsion-solvent evaporation method with different ratios of glycerol to PVA aqueous solution (v/v) in the external aqueous phase, only 1 mL of distilled water was used without adding epirubicin in the inner aqueous phase. (a), (b) 1:5. (c) 1:4. (d) 1:2.

aggregated to the regular hexapod assemblies by increasing the viscosity of the system further. Similarly, microarchitectures ranged from lath- to star-like aggregates accordingly. Therefore, we conclude that glycerol not only can be a stabilizer in double emulsion system but also may control PDLLA assembly morphologies because assembly of polymer can be assisted by additional external small molecules and viscosity effect [18,19,22,23].

3.3. Effect of epirubicin on PDLLA microarchitectures

Fig. 3 shows the typical TEM and CLSM images of the sheaf-like morphology of PDLLA microarchitectures when various concentrations of epirubicin (5, 10 and 20 mg mL^{-1}) were added into the inner aqueous phase. The sheaf-like microarchitectures are ca. 1–3 μm in length. Fig. 3c reveals that the microarchitectures consist of several sheaves in comparison with Fig. 3b in size and the sheaf-like microarchitectures coexisted with microspheres in the product. Since PLA is not fluorescent, the bright colored area qualitatively represents epirubicin, the larger size sheaf-like microarchitectures is selected for clarity. It is clear that the distribution of epirubicin in sheaves is uniform. Zhang et al. reported that the presence of carboxylic group in hydrophobic drug molecules seemed to be a prerequisite for morphology variation of amphiphilic copolymer assemblies [19]. Therefore, we believe that epirubicin is not only a kind of good model drug for sustained drug release but also induces the assembly of PDLLA due to its special polyhydroxyl structure.

3.4. Effect of molecular weight of PDLLA on microarchitectures

In order to verify the generality of the above results (PDLLA, M_n 56,000), we also investigated the assembly of PDLLA with molecular weight M_n 5000. Fig. 4a–c shows a series of TEM images of PDLLA (M_n 5000) microarchitectures. As is shown, nanofilaments, sheaf-like microarchitectures, even hexapod aggregates of PDLLA could be formed when the volume ratio of glycerol to PVA aqueous solution was 1:5. The average diameter of the nanofilaments about 10–15 nm is in good agreement with that of the arms of the sheaf-like microarchitectures. This series of images verifies visually our assumption that sheaf-like microarchitectures were assembled from well-aligned individual nanofilaments. Moreover, the viscosity of the system is in favour of the formation of the regular hexapod aggregates from sheaf-like microarchitectures spontaneously. Fig. 4d shows that the sheaf-like microarchitectures of PDLLA

