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# A facile preparation of highly interconnected macroporous poly(D,L-lactic acid-*co*-glycolic acid) (PLGA) scaffolds by liquid–liquid phase separation of a PLGA–dioxane–water ternary system

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**Abstract**

Regular and highly interconnected macroporous scaffolds ranging in size from 50 to 150 μm were fabricated from poly(D,L-lactic acid-*co*-glycolic acid) (PLGA)–dioxane–water ternary systems via thermally induced phase separation (TIPS) without any surfactant or other additives. The effect on scaffold morphology of processing parameters including quenching temperature, polymer concentration, solvent composition and molecular weight, was investigated as a function of quenching time. The cloud-point temperature of the polymer solution was found to depend on polymer concentration, solvent composition, and polymer molecular weight. The water content in the solvent mixture had the greatest effect on the cloud-point temperature. The optimal quenching temperature for preparing macroporous inter-connected scaffolds from a 9 wt% PLGA solution (dioxane–water = 87/13, by wt) was less than −7 °C. In low viscosity PLGA solutions, sedimentation of the polymer rich phase occurred due to the segregation of the separated phases under gravity. This led to the formation of scaffolds with irregular and closed pores.
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*Keywords:* PLGA scaffolds; thermally induced phase separation; macroporous inter-connected structure

## 1. Introduction

Over the past decade, many scientists have been involved in research into the generation of natural tissue replacements for defect tissues or organs by creating an extra cellular matrix (ECM) composed of selective tissue-specific cells on synthetic biodegradable polymeric scaffolds [1–6]. These scaffolds are porous materials that provide a three-dimensional framework for selective cell penetration and facilitate the formation of new tissue [7,8]. Ideally, scaffolds should be highly porous, have adequate mechanical stability and strength, and contain interconnected pores to permit cell penetration and ingrowth into the implanted structures [9,10]. The surface properties of the scaffolds usually determine the cell adhesion behavior and the activation of cell-substratum events [10]. Pore size is an important consideration for selective cell culture; for instance, the optimal pore size is 20 μm for the ingrowth of fibroblasts and hepatocytes, between 20 and 150 μm for skin regeneration, and in the range of 100–250 μm for bone regeneration [11,12]. In the search for synthetic materials suitable for use in tissue replacement, aliphatic polyesters such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and its copolymers have been extensively investigated as analogues of the native matrix because of their good biocompatibility and biodegradability [13,14]. Heat-compressed non-woven fiber meshes of PGA have been widely used in soft tissue regeneration. However, the impact strength and elasticity of poly(L-lactic acid) (PLLA) and PGA scaffolds are usually insufficient for these materials to be used in implantation devices, especially devices for soft tissue regeneration [15]. In addition, macroporous scaffolds with highly interconnected networks of pores ranging in size from 50 to 150 μm are usually required for soft-tissue cell penetration.

Numerous techniques have been developed for fabricating polyester scaffolds, including porogen leaching/salt

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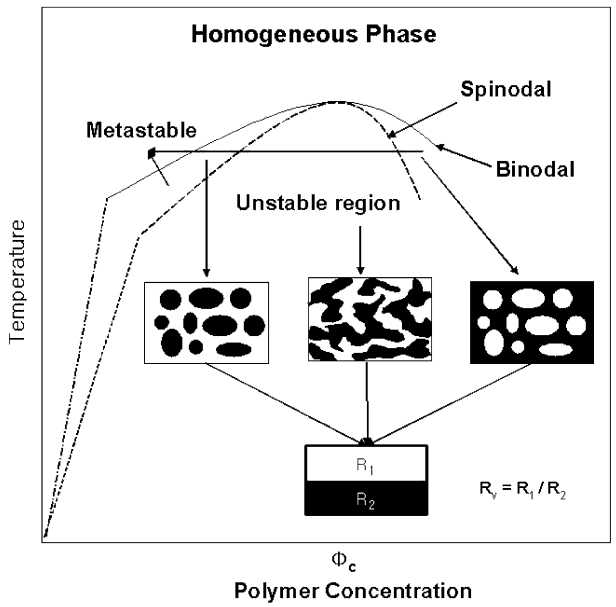



Fig. 1. Schematic representation of a typical polymer–solvent–non-solvent ternary phase diagram. (R1: polymer lean phase, R2: polymer rich phase, Rv: volume ratio).

