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Annealing regulates performances of electrospun poly(ε-caprolactone) membrane to accommodate tissue engineering†

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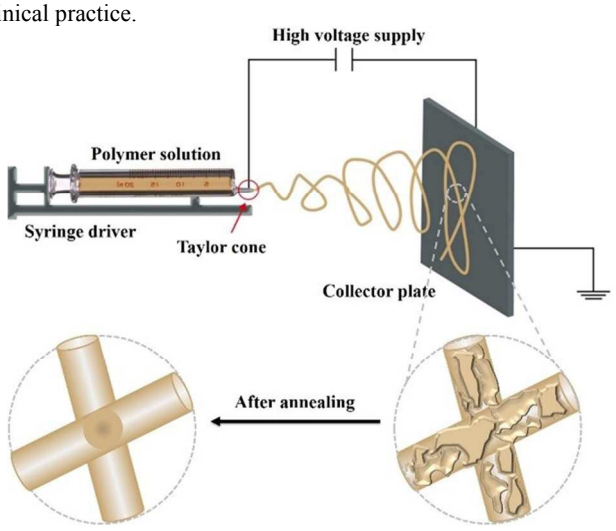
The profound effect of annealing on the electrospun poly(ε-caprolactone) scaffold is comprehensively investigated for the first time. Interestingly, after annealing at 42 °C, the contact angle of sample decreases from (133.39 ± 3.20) to (124.11 ± 2.08)º, meanwhile the Young's modulus dramatically increases from 8.41 ± 1.64 to 11.27 ± 2.41 MPa, which are attributed to the improved crystallinity. The above results and additional excellent cellular proliferation demonstrate that the annealed matrix possesses better physical and biological performances as a tissue engineering scaffold.

Thermal annealing belongs to an effective post-treatment approach to fabricate the products with excellent structures and physical performances. Zhu, Fong, and co-workers found that, upon annealing nylon-6 nanofibers at above 150 °C, polymer chains were mobile and recrystallized towards an equilibrium state of stable α-form crystals.[1] Tan *et al* reported that the annealing process could cause the increased Young's modulus of electrospun poly(L-lactic acid) (PLLA) nanofibers because of their improved crystallinity.[2] In comparison with most of the reported post-treatment methods, thermal annealing has great potential to be utilized in some biomedical fields due to its mild processing condition and elimination of the organic agents,[3] such as the tissue engineering and drug delivery.[4,5] In spite of these studies, the profound effect of thermal annealing on the surface morphology, thermal behavior, mechanical performance, and cell behavior of the tissue engineering scaffold is still not evaluated systemically.

Electrospinning technology is an attractive tool for the production of tissue engineering scaffolds because of its ability to mimic the geometry and surface topography of extracellular matrix.[6–8] Moreover, the electrospun scaffolds possess an interconnected three-dimensional (3D) structure, a high surface-to-volume ratio, and a variable fiber diameter. Although the electrospun scaffolds have already fulfilled many requirements for tissue engineering applications, they still lack the necessary biomechanical properties for the reparation of hard-mineralized defects. Generally, the stiffness and strength of scaffolds must be high enough to withstand the forces, so that the porous structure will not collapse during the patient's normal activities.[9,10] However, scaffolds with the capacity to withstand high load are always at the expense of losing the interconnected porous structure, which finally hinders the internal flow required to cell colonization. In order to solve the problem, several research groups have proposed to induce the interfiber bonding among the electrospun nanofibers.[11] However, this approach leads to the morphological changes of porous structure, including the gross appearance, porosity, fiber diameter, and so on. The addition of tricalcium phosphate, fibers, and some other reinforced fillers into the polymeric matrices is another enhancement method.[12] Nevertheless, the problems of interfacial compatibility along with biodegradability make the modification not efficient in clinical practice.



Scheme 1 Schematic illustrations of electrospinning process to prepare PCL membrane, and improvement of surface smoothness and fiber bonding after thermal annealing.

In the present work, thermal annealing is firstly employed to optimize the physical properties of electrospun PCL, with regard to surface roughness, mechanical properties, and thermal and degradation behaviors, meanwhile the relationship between structural organization and mechanical performance is revealed. It is of great interest to find that the electrospun matrix presents smoother surface and smaller contact angle after annealing. More importantly,

their Young's modulus and tensile strength increase drastically due to the improved crystallinity. Not only a comparative analysis of the physical changes is presented, but also the additional data are exhibited on the *in vitro* response of cells to these structural signals. Taken together, the improved physical properties and excellent cell proliferation lay a critical basis when the annealed electrospun PCL mat is being considered as a well-suited candidate of tissue engineering scaffolds.

