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Nanofibrous membrane of collagen–polycaprolactone for cell growth and tissue regeneration

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Abstract Nanofibrous substrates of synthetic polymers including polycaprolactone (PCL) have shown considerable potential in tissue regeneration. This paper reports the use of PCL/collagen nanofibers to improve the in vitro osteoblastic responses for the applications in bone regeneration area. Collagen and PCL were dissolved in a co-solvent, and the resulting solution was electrospun into a nanofibrous web. Nonwoven fibrous matrices were successfully produced at various compositional ratios (PCL/collagen = 1/3, 1 and 3 by weight). Although the PCL nanofiber was hydrophobic, the presence of collagen significantly improved the water affinity, such as the water contact angle and water uptake capacity. Tensile mechanical tests showed that the collagen–PCL nanofiber had a significantly higher extension rate (approximately 2.8-fold) than the PCL while maintaining the maximum tensile load in a similar range. The osteoblastic cells cultured on the collagen–PCL nanofibrous substrate showed better initial adhesion and a higher level of growth than those cultured on the PCL nanofiber. Furthermore, real-time RT-PCR revealed the expression of a series of bone-associated genes, including osteopontin, collagen type I and alkaline phosphatase. The expression of these genes was significantly higher on the collagen–PCL nanofiber than on the PCL nanofiber. When subcutaneously implanted in mouse the collagen–PCL membrane facilitated tissue cells to well penetrate into the nanofibrous structure at day 7, whilst no such cell penetration was noticed in the pure PCL nanofiber. Overall, the presence of collagen within the PCL nanofiber improves the water affinity, tensile extension rate, and the tissue cell responses, such as initial adhesion, growth, penetration and the expression of bone-associated genes. Therefore, the collagen–PCL nanofibrous membrane may have potential applications in the cell growth and bone tissue regeneration.

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1 Introduction

The recent development of medical polymers has created new scaffolds with a nanofibrous structure, known as 'nanofibers', using electrospinning [1, 2]. A range of degradable polymers in the synthetic base, including poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(caprolactone) (PCL), and their copolymers, have been produced into a fibrous structure with diameters ranging from tens of nanometers to a few micrometers [1–5]. Many studies have reported the performance of polymeric nanofibers as a matrix for the regeneration of defective or degenerative tissues, including vascular grafts, skin substitutes, peripheral nerve regeneratives, bone tissue engineering matrices and periodontal membranes [3–10].

The degradation rate and cell compatibility of the polymeric materials are important parameters to consider, and are dependent mainly on the composition of the polymers. Therefore, tuning of the internal and/or surface

composition to the properties of tissues to be recovered is a critical issue in the development of tissue regenerative matrices [11, 12]. Unfortunately, synthetic polymers produced thus far have innate hydrophobicity that is unsuitable for guiding initial protein adsorption, cell adhesion and subsequent cell growth [13, 14]. In practice, confirming the cell population is essential before the polymeric nanofibers can be used as a matrix for tissue engineering [15, 16].

Some studies have modified the surface and internal properties of the polymeric nanofibers to improve the cell compatibility and tissue regeneration potential. The addition of hydrophilic polymers, such as polyethylene oxide and poly(vinyl alcohol), has shown enhanced cell adhesion and activity [17]. Moreover, coating the surface with gelatin or collagen was reported to improve the responses of endothelial cells [18, 19]. The authors recently reported that the addition of gelatin to PLA improved the cell responses significantly, such as the initial cell adhesion and osteoblastic activity [20].

Based on previous studies, we herein incorporated collagen within a PCL solution and generated a nanofiber with a collagen–PCL composition using an electrospinning process. The effects of collagen on the nanofiber properties and tissue cell behaviors including cell population and osteogenic differentiation in vitro and cell penetration in vivo were investigated.