leaching, emulsion freeze-drying, gas expansion, fiber bonding and phase separation [16–20]. Recently, the method of freeze-drying via thermally induced phase separation (TIPS) was developed for the preparation of polyester scaffolds [18,20–25]. Solid–liquid phase separation of a PLA or PLGA binary solution has been used to prepare macroporous scaffolds with non-uniform ladder-like structures [21]. An approach that has shown great promise for the preparation of open macroporus scaffolds ranging in size from 20 to 300 μm is to arrest the morphology during the spinodal phase separation stage of liquid–liquid demixing of a PLA–dioxane–water ternary system, followed by freeze-drying to obtain the scaffold [22–25]. In principle, the final porous morphology is thought to rely on the thermodynamic state of the solution to be quenched, as schematized in the temperature-composition phase diagram in Fig. 1. A nucleation and growth mechanism of phase separation results in a poorly connected stringy or beady structure. In contrast, spinodal phase separation is expected to give rise to a highly interconnected structure. The initial pore size is determined by the concentration fluctuations induced by the quenching, the polymer concentration in the solution, and fluctuations associated with thermal flow. Therefore, the final porous morphology can be controlled to some extent either by varying processing parameters such as the quenching rate, quench temperature exposed to the atmosphere, and quenching period, or by varying formulation parameters such as the polymer concentration, solvent composition, crystalline and molecular. The coarsening effect can be utilized to increase pore size. However, the solution finally precipitates to form two separated phases with a boundary line due to the separation under gravity of phases with different densities (see Fig. 1). Park prepared an interconnected PLGA scaffold with a pore size of less than 50 μm by quenching a PLGA solution (dioxane–water = 87/13) into liquid nitrogen in the presence of F-127 [23]. Teyssie showed that PLLA scaffolds with an interconnected pore structure could be prepared from solutions of PLLA in polyisostyrene–cyclohexane binary systems by arresting the crystallization of the semicrystalline PLLA during spinodal decomposition [25,26].

Our group has focused mainly on developing techniques based on liquid–liquid TIPS of polyester ternary systems to prepare pore-designed PLA or PLGA macroporous scaffolds with highly interconnected structures and pore sizes ranging from 50 to 300 μm suitable for certain selective cell cultures. Previously, we successfully prepared highly interconnected macroporous PLLA scaffolds with pore sizes of 50–150 μm by controlling the crystallization of PLLA during the coarsening process [27,28]. In other work, we found that the pore size in PLLA scaffolds with interconnected structures could be increased to greater than 200 μm by adding certain additives such as ionic compounds, surfactants and Pluronics [29,30].

In the present study, regular and highly interconnected macroporous PLGA scaffolds with pore sizes greater than 50 μm were fabricated facily from PLGA–dioxane–water ternary systems with free surfactant or other additives by means of liquid–liquid TIPS. The effects of a range of processing and formula parameters on the phase diagram of this polymer system and the porous morphology of the resulting scaffold were investigated.

## 2. Experimental

### 2.1. Materials

Two poly(D,L-lactic acid-*co*-glycolic acid)s (PLGAs), referred to as PLGA1 and PLGA2, were purchased from

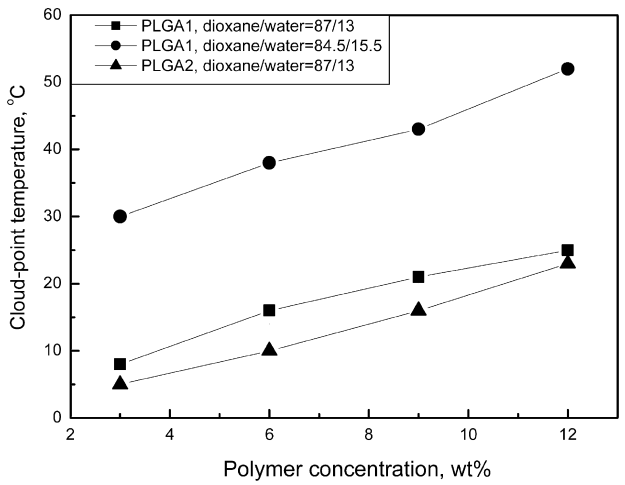



Fig. 2. Phase diagram of cloud-point temperature versus polymer composition of the PLGA1 or PLGA2–dioxane–water ternary system.

Alkermes Co. The inherent viscosities (chloroform) of PLGA1 and PLGA2 were about 0.71 and 0.57 dL/g, respectively. Diethylene dioxide (1,4-dioxane), a good solvent for PLGA, was of analytical grade and distilled before use. Twice-distilled deionized water was used.

### 2.2. Cloud-point temperature measurements

The cloud-point temperatures of the ternary systems were determined by visual turbidimetry. PLGA was dissolved in a 1,4-dioxane–water (87/13 or 84.5/15.5, by wt) mixture in a 4 ml vial with a magnetic stirrer by heating at 60 °C for 2 h. The sample was then maintained as a clear solution at a temperature ca. 15 °C above the expected cloud-point temperature. This solution was then slowly cooled in steps of 1 °C, equilibrating the system for 10 min at each new temperature. The cloud-point temperature was taken as the temperature at which the clear solution became turbid, as determined by visual inspection. The error range was approximately ±1 °C.

### 2.3. Scaffold preparation

PLGA solutions (6, 9 and 12 wt%) were prepared by adding appropriate quantities of PLGA to 4 ml vials filled with a mixture of 1,4-dioxane and water (84.5/15.5 or 87/13, by wt). Samples were dissolved as described in Section 2.2. The tube of dissolved sample was then heated to 15 °C above the measured cloud-point temperature, after which it was quickly dipped into a constant temperature bath maintained at the desired quenching temperature (19, 13, 3, −2, −7 or −17 °C). The tube was removed from the quenching bath after 1, 2, 5, 10, 30 or 60 min to observe the coarsening effect. The annealed sample was immediately immersed in liquid nitrogen to be fast-frozen. One small pore was cut in the tube using an electronic knife to allow the solvents to escape. Samples were freeze-dried at −77 °C and 7 mmTorr for 3 days to remove the solvents, yielding the porous scaffolds.