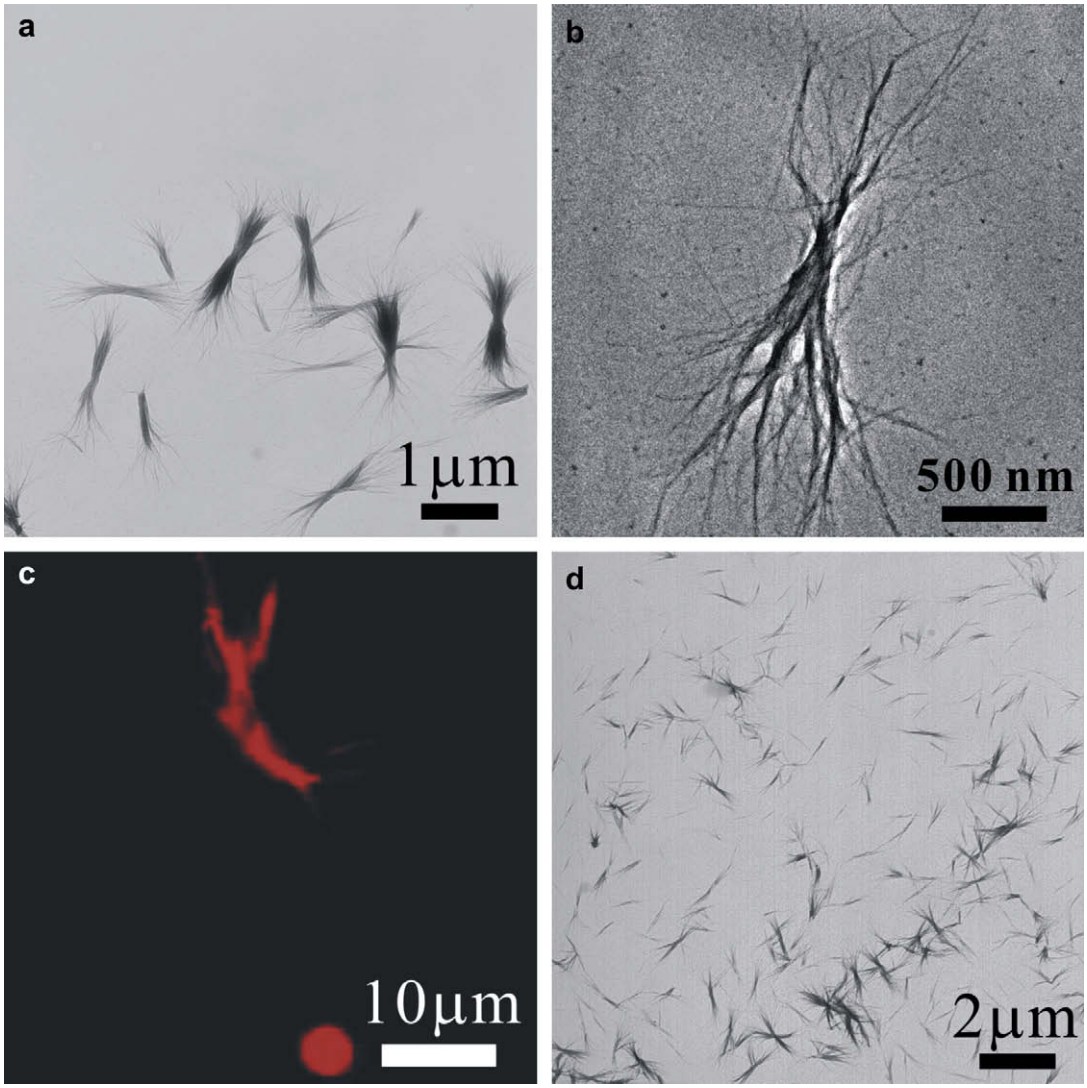


Fig. 3. TEM (a), (b), (d) and CLSM (c) images of PDLLA microarchitectures containing epirubicin fabricated by the double emulsion-solvent evaporation method without glycerol in the external aqueous phase. The concentrations of epirubicin were as follows: (a) 5 mg mL^{-1}; (b), (c) 10 mg mL^{-1}; (d) 20 mg mL^{-1}.

(M_n 5000) were also assembled when 1 mL of epirubicin solution (5 mg mL^{-1}) was as the inner aqueous phase. As a result we believe that glycerol or epirubicin might induce amorphous PDLLA to form non-spherical microarchitectures.

3.5. Structure characterization

To investigate the structure of these assemblies, HRTEM was used to characterize the lath- and sheaf-like microarchitectures which were prepared with the ratio of glycerol to PVA aqueous solution as 1:4. Fig. 5a–c and d–f is the TEM and HRTEM images of lath- and sheaf-like microarchitectures, respectively. In Fig. 5a and d, lots of nanoparticles of ca. 5 nm were absorbed on the surface of lath- and sheaf-like microarchitectures. The lattice distance is measured to be 0.244 and 0.257 nm (Fig. 5c) and that shown in Fig. 5f is 0.217, 0.244 and 0.257 nm. Fig. 5b shows crystal lattice and amorphous state. In addition, the lath- (data not shown) and sheaf-like polymeric microarchitectures were destroyed by electron beam in HRTEM observation (Fig. 5e). The results indicate that the microarchitectures containing nanoparticles were composed of amorphous and crystalline substances.

HRTEM characterizations of sheaf-like polymeric microarchitectures induced by epirubicin (Fig. 6) revealed that their structure and composition were similar to those induced by glycerol. Fig. 6a shows that many nanoparticles were absorbed on the surface of sheaf-like microarchitectures or scattered around them. The lattice distance is also 0.217, 0.244 and 0.257 nm (Fig. 6b). The microarchitectures were destroyed by electron beam after HRTEM analysis (Fig. 6c). That is to say, the sheaf-like microarchitectures absorbed with nanoparticles also might be consisted of different amorphous and crystallized substances.

Because PVA can be crystallized through stirring its dilute solution [30], we did the control experiments in the absence of PDLLA. Fig. 7a shows the typical TEM image of PVA nanoparticles with ca. 50 nm diameter when glycerol was added in the external aqueous phase with the volume ratio of glycerol to PVA aqueous solution as 1:5. By further magnification, we observed smaller nanoparticles with ca. 5 nm diameter absorbed on the surface (Fig. 7b), which are consistent with those nanoparticles shown in Fig. 5a and d in size. Fig. 7d and e shows that the same shape and size of nanoparticles formed when only epirubicin was added in the inner aqueous phase. The lattice distances shown in Fig. 7c and f are measured to be 0.217, 0.244 and 0.257 nm.