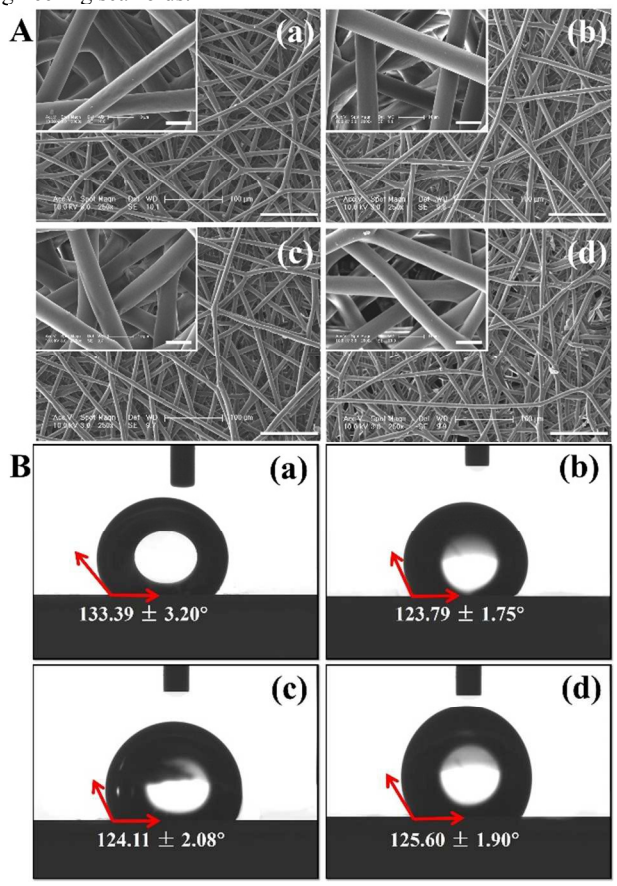


Fig. 1 Representative SEM micrographs (A) and contact angle images (B) of electrospun PCL membranes without annealing (a) and with annealing under different annealing temperatures: T_a = 35 °C (b), T_a = 42 °C (c), and T_a = 50 °C (d). Scale bar represented 10 and 100 μm in the enlarged and original micrographs, respectively.

The chloroform solution of poly(ε-caprolactone) (PCL) (10 wt.%) was electrospun with an injection rate of 0.1 mm min^{-1} and an electrical field of 15 kV (Scheme 1). Before characterization, these electrospun membranes were annealed at different temperatures of 35, 42, and 50 °C for 12 h. The choice of these experimental temperatures is dependent on the fact that the recrystallization of some microcrystals and the formation of perfect crystallites are always realized within such temperature range, and the long annealing time is to ensure complete rearrangements of polymer chains. The effect of thermal annealing on the physical and biological properties of scaffolds is analyzed, including surface roughness, tensile strength, thermal property, degradation behavior, and *in vitro* response of cells to these physical changes.

For many processing methods that involved annealing course, it is always problematic to assume that the surface properties of the resultant products are equivalent to those of the unannealed ones. Fortunately, the results in this work reveal the different phenomena. Fig. 1A shows the typical morphologies of electrospun PCL membranes without and with annealing at various temperatures. The fiber diameter and its distribution are not significantly different before and after thermal annealing, which suggests that thermal annealing is quite a mild post-treatment method and would not cause severe damages to the fibrillar structure. Noticeably, the as-spun sample exhibits relatively rough and uneven surface, while all mats after annealing present smooth surface morphologies. The driving force for the reduction in roughness is the diminishment of the contact surface area between polymer and air in the process of annealing.[13]

It is well-known that the roughness of a solid surface can affect the hydrophilicity. In this work, the hydrophilicity of electrospun PCL membranes without and with annealing at 35, 42, and 50 °C is quantitatively analyzed by the contact angle measurement, as illustrated in Fig. 1B. Before thermal annealing, the contact angle of the as-spun membranes is (133.39 ± 3.20)°. When the annealing temperature ranges from 35 – 50 °C for 12 h, the contact angles decrease and maintain at around 124°, indicating the increase of hydrophilicity. It should be noted that the distinction in the hydrophilicity is quite inconspicuous among the electrospun films annealed under different temperatures. It has been reported that the contact angles of as-spun mats depend on the sizes of the polymer fibers.[14] In this regard, the electrospun PCL mats prepared under the same electrospinning condition but various annealing temperatures possess similar fiber diameters and distributions, so their contact angles exhibit no obvious difference.