### 2.4. Morphology characterization

The morphology of the scaffolds was investigated by scanning electronic microscopy (SEM, Hitachi S-2150). Fracture-frozen cross sections of the scaffold, taken either in a longitudinal or transverse way, were coated with Pt and examined.

### 2.5. Differential scanning calorimetry (DSC) measurements

Polymer solutions (PLGA1 or PLGA2) of concentration 6 and 9 wt% in dioxane–water mixture (87/13 by wt) were prepared as mentioned above and placed in 4 ml vials. The tubes were heated to 15 °C above the measured cloud-point temperature and immediately fast-frozen by immersion in liquid nitrogen. A small amount of the quenched sample was taken quickly out of the tube and transferred into a cell. The sample in the cell was then subjected to DSC (TA 2910) measurements at a heating rate of 10 °C/min from −80 to 70 °C, followed by a second scan at a cooling rate of −10 °C/min from 70 to −40 °C.

## 3. Results and discussion

### 3.1. Phase diagram

The cloud-point temperature of each PLGA–dioxane–water ternary system was determined as the temperature at which a slowly cooled solution was visually judged to have become turbid. The sharp change in turbidity at the cloud-point temperature observed experimentally is the result of liquid–liquid demixing. The cloud-point temperatures of the PLGA solutions considered in this study are presented in Fig. 2. For all of the ternary systems, the cloud-point temperature increases slightly with increasing polymer concentration. Of particular note is the dramatic increase in the cloud-point temperature (~20 °C) of the PLGA1 system when the water content in the solvent mixture is increased 2.5%. This indicates that liquid–liquid demixing depends mainly on the water content of the solvent mixture; similar behavior has been observed previously in the PLLA–dioxane–water ternary system [27]. Increasing the molecular weight of the PLGA reduces the polymer–solvent interactions, which should lead to an increase in the cloud point temperature. Consistent with this, ternary mixtures with PLGA2, which has a lower molecular weight than PLGA1, have cloud-point temperatures up to 6 °C lower than those of the corresponding PLGA1 mixtures, in accordance with the predictions of Witte [31,32].

Previously, we showed that PLLA solutions with concentrations above the sedimentation boundary concentration form gels when the temperature is lowered to below the gelation point [27,28]. This gel formation is due to the crystallization of PLLA. When the PLLA concentration was below the boundary concentration (<4.5 wt%), sedimentation occurred leading to the formation of two layers during the quenching process. For the PLGA solution system considered here, phase separation was observed over the concentration range of 3–12 wt% when the mixture was cooled below the cloud-point temperature. However, gelation was not observed even on cooling to 0 °C. In addition, at long times during the coarsening process the mixture separated into two layers as a result of sedimentation under gravity, which separated the coarsened phases on the basis of their density.

### 3.2. Quenching temperature

As mentioned in Section 1, the ultimate porous morphology of a scaffold, including pore size, pore shape and porosity, is determined by the thermodynamic state of