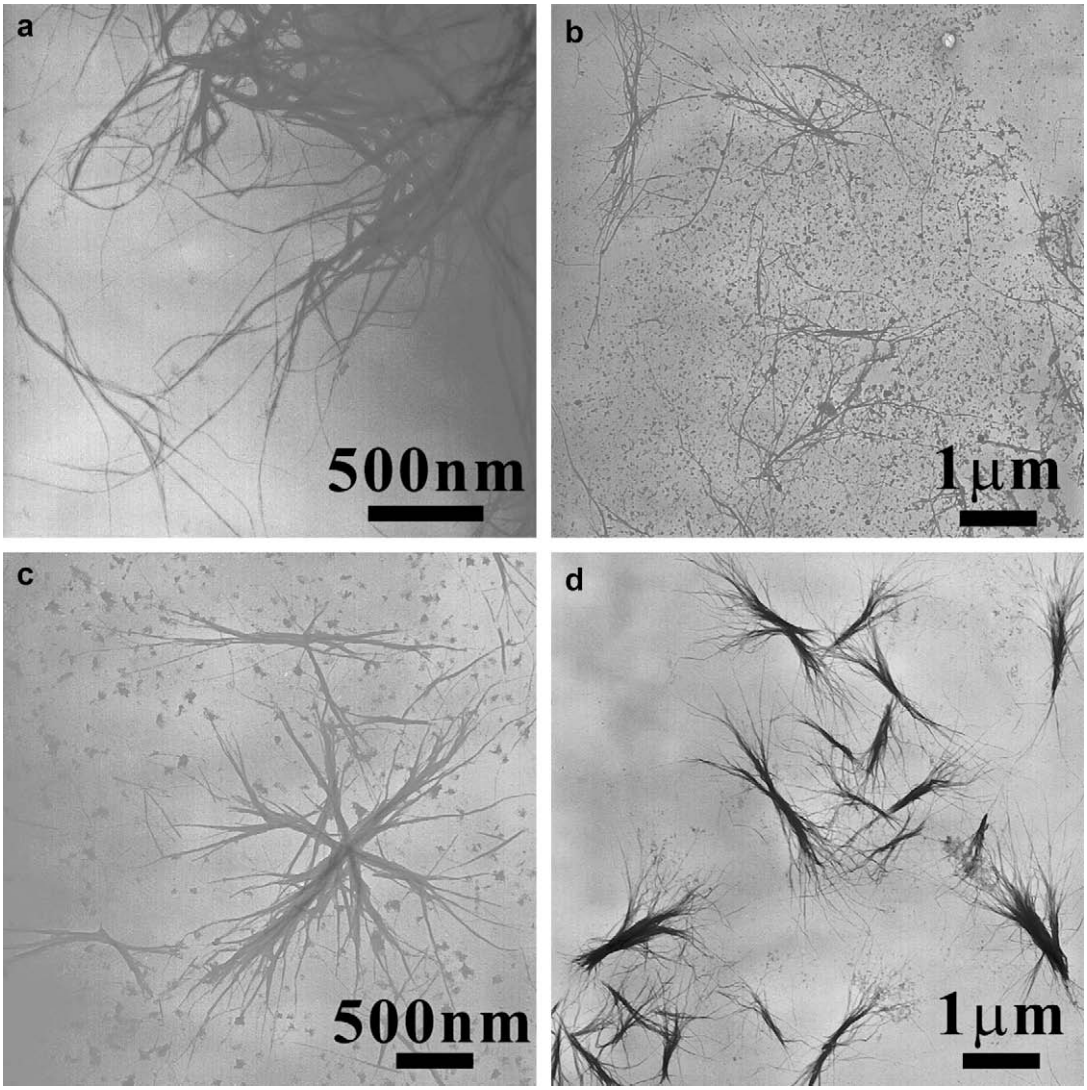


Fig. 4. TEM images of PDLLA (M_n 5000) microarchitectures fabricated by the double emulsion-solvent evaporation method. From nanofilaments (a) to sheaf-like microarchitectures (b), to hexapod aggregates (c), the ratio of glycerol to PVA aqueous solution in the external aqueous phase was 1:5, only 1 mL of distilled water was used without adding epirubicin in the inner aqueous phase; (d), sheaf-like microarchitectures, 1 mL of epirubicin solution (5 mg mL^{-1}) was used as the inner aqueous phase without glycerol in the double emulsion system.

Fig. 8 shows a typical XRD pattern of the raw material of PVA. There are three diffraction peaks located at $2\theta = 19.6°$, 23.5° and 41.1°, which are in agreement with the lattice distance measured in Fig. 7c and f and the lattice distance shown in Fig. 5c and f and Fig. 6b. In the control experiments we did not observe the lath- or sheaf-like microarchitectures in the absence of PDLLA. Based on the above results we verify that the lath- and sheaf-like microarchitectures are amorphous PDLLA and the crystallized nanoparticles absorbed on or around these non-spherical microarchitectures are PVA.

3.6. Hydrogen bond formation

IR spectroscopy is used to investigate the intermolecular and intramolecular interactions in polymers. Fig. 9 presents IR spectra of PDLLA, PDLLA microarchitectures induced by glycerol or epirubicin. Several characteristic bands of PDLLA are located at 753 and 862 cm^{-1} (CH bend); 1050, 1088, 1134, 1191 and 1270 cm^{-1} (=C–O stretch); 1383 cm^{-1} (CH_2 wag); 1456 cm^{-1} (CH_3 bend); 1760 cm^{-1} (C=O stretch, ester group); 2945 cm^{-1} (CH stretch); 2995 cm^{-1} (CH_3 stretch); 3508 and 3668 cm^{-1} (OH stretch, end group). It can be observed that several changes occur in the spectra of PDLLA microarchitectures induced by glycerol or epirubicin in comparison with the spectrum of PDLLA. For PDLLA microarchitectures induced by glycerol, the original bands of PDLLA at 3508 and 3668 cm^{-1} for OH stretch, 1191 cm^{-1} for carboxyl stretch are shifted to 3492, 3650 and 1185 cm^{-1}, respectively. While for PDLLA microarchitectures induced by epirubicin, the band of PDLLA at 3508 cm^{-1} disappears and the bands at 3668, 1191 cm^{-1} are shifted to 3651, 1185 cm^{-1}. Moreover, it can be observed that the bands centered near 3500 cm^{-1} become wider pronouncedly in the spectra of PDLLA microarchitectures induced by glycerol or epirubicin.