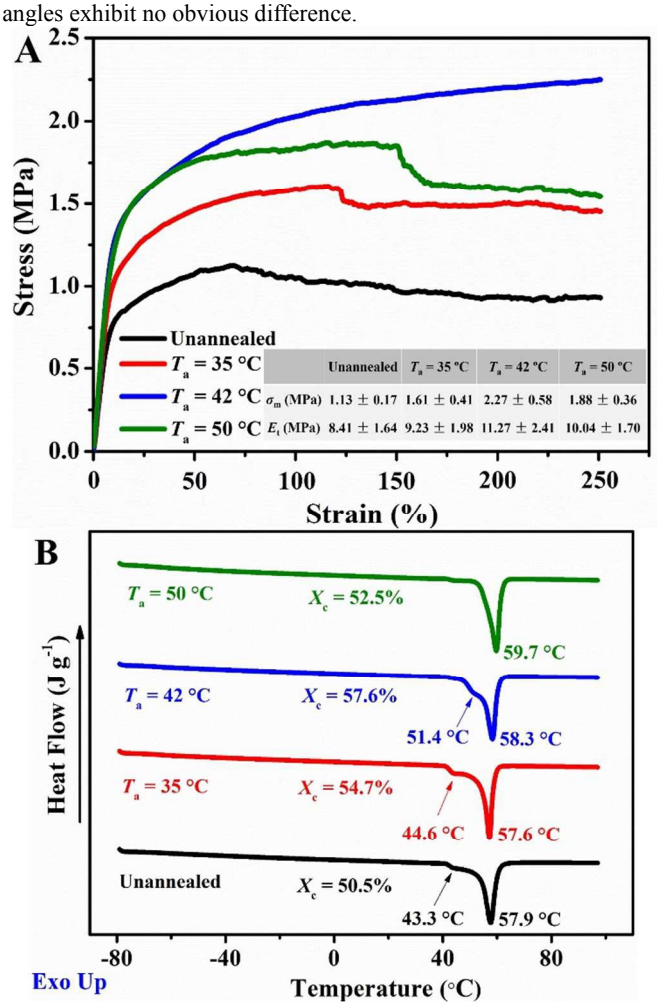


Fig. 2 Representative stress-strain curves (A) and DSC scans (B) of electrospun PCL membranes without and with annealing at different temperatures.

The mechanical properties of electrospun PCL mats without and with thermal annealing at different temperatures are shown in Fig. 2A. It is clearly seen that the Young's modulus as well as the breaking strength of membranes are dramatically improved after annealing. Especially, when the sample is annealed at 42 °C, the Young's modulus rises from 8.41 ± 1.64 to 11.27 ± 2.41 MPa, and the breaking strength (*i.e.*, 2.27 ± 0.58 MPa) is approximately 2 times higher compared with that of the unannealed one (*i.e.*, 1.13 ± 0.17 MPa). The dramatic increase in mechanical performance can be explained from the change in crystallinity, as shown in Fig. 2B. With the treatment of thermal annealing, the crystallinity of electrospun PCL film increases promptly. After annealing at 42 °C, the crystallinity increases from 50.5% to a high level of 57.6%. It can be inferred from the thermograms that a higher crystallinity obtained after annealing is mainly due to the crystallization of noncrystalline region. Electrospinning is a rapid process that does not provide sufficient time for crystallization. Some of the amorphous chains rearrange their configuration to form more closely-packed crystallites during the annealing course.[15] With the increase of annealing temperature from 35 to 42 °C, a more flexible mobility of polymer chain results in a higher recrystallization of some microcrystals and the upregulated degree of crystal perfection. While, in terms of the electrospun membranes annealed at 50 °C, the disentanglement of PCL molecular chain hinders the nucleation and subsequent crystal growth at a degree. Apart from the increase of crystallinity, an obvious change in the melting behavior is also presented. In terms of the unannealed sample, a low-temperature melting peak of 43.3 °C is shown, which might be attributed to the imperfection of crystallites. Whereas the position of such melting peak slightly shifts to a higher temperature when the mats are annealed, further verifying that larger and more perfect crystallites are formed. On the whole, annealing is an attractive method for the preparation of scaffolds with ordered microscopic structures and enhanced mechanical properties.