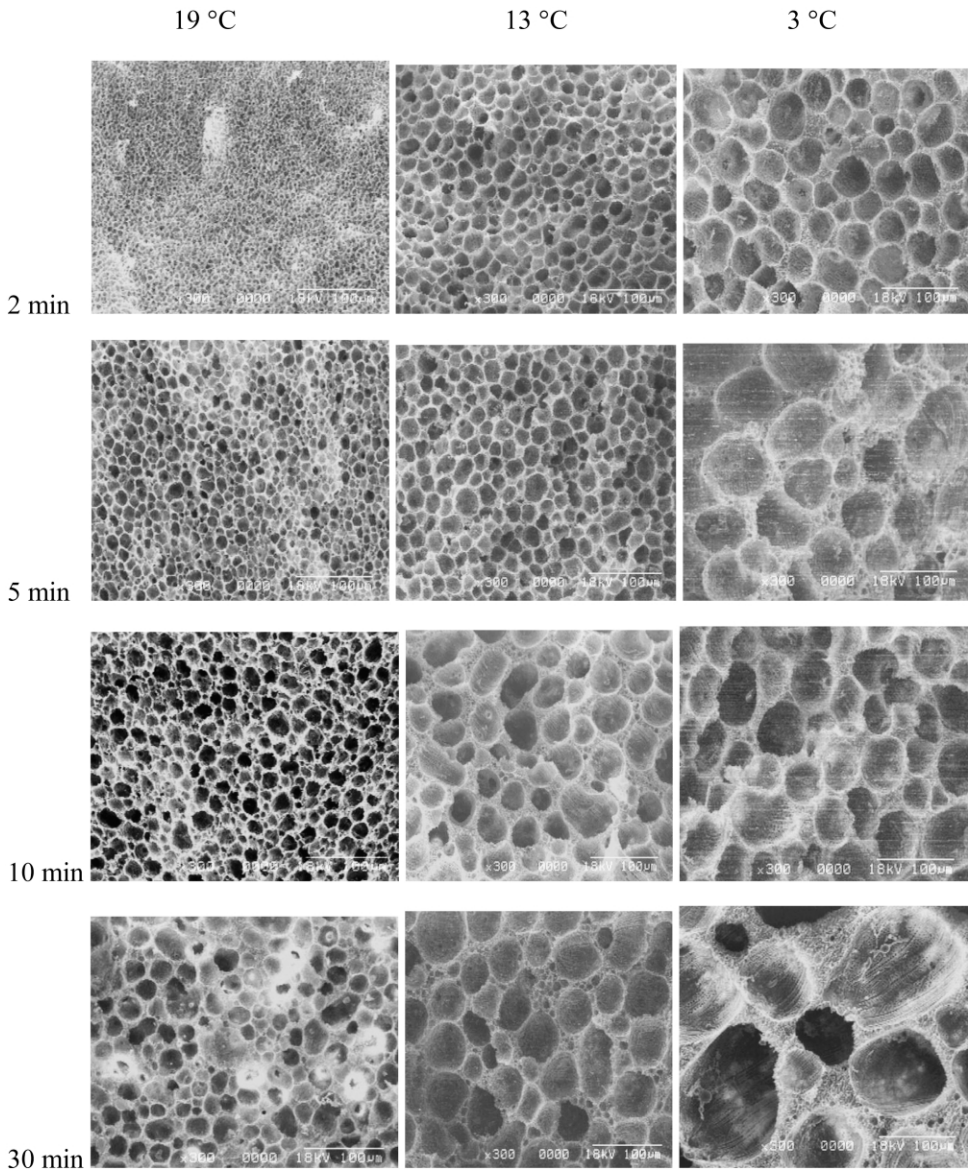



Fig. 3. SEM micrographs of scaffolds prepared by quenching a 9 wt% PLGA1 ($M_w$ = 130,000) in dioxane–water (87/13, by wt) mixture at three different temperatures as a function of aging time.

the solution prior to freeze-drying. Phase separation is induced by binodal or spinodal decomposition on the basis of the location of the system in the miscibility gap in the phase diagram. The morphology of the final scaffold depends on processing and formula parameters such as the quenching temperature, polymer concentration, and solvent composition, as well as the aging time.

Fig. 3 presents three sets of SEM micrographs showing the structural evolution of a solution of 9 wt% PLGA1 in dioxane–water (87/13) quenched to 3, 13, and 19 °C. When the solution was quenched to 19 °C, which is located just below the cloud point temperature in the metastable region of the phase diagram (Fig. 2), binodal phase separation occurred. As the system was aged at this temperature, small pores gradually increased in size such that after 30 min of aging an irregular structure of non-uniform pores (size $<20$ μm) was produced. This is due to development of the nucleation and growth of polymer-lean phase. When the solution was quenched to 13 or 3 °C (quenching depths of 8 and 18 °C, respectively), spinodal phase separation was expected. As can be seen in Fig. 3, open porous structures are observed during the early stage of phase separation ($<2$ min), and the pore size is greater on quenching to 3 °C. Generally, after a short time ($<2$ min), phase separation entered the coarsening process during which the thermodynamic driving force comes from minimizing the interfacial tension. After 30 min of aging at 3 °C, the pores developed into large, closed, and irregular structures with sizes of more than 150 μm. This structure develops because coarsening occurs via a coalescence mechanism in which larger porous structures are produced by the combination of smaller porous structures.