To observe the changes of the C=O stretch more clearly, the corresponding IR spectra with higher resolution are provided in Fig. 10. The original strong band of PDLLA at 1760 cm^{-1} for the ester group becomes significantly wider and is shifted to 1759 or 1751 cm^{-1} for PDLLA microarchitectures induced by glycerol or by epirubicin, respectively. These registered events indicate that there are obviously hydrogen bonds formation between hydroxyl (in

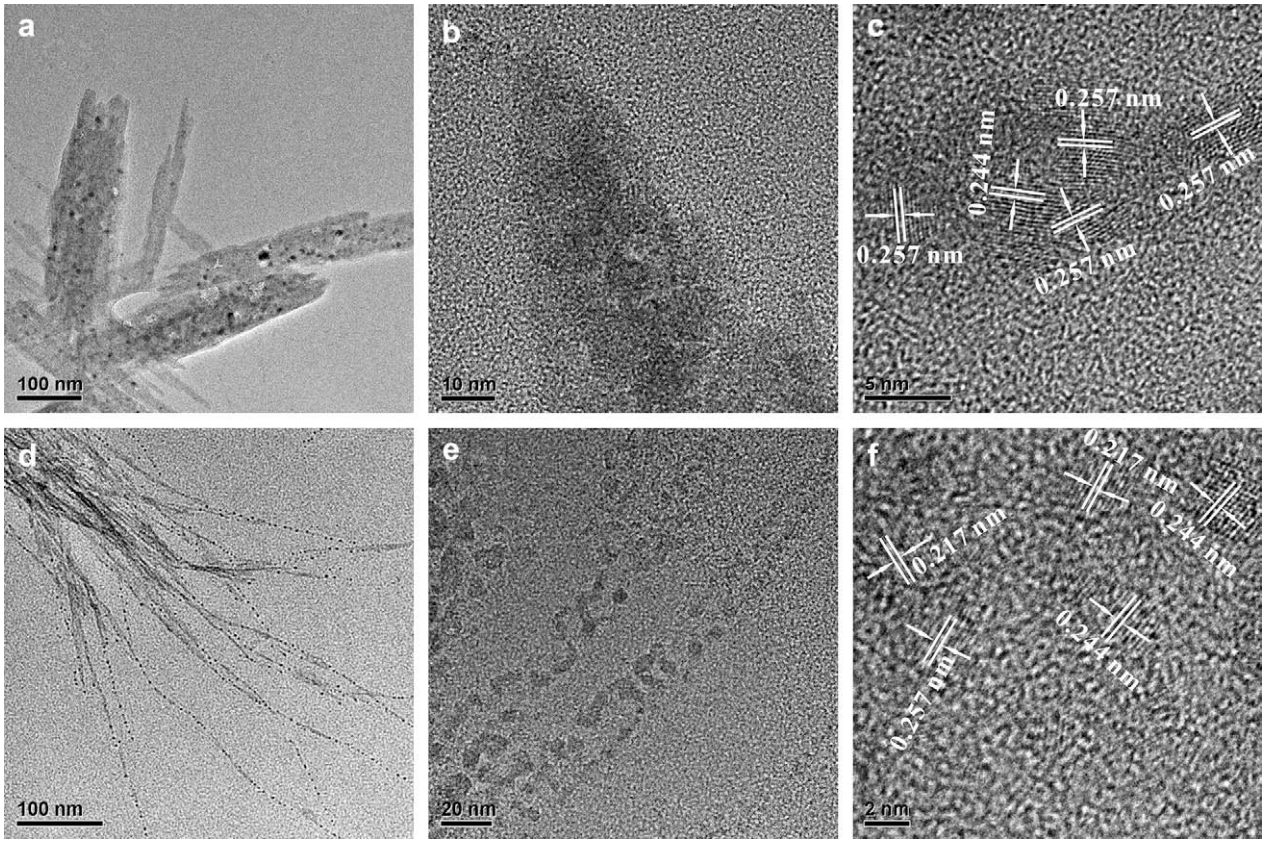


Fig. 5. TEM and HRTEM images of lath- (a–c) and sheaf-life (d–f) microarchitectures absorbed PVA nanoparticles fabricated with the ratio of glycerol to PVA aqueous solution as 1:4, (e) shows that the sheaf-like microarchitecture was destroyed by electronic beam after HRTEM detection.

glycerol or epirubicin) and carboxyl / hydroxyl groups (in PDLLA) or amino (in epirubicin) and carboxyl / hydroxyl groups [36].

3.7. Formation mechanisms

On the basis of the above experimental results, we suggest that the formation mechanisms of the microspheres and non-spherical microarchitectures in the presence of glycerol or epirubicin are as follows:

i) The mechanism for the formation of the microspheres is as schematically illustrated in Scheme 2a. Hydrogen bonds between the hydroxyl (in glycerol or epirubicin), amino (in epirubicin) and carboxyl / hydroxyl groups (in PDLLA) made different PDLLA chains associate with each other through small organic molecule linkages when glycerol or epirubicin solution was added into the W/O/W system. Surfactants played crucial roles in stabilizing the predominant emulsion droplets in the system. Along with the evaporation of organic solvent, PDLLA microspheres were obtained after shrinkage and solidification of the emulsion droplets [37,38].

ii) Based on the IR spectra, TEM and HRTEM images, we assume the mechanisms for the formation of non-spherical microarchitectures as illustrated in Scheme 2b and c. PDLLA chains associated together by means of the small organic molecule linkages due to the formation of hydrogen bonds between