By and large, a tunable degradation rate of scaffold needs to be similar to the rate of tissue formation, which provides the necessary three-dimensional space for a new tissue. In this work, the short-term *in vitro* biodegradation of electrospun PCL scaffolds is carried out in phosphate-buffered saline (PBS) containing α-chymotrypsin (Fig. 3A). In comparison with the as-spun mats, the biodegradation rates of the annealed PCL samples exhibit a trend of decrease. In detail, the weight loss of unannealed membrane is nearly 11% after 6 days incubation, whereas the annealed samples undergo a relatively slower degradation rate in the range of 6.7 – 9.2% under the same condition. The surface morphologies of electrospun PCL membranes change upon biodegradation and are shown in Fig. 3B. Consistently with the weight loss data, the unannealed membrane presents a significant α-chymotrypsin-catalyzed biodegradation, as evidenced by the appearance of thinner fiber and porous structure. On the contrary, fabrics with annealing treatment just reveal slight surface erosion, where only some tiny pores are observed. As once reported by Kikkawa *et al.*, a preferential hydrolysis took place in the free amorphous region rather than in the restricted orderly region,[16] because a more flexible polymer chain could adapt to an enzyme conformation better than a more rigid polymer chain for the enzyme-catalyzed biodegradation. Thus, it is reasonably concluded that the degradation performances of electrospun PCL scaffolds can be regulated and controlled by varying the annealing conditions.

The cellular adhesion ability is critical to the electrospun PCL scaffolds that used for tissue engineering. In this work, the adhesion of osteoblast cells (*i.e.*, MC3T3-E1 cells) on the electrospun membrane without or with annealing under different conditions is compared after culture for 24 h (Fig. S1, ESI†). It is observed that all the cells adhere effectively on the films and grow well. Especially for the annealed electrospun membranes, more dense cells are presented on its surface compared with the as-spun mat, which is ascribed to the improved surface morphologies and enhanced interfacial adhesion.[17] In order to detect the long-term cytocompatibility of various electrospun PCL scaffolds, the proliferation of MC3T3-E1 cells is examined by both live/dead staining (Fig. 4A) and Cell Counting Kit-8 (CCK-8) assays (Fig. 4B). No dead cells are observed during the proliferation course, indicating that the fabricated PCL scaffolds possess excellent cellular viabilities and great potential for the application in tissue engineering. It is worthy to note that the cell number on the membrane annealed at 42 °C is the most among all situations, which exhibits above 9.5 times increase of cellular number in relative to that of the first day. The improved hydrophilicity and the highest mechanical properties of scaffolds are two key factors to accelerate cell proliferation.[18]