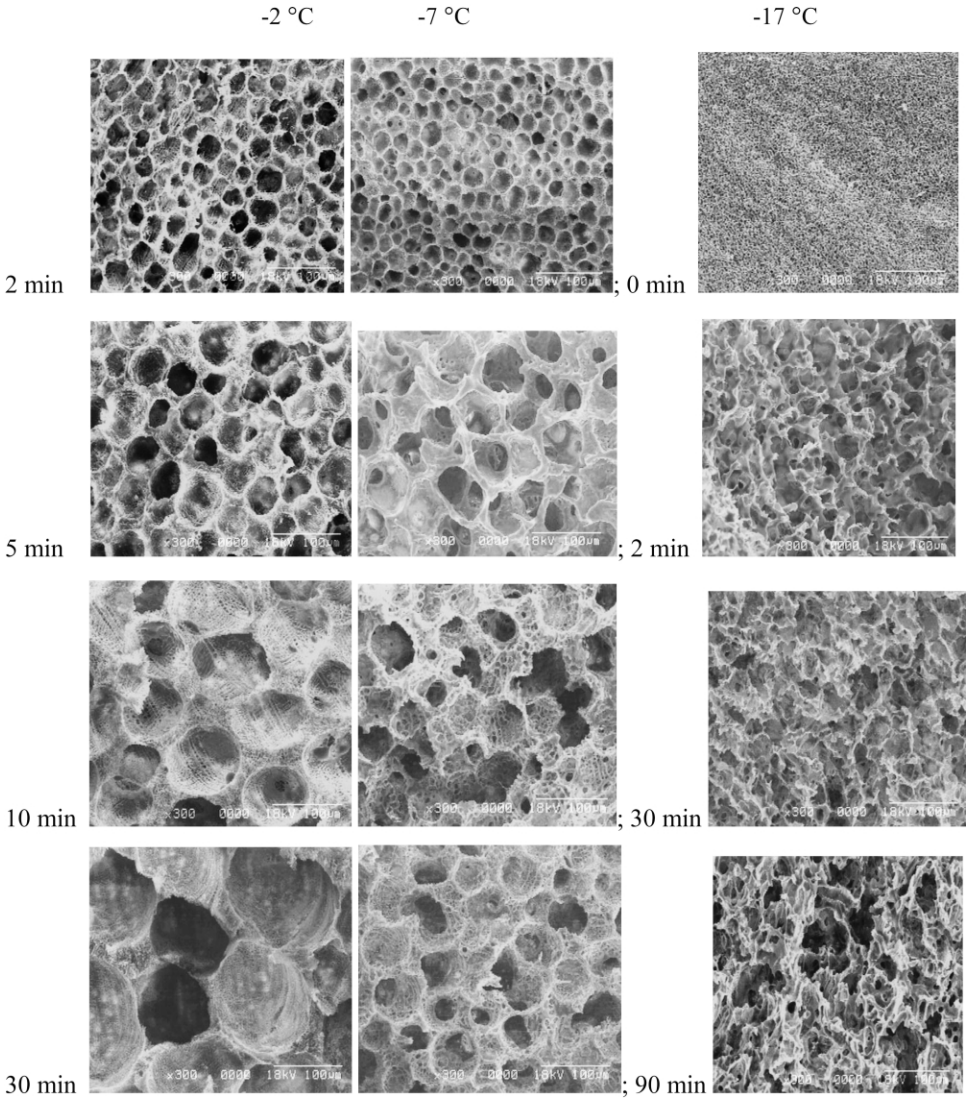



Fig. 4. SEM micrographs of scaffolds prepared by quenching a 9 wt% PLGA1 solution at lower temperatures as a function of aging time.

It is interesting to note that the variation of morphology is out of our expectation, when the quench process was carried out at further lower temperatures, such as −7 or −17 °C as shown in Fig. 4 of time-sequences of SEM micrographs taken of the same PLGA1 system quenched to −2, −7, and −17 °C. When the system was quenched to −7 °C, a regular and highly interconnected macroporous structure with a pore size of greater than 70 μm was obtained after 5 min of aging. This interconnected structure was maintained throughout the 30 min that the coarsening process was monitored. After more than 10 min of coarsening at this temperature, unusual ladder-like structures formed in the pore walls. Such ladder-like structures are generally thought to result from solid–liquid phase separation [27]. Considering that PLGA is amorphous, the solid–liquid phase separation most likely originates from crystallization of the solvent molecules when the temperature is well below the melting points of pure dioxane and water (11 and 0 °C,

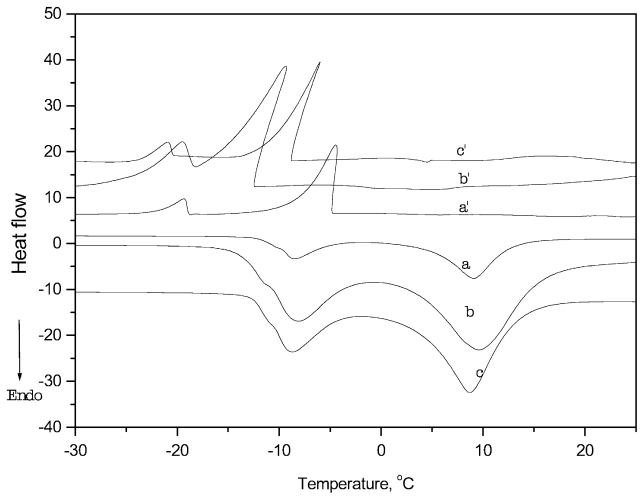



Fig. 5. DSC thermograms of PLGA solutions in dioxane–water (87/13, by wt) measured in situ. (a, a′) 6 wt%, PLGA1; (b, b′) 9 wt%, PLGA1; (c, c′) 9 wt%, PLGA2. (a, b, c) first scan at a heating rate of 10 °C/min; (a′, b′, c′) subsequent scan at a cooling rate of −10 °C/min.