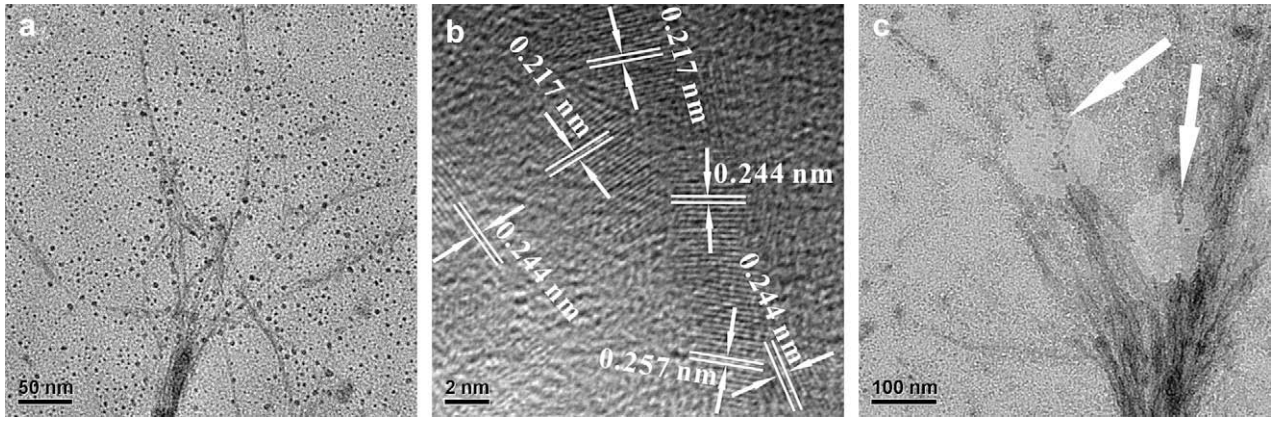


Fig. 6. TEM (a) and HRTEM (b) images of PDLLA sheaf-like microarchitectures absorbed PVA nanoparticles fabricated with the epirubicin concentration of 10 mg mL^{-1}. (c) shows that the sheaf-like microarchitecture was destroyed by electronic beam after HRTEM detection.

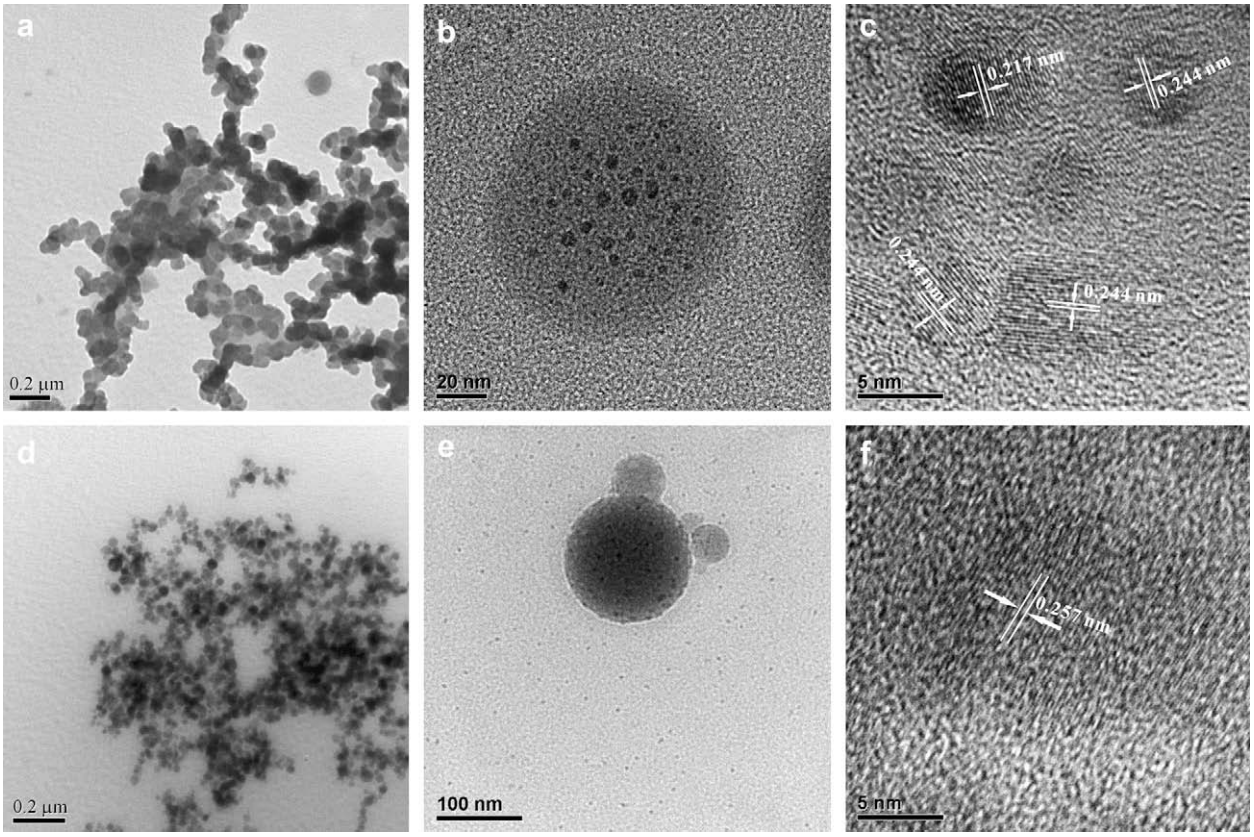


Fig. 7. TEM and HRTEM images of PVA nanoparticles fabricated by the double emulsion-solvent evaporation without PDLLA in the system as the control experiments. (a)–(c), the ratio of glycerol to PVA aqueous solution was 1:5 and the inner aqueous phase was 1 mL distilled water; (d)–(f), the epirubicin concentration was 10 mg mL^{-1} and the external aqueous phase was PVA aqueous solution only.