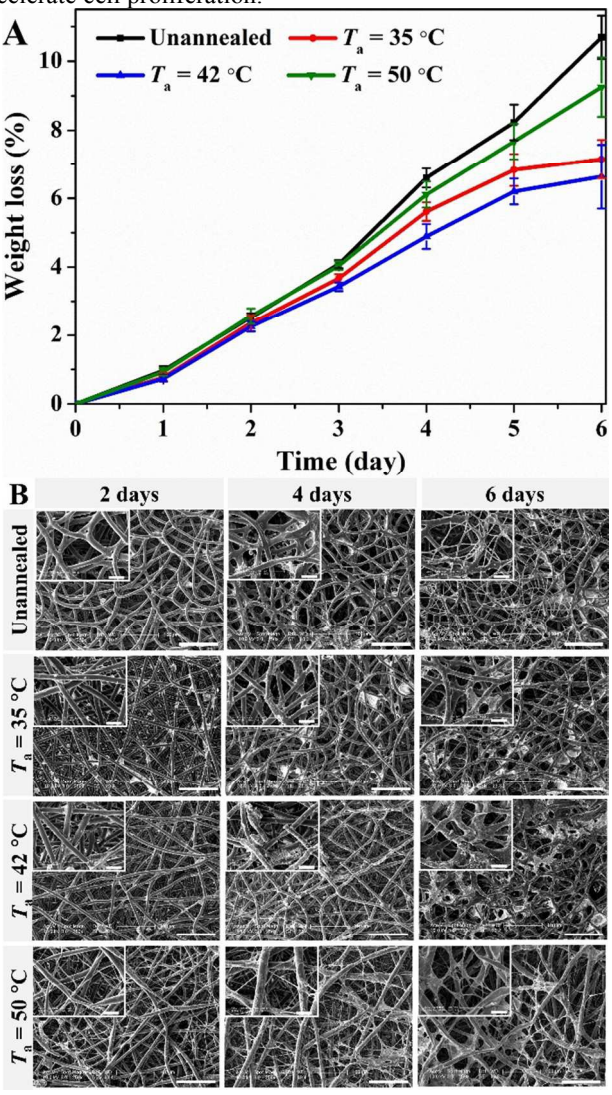


Fig. 3 Effect of annealing temperature on enzyme-catalyzed biodegradation properties of electrospun PCL membranes (A); SEM microimages of morphological changes of electrospun PCL membranes without and with annealing at various temperatures after incubation for 2, 4, or 6 days (B). Scale bar represented 20 and 100 μm in the enlarged and original micrographs, respectively. The statistic data were represented as mean ± standard deviation (n = 3).

Conclusions

For bone reconstruction, the fabrication of biodegradable scaffolds with an interconnected porous structure and adequate mechanical strength has been a formidable challenge nowadays. Annealing is a reliable modification approach due to its mild processing conditions and effective elimination of the organic solvents, which is developed to optimize the biomechanical properties of electrospun PCL scaffolds in this study. It is demonstrated that the annealing process does not cause any severe damages to the fibrillar structure, including fiber diameter and porosity of the scaffolds. Interestingly, the scaffolds are effectively achieved with improved hydrophilicity, higher crystallinity, enhanced mechanical strength, and optimized cell proliferation rate by annealing the samples under different temperatures, which may be an important technique for creating scaffolds with greater success on bone tissue reparation.

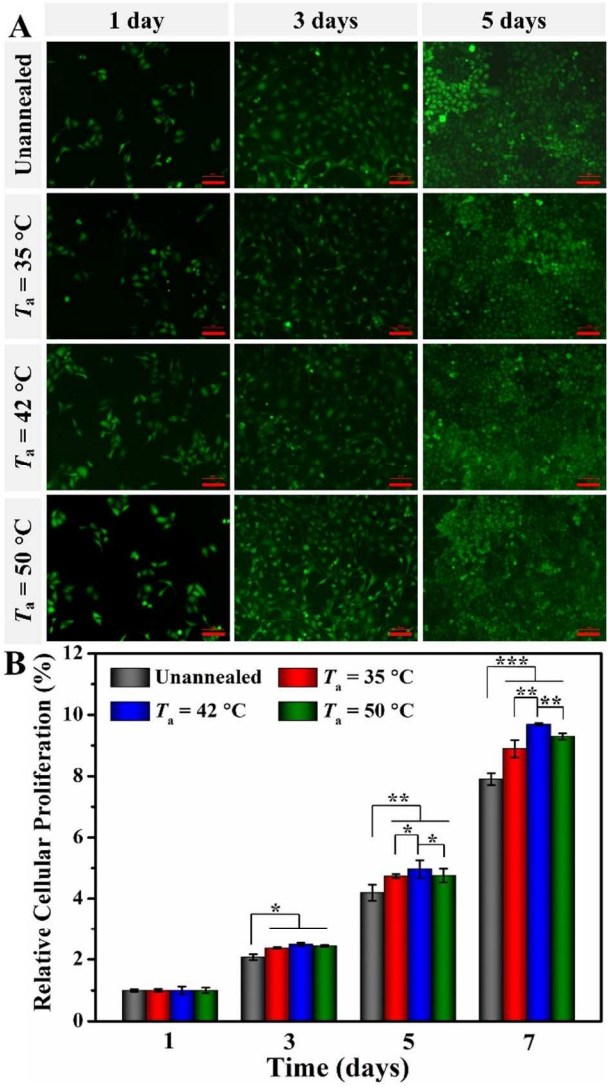


Fig. 4 Fluorescence microscope photographs (A) and relative cellular proliferation (B) of MC3T3-E1 cells on electrospun PCL scaffolds without or with annealing at different temperatures cultured *in vitro* for 1, 3, 5 or 7 days (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$). Viable cells were stained green with calcein-acetoxymethyl ester (calcein AM), and dead cells were dyed red by propidium iodine (PI). Scale bar represented 100 μm in the micrographs. The data were represented as mean ± standard deviation ($n = 3$).

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Notes and references

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