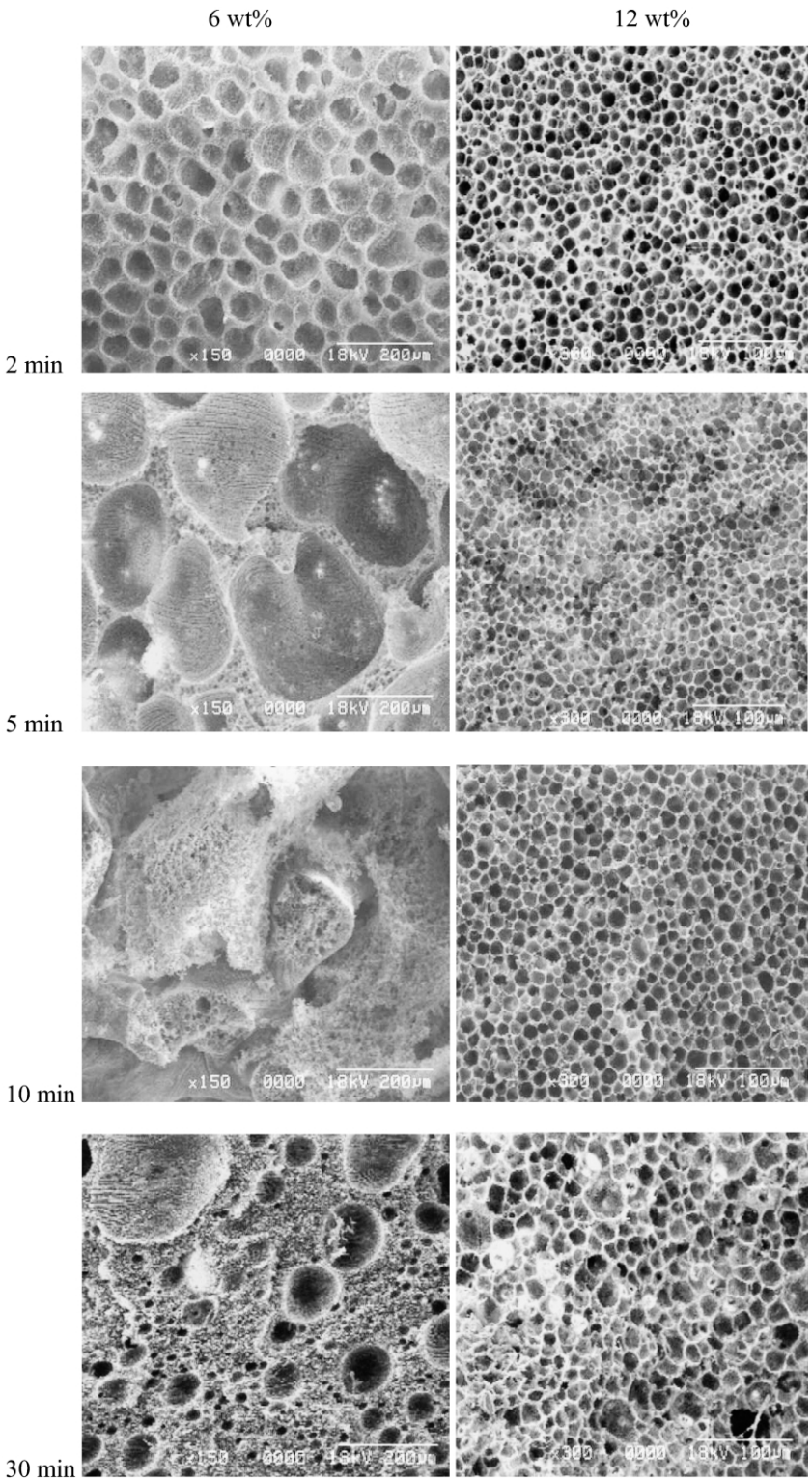



Fig. 6. SEM micrographs of scaffolds prepared by quenching a mixture of PLGA1 in dioxane–water (87/13, by wt) to 3 °C as a function of aging time with different polymer concentrations.

respectively). Furthermore, the pore size at −7 °C is smaller than that at 3 °C, despite the larger quenching depth. This can be explained in terms of the prevention of phase separation at the lower temperature due to the viscosity increase caused by solvent crystallization. This effect caused the pores to develop at a very slow rate, attaining sizes of only 90 μm after 30 min of aging. The effect of the prevention of phase separation as a result of solvent crystallization was evident in the system quenched to −17 °C (Fig. 4), in which the morphology barely changed

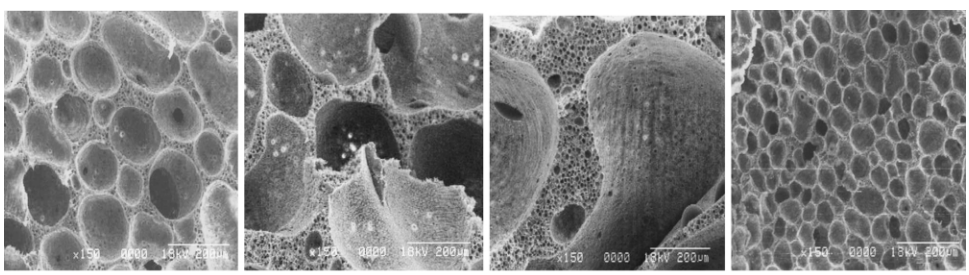
during the coarsening process from 2 to 90 min. This indicates that the phase separation development or coarsening process is fixed with the maximum viscosity due to quick solvents crystallization under −17 °C. However, when the solution was directly quenched in liquid nitrogen, a typical nucleation structure was obtained (see 0 min at −17 °C in Fig. 4) because the cooling rate was too fast for phase separation to occur. When the system was quenched to −2 °C, on the other hand, the crystallization of the solvents was too slow to prevent the coalescence of the pores. Thus, irregular and close pores are observed after 30 min of aging at this temperature (Fig. 4).

Thermal analysis of the PLGA solution was performed with a first scan from −80 to 60 °C at a heating rate of 10 °C/min and a subsequent scan from 60 to −40 °C at a cooling rate of 10 °C/min. During the first scan (Fig. 5b), melting peaks appeared at 10 and −8 °C. These peaks can be respectively assigned to the melting of dioxane and water (melting point 11 and 0 °C). The small difference between the peak positions and the melting points of the pure solvents results from the depression of the solvent melting points due to amorphous PLGA or as impurity intertwined into crystal phase. During the subsequent cooling process (Fig. 5b), crystallization peaks appeared at −12 and −20 °C corresponding to the crystallizations of the dioxane and water. Comparing a, a′ and c, c′ with b, b′ in Fig. 5, the large disparity between the peak positions observed during cooling and the crystallization temperature expected for the pure solvent was due to the fast cooling rate used in the experiment. Emphasis should be paid on crystallization process of solvents during quenching process for the phase separation. The crystallization temperature depends on the cooling rate, polymer concentration and solvent composition in the mixture as well as the quenching temperature. For quenching to −7 and −17 °C, the solvents crystallize to a greater extent at long aging times than is the case in systems quenched to −2 °C. This crystallization at the lower quenching temperatures increases the viscosity of the system, which prevents phase separation due to spinodal decomposition, as shown in Fig. 4.