small organic molecules containing polyhydroxyl structure (glycerol or epirubicin) and PDLLA main chains. A portion of emulsion droplets was elongated by the reasonable shear stress, and the elongated droplets were broken by the high shear stress further when stirred at the fast rate. Several individual PDLLA chain associations formed nanofilaments (Fig. 4a) with low curvature finally. Individual nanofilaments are in favour of the formation of sheaf-like microarchitectures with the evaporation of organic solvent [15]. The sheaf-like microarchitectures can be induced by either glycerol or epirubicin based on hydrogen bonds under severe shear stress. Only due to the viscosity of glycerol, more individual chains associated together to form lath-like microarchitectures based on weak interactions and severe shear stress.

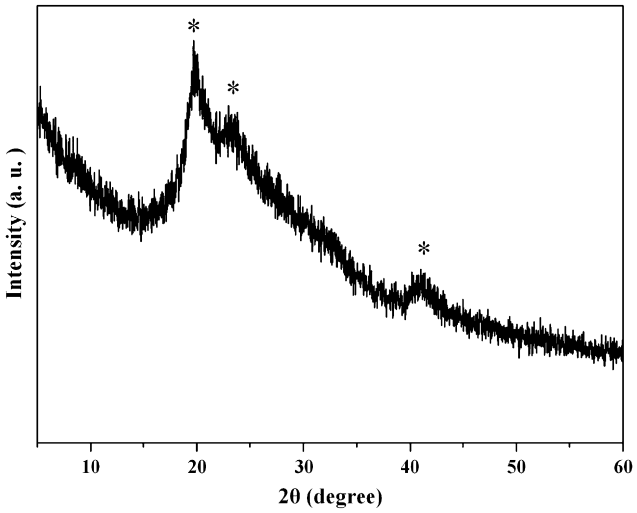


Fig. 8. XRD pattern of the raw material of PVA. The three diffraction peaks labelled with asterisks are at $2\theta = 19.6°$, 23.5° and 41.1°.

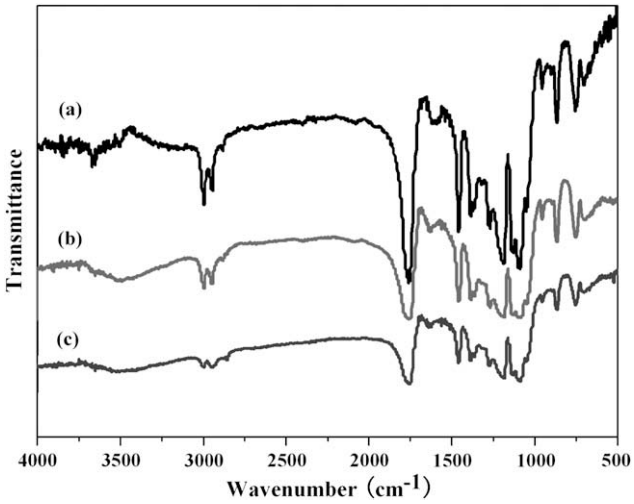


Fig. 9. FTIR spectra of PDLLA (M_n 56,000) (a) and its microarchitectures induced by glycerol (b) or by epirubicin (c) in solid state.

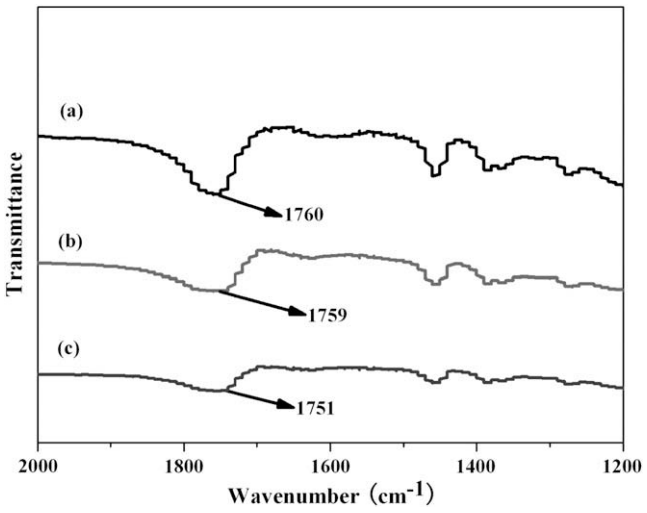


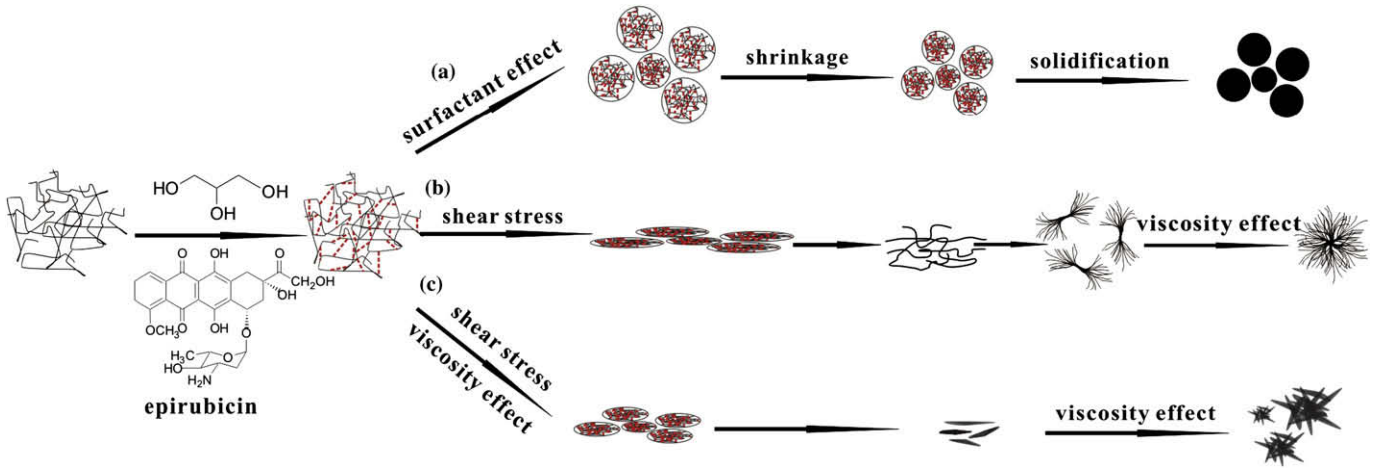
Fig. 10. FTIR spectra of PDLLA (M_n 56,000) (a) and its microarchitectures induced by glycerol (b) or by epirubicin (c) in solid state with higher resolution.

Furthermore, the lath-like microarchitectures (Fig. 2a) aggregated to the star-like assembly (Fig. 2c) on increasing the viscosity via increasing the amount of the glycerol in the system. Similarly, sheaf-like microarchitectures spontaneously self-assembled to the regular hexapod aggregates because of viscosity effect (Fig. 2d). Compared with glycerol in the system, the viscosity of the system was relatively lower when only epirubicin was added into the double emulsion system, therefore, we could not observe any hexapod aggregates except sheaf-like microarchitectures (Fig. 3). Therefore, we suggest that the viscosity of droplets would play the pivotal role in the shear response to result in the lath-like, even star-like microarchitectures and hexapod aggregates based on weak interactions [22,23]. Moreover, it should be mentioned here that the chirality of PDLLA is probably one important factor in favour of the formation of these anisotropic non-spherical microarchitectures according to the explanation of the assembly of PHB-*block*-mPEG sheaf- and star-like microarchitectures [23].

PVA is a traditional surfactant in emulsion, nevertheless, PLLA short fiber formation was explained by Mizutani et al. that droplets of the PLLA solution formed spheres coated with PVA membrane with sodium tripolyphosphate as a coagulation agent due to hydrogen bond formation, which were then deformed into fibrous shapes due to stirring [9]. Moreover, Qian et al also reported that CdSe and CdTe anisotropic nanowires were prepared through a PVA assisted solvothermal method due to the oriented attachment of PVA [39]. However, in the blank experiment we did not observe PDLLA lath- or sheaf-like non-spherical microarchitectures. In addition, PVA membrane did not form at the interface between the methylene chloride and the PVA solution containing glycerol or epirubicin, it is different from the mechanism of short fiber formation of crystallized PLLA due to the PVA effect, whereas the structures of glycerol and epirubicin are similar to sodium tripolyphosphate according to the number of oxygen atoms [9]. Small organic molecules containing polyhydroxyl structure (glycerol or epirubicin) play a more vital role than PVA obviously and the effect of PVA nanoparticles on the formation of these non-spherical microarchitectures needs further study. In one word, the mechanisms of assembly of PDLLA are different from amphiphilic block/graft copolymers or crystalline homopolymers, which cannot be ascribed to the crystallization or/and the amphiphilic properties of the polymer.

4. Conclusions

In summary, some novel PDLLA non-spherical microarchitectures were obtained by the double emulsion-solvent evaporation method in the presence of glycerol or epirubicin. We investigated the effects of glycerol, epirubicin and molecular weight of PDLLA on the formation of PDLLA microarchitectures. Without glycerol or epirubicin, only PDLLA microspheres were obtained. When glycerol was added into the external aqueous phase, PDLLA lath-, star-, sheaf-like microarchitectures and hexapod aggregates besides microspheres can be obtained. When epirubicin was added into the inner aqueous phase, the sheaf-like morphology of PDLLA assembly was the only non-spherical form. Based on weak interactions between small organic molecules containing polyhydroxyl structure and PDLLA chains, PDLLA assembled to nanofilamentous, even sheaf-like microarchitectures by applying shear stress. With the viscosity of the system, PDLLA



Scheme 2. Schematic illustration for the formation of (a) microspheres with glycerol or epirubicin in the system; (b) nanofilaments, sheaf-like, even hexapod aggregates with glycerol or epirubicin in the system; (c) lath-, star-like aggregates with glycerol in the system. ..... represents the linkage between PDLLA main chains via small organic molecules containing polyhydroxyl structure due to hydrogen bonds formation between hydroxyl (in glycerol or epirubicin) and carboxyl / hydroxyl groups (in PDLLA) or amino (in epirubicin) and carboxyl / hydroxyl groups.

also assembled to lath-, even star-like microarchitectures and hexapod aggregates. The formation mechanism is different from that of well-defined amphiphilic or crystalline polymers. These non-spherical microarchitectures of PDLLA, especially sheaf-like assemblies will have promising applications in novel drug delivery system as particle shape can significantly impact the performance of polymer drug carriers [35,40,41].