### 3.3. Polymer concentration, solvent composition and polymer molecular weight

The effect of polymer concentration in solution (PLGA1 in dioxane–water, 87/13) on scaffold morphology was investigated by taking SEM images of the time evolution of the structure in systems with 6 and 12 wt% PLGA1 quenched to 3 °C. These images, shown in Fig. 6, can be compared with the results for the 9 wt% PLGA1 system presented in Fig. 3. For the system with lower polymer concentration (6 wt%) quenched to 3 °C, sedimentation occurred after 5 min of aging. During the starting period of phase separation (<5 min), the pore size in the 6 wt% system is larger than that in the 9 wt% system, although the quenching depth (the temperature difference between cloud point temperature and experimental temperature) of the 6 wt% system is slightly smaller than that of the 9 wt% system (see Fig. 2). The smaller quenching depth of the 6 wt% system is related to its lower polymer concentration as well as the lower viscosity of this solution. During the aging process in the spinodal region, the polymer distribution and volume ratio of the two separated phases is almost constant at short aging times (<2 min). The volume ratio of the polymer rich phase to the polymer lean phase for the 6 wt% system is lower than that at 9 wt% theoretically. After 5 min of aging of the 6 wt% system, coalescence occurs and the morphology becomes irregular. This occurs because the polymer lean phase is so large as to segregate into two layers by gravity force for the lower viscosity system (6 wt%). For the system with a higher concentration of polymer (12 wt%), the pore size is small and increases gradually to a size of only 10 μm after 30 min of aging because of the higher viscosity and volume ratio of the polymer rich phase.

As we illustrated previously [27], solvent composition strongly influences scaffold morphology. Fig. 7 shows SEM micrographs taken of a mixture made up of 9 wt% PLGA1 in dioxane–water (84.5/15.5). As mentioned in Section 3.1, increasing the water content from 13 to 15.5 wt% caused the cloud-point temperature to increase by 20 °C. Hence, the solution (dioxane–water, 84.5/15.5) was quenched to 23 °C



2 min 5 min 10 min 30 min

Fig. 7. SEM micrographs of scaffolds prepared by quenching a 9 wt% PLGA1 solution with a different solvent composition (84.5/15.5, by wt) to 23 °C as a function of aging time.