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Appendix. Supplementary data

Characterizations (homonuclear decoupled ^{1}H NMR, XRD and DSC) of the raw materials of PDLLA and the detailed experiment of PVA membrane formation. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.polymer.2009.05.047.

References

[1] Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Chem Rev 1999;99:3181–98.
[2] Auras R, Harte B, Selke S. Macromol Biosci 2004;4:835–64.
[3] Fujiwara T, Kimura Y. Macromol Biosci 2002;2:11–23.
[4] Liu M, Zhou ZM, Wang XF, Xu J, Yang K, Cui Q, et al. Polymer 2007;48:5767–79.
[5] Cui FD, Cun DM, Tao AJ, Yang MS, Shi K, Zhao M, et al. J Controlled Release 2005;107:310–9.
[6] Tsuji H. Macromol Biosci 2005;5:569–97.
[7] Hu JL, Tang ZH, Qiu XY, Pang X, Yang YK, Chen XS, et al. Biomacromolecules 2005;6:2843–50.
[8] Portinha D, Boué F, Bouteiller L, Carrot G, Chassenieux C, Pensec S, et al. Macromolecules 2007;40:4037–42.
[9] Mizutani Y, Hattori M, Okuyama M, Kasuga T, Nogami M. Polymer 2005;46:3789–94.
[10] Zhang LF, Eisenberg A. Science 1995;268:1728–31.
[11] Rodríguez-Hernández J, Chécot F, Gnanou Y, Lecommandoux S. Prog Polym Sci 2005;30:691–724.
[12] Förster S, Plantenberg T. Angew Chem Int Ed 2002;41:688–714.
[13] Zhang WD, Zhang W, Zhou NC, Cheng ZP, Zhu J, Zhu XL. Polymer 2008;49:4569–75.
[14] Won YY, Davis HT, Bates FS. Science 1999;283:960–3.
[15] Resendes R, Massey JA, Temple K, Cao L, Power-Billard KN, Winnik MA, et al. Chem Eur J 2001;7:2414–24.
[16] Liu WY, Liu RG, Li YX, Kang HL, Shen DW, Wu M, et al. Polymer 2009;50:211–7.
[17] Sunintaboon P, Ho KM, Li P, Cheng SZD, Harris FW. J Am Chem Soc 2006;128:2168–9.
[18] Cui HG, Chen ZY, Zhong S, Wooley KL, Pochan DJ. Science 2007;317:647–50.
[19] Zhang JX, Li SH, Li XD, Li XH, Zhu KJ. Polymer 2009;50:1778–89.
[20] Wu JH, Tang QW, Li QH, Lin JM. Polymer 2008;49:5262–7.
[21] Liu Y, Zhao DY, Ma RJ, Xiong DA, An YL, Shi LQ. Polymer 2009;50:855–9.
[22] Meng FT, Ma GH, Qiu W, Su ZG. J Controlled Release 2003;91:407–16.
[23] Ravenelle F, Marchessault RH. Biomacromolecules 2003;4:856–8.
[24] Bondzic S, Polushkin E, Ruokolainen J, Brinke GT. Polymer 2008;49:2669–77.
[25] He LL, Zhang LX, Liang HJ. Polymer 2009;50:721–7.
[26] Okada H. Adv Drug Deliv Rev 1997;28:43–70.
[27] Lu Z, Bei JZ, Wang SG. J Controlled Release 1999;61:107–12.
[28] Yamaguchi Y, Takenaga M, Kitagawa A, Ogawa Y, Mizushima Y, Igarashi R. J Controlled Release 2002;81:235–49.
[29] O'Donnell PB, McGinity JW. Adv Drug Deliv Rev 1997;28:25–42.
[30] Dai LX, Ide K, Yamaura K. J Appl Polym Sci 1997;64:1337–44.
[31] Birtle AJ. Clin Oncol 2000;12:146–52.
[32] Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Pharmacol Rev 2004;56:185–229.
[33] Fujiwara K, Hayakawa K, Nagata Y, Hiraoka M, Nakamura T, Shimizu Y, et al. Cardiovasc Intervent Radiol 2000;23:218–23.
[34] Birnbaum DT, Brannon-Peppas L. J Biomater Sci Polym Ed 2003;14:87–102.
[35] Champion JA, Mitragotri S. Proc Natl Acad Sci USA 2006;103:4930–4.
[36] Wan Y, Wu H, Yu AX, Wen DJ. Biomacromolecules 2006;7:1362–72.
[37] Garti N. Lebensm-Wiss u-Technol 1997;30:222–35.
[38] Desgouilles S, Vauthier C, Bazile D, Vacus J, Grossiord JL, Veillard M, et al. Langmuir 2003;19:9504–10.
[39] Yang Q, Tang KB, Wang CR, Qian YT, Zhang SY. J Phys Chem B 2002;106:9227–30.
[40] Geng Y, Dalhaimer P, Cai SS, Tsai R, Tewari M, Minko T, et al. Nat Nanotechnol 2007;2:249–55.
[41] Decuzzi P, Ferrari M. Biomaterials 2006;27:5307–14.