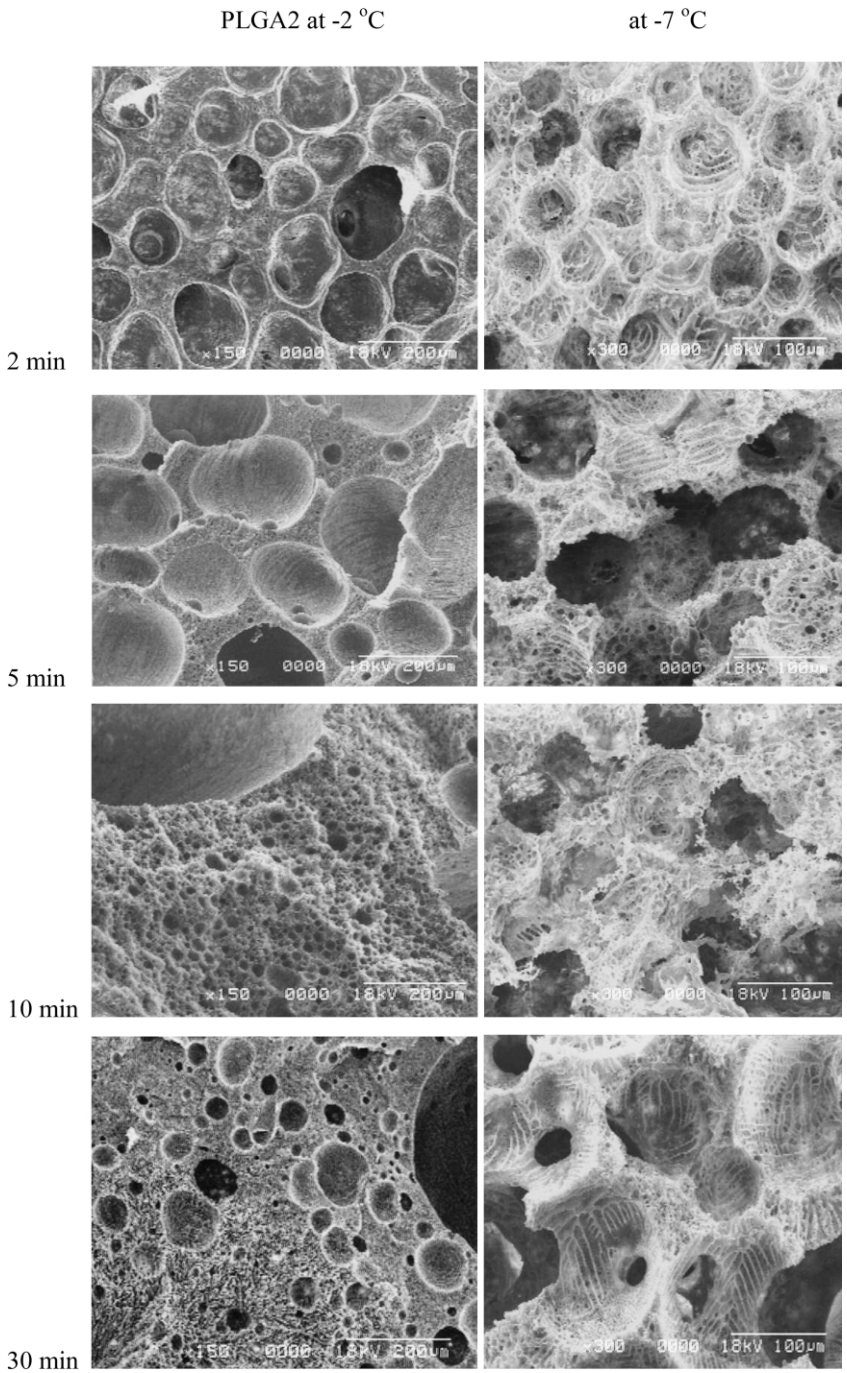



Fig. 8. SEM micrographs of scaffolds prepared by quenching a 9 wt% PLGA2 ($M_w$ = 80,000) solution (87/13, by wt) to different temperatures as a function of aging time.

(quenching depth: 18 °C) in order to compare it with the 87/13 system quenched at 3 °C (quenching depth: 18 °C) shown in Fig. 3. Sedimentation was observed after 10 min of aging. Consistent with our previous results for the PLLA–dioxane–water system (84.5/15.5) [27], increasing the water content in the solvent caused the sedimentation boundary to shift to higher polymer concentration. The effect of viscosity on phase separation should be especially important in these systems, given the long times required for the coarsening process. Increase of the amount of water, as non-solvent will decrease the viscosity because small molecules serve as a friction. In addition, the higher quenching temperature used for the 84.5/15.5 system (23 °C) will lower the viscosity of the solution. The reduction in the viscosity due to these factors enhances the coalescence during phase separation and sedimentation of the polymer rich phase.

The effect of polymer molecular weight on scaffold morphology and phase separation during aging is illustrated in Fig. 8, which shows SEM micrographs taken of a 9 wt%

solution of the lower-molecular-weight polymer PLGA2 ($M_w = 80{,}000$). During the early stage of phase separation, the size of the pores in the PLGA2 system is greater than 120 μm. This is greater than the pore size observed in the corresponding PLGA1 ($M_w = 130{,}000$) solution. The PLGA2 solution produces larger pores due to its lower viscosity and lower volume ratio of the polymer rich phase. As shown in Fig. 2, the higher molecular weight polymer, PLGA1, has a higher cloud-point temperature in solution by 5 °C. So, for the 9 wt% PLGA2 solution system, the quench was carried out at −2 °C with the same quenching depth (18 °C). In this case, a decrease of quenching temperature could cause dropping the volume ratio of polymer rich phase and polymer lean phase. Increasing solvent phase constructed larger space in scaffold, meaning increase of pore size. Furthermore, the viscosity for PLGA2 system decreased more, due to lower polymer molecular weight by 8/13 times compared with PLGA1. This decrease in viscosity can be outweighed by the slight increase in viscosity due to lowering the temperature by 5 °C, because the dependence of viscosity on molecular weight follows a power law with an exponent of approximately 0.7–0.9 [32]. However, this system (PLGA2, 9 wt%) was quenched to −7 °C and an interesting morphology appeared, as shown in Fig. 8. The increased viscosity of this system due to solvent crystallization meant that sedimentation was prevented and partial interconnection between pores was maintained during the coarsening process (30 min). The crystallization of the solvent in the PLGA2 system quenched to −7 °C is plausible, given that the DSC thermogram of the 9% PLGA1 solution (87/13) (Fig. 5c) showed a crystallization peak close to −10 °C.

## 4. Conclusion

The phase diagram of a PLGA–dioxane–water ternary system was carefully measured. The cloud-point temperature was found to depend on polymer concentration, solvent composition, and polymer molecular weight. A solution of 9 wt% PLGA1 ($M_w = 130{,}000$) in dioxane–water (87/13) produced a regular and highly interconnected macroporous scaffold with a pore size of over 70 μm via a liquid–liquid spinodal phase separation process. Optimally, the quenching temperature should be ≤ −7 °C so as to induce crystallization of the solvents, which prevents further phase separation after spinodal separation. The interconnection was constricted during coarsening process because of the maximum viscosity induced by the solvent crystallization. For the PLGA1 system with a lower polymer concentration (6 wt%), sedimentation occurred due to the lower viscosity and lower volume ratio of the polymer rich phase in this system. This phenomenon was also observed for the system with high water content (84.5/15.5) in the solvent.

For solutions of the lower-molecular-weight polymer PLGA2 (87/13), sedimentation tended to occur due to the low viscosity of this solution, the exception being when it was quenched to lower temperatures (≤ −7 °C). Most commercial PLGAs have different characteristics including molecular weights and LA/GA ratios, and are therefore expected to exhibit different phase transition and solution properties. Hence, the optimum quenching conditions should be tested for each PLGA in order to effectively fabricate porous scaffolds for cell culture. This work is in progress in our lab.

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