2 Materials and methods

2.1 Preparation of nanofibers

Collagen type I (ateolocollagen, RegenMed Co., Korea) and poly(ε-caprolactone) (PCL, $M_w = 80{,}000$, Aldrich) were dissolved separately in 2,2,2-trifluoroethanol (TFE, Aldrich). The concentrations of collagen and PCL were 5 wt% and 10 wt%, respectively. Each solution was mixed at various volume ratios, collagen: PCL = 1/3, 1 and 3. The mixtures were stirred vigorously for 24 h and subjected to electrospinning. The electrospinning conditions were as follows: injection rate of 0.2 ml/h, a voltage of 15 kV and a distance of 10 cm. After electrospinning 10 ml of each solution, the collected products were dried under vacuum overnight to evaporate the remnant solvent.

2.2 Characterization

The morphology of the electrospun samples was examined by scanning electron microscopy (SEM, Hitachi). The diameter of the nanofibers was calculated from the SEM images. The water affinity of the nanofibers was examined by measuring the contact angle in response to distilled water. The water uptake capacity of the electrospun samples was examined by immersing the samples in a phosphate buffered solution (PBS) at 37°C for up to 24 h. The degradation behavior in PBS was also observed for up to 56 days.

2.3 Mechanical tensile test

The samples were prepared with dimensions of 3 mm × 25 mm (a gauge length of 20 mm) for the mechanical test using Instron (Model 3344). A tensile load was applied at a stretching speed of 2 mm/min in ambient conditions. The load-displacement curve of each sample was recorded, and the maximum load and extension at failure were obtained. A total of ten specimens were tested for each condition.

2.4 In vitro cell culturing

The pre-osteoblastic cell line, MC3T3-E1, was used to examine the cell behavior on the nanofibrous membranes. The cells were cultured in α-modified minimum essential medium (α-MEM) supplemented with 10% fatal bovine serum (FBS) and containing 100 units/ml penicillin G sodium, 100 μg ml^{-1} streptomycin sulfate, and 0.25 μg ml^{-1} amphotericin B in an incubator humidified containing a 5% CO_2/95% air atmosphere at 37°C. The nanofibrous samples were sterilized with 70% ethanol and placed onto the culture well. The cells were seeded on each sample at a density of 2×10^4 cells/ml and cultured for different periods.

2.5 Cell adhesion and viability

The cells adhered to and grown on the nanofibrous membranes (size of 10 mm × 10 mm) were observed by confocal laser scanning microscopy (Carl Zeiss LSM 510) at 3 h and 3 days. The cell viability was measured from the mitochondrial NADH/NADPH-dependant dehydrogenase activity using a cell proliferation assay kit (CellTiter 96 Aqueous One Solution, Promega). At each culturing time (3, 7, 14 days), the cells were harvested, the MTS reagent was added, and subjected to a colorimetric measurement using a spectrophotometer at A490 nm. Data were averaged from triplicate tests.

2.6 Gene expression by real-time PCR

The expression of the bone-associated genes, including collagen type I (Col), osteopintin (OPN), alkaline phosphatase (ALP), was confirmed by quantitative real-time polymerase chain reaction (PCR). Cells were seeded on

triplicate samples for each group and tested at the culturing times of 1, 3 and 5 days. The first strand cDNA was synthesized from the total RNA (2 μg) using a SuperScript first strand synthesis system for real-time PCR (Invitrogen) according to the manufacturer's instruction. The reaction mixture was made up to 50 μl. Real-time PCR was performed using SYBR GreenER qPCR SuperMix reagents (Invitrogen) and a Bio-Rad iCycler. The relative transcript quantities were calculated using the ΔΔCt method with GAPDH, as the endogenous reference gene amplified from the samples. The primer sequences of the genes are summarized in Table 1.

Table 1 Primer sequences of the genes for the real-time RT-PCR

Gene	Primer sequence
ColI	(f) 5′-GCATGGCCAAGAAGACATCC-3′
	(r) 5′-CCTCGGGTTTCCACGTCTC-3′
OPN	(f) 5′-GACCACATGGACGACGATG-3′
	(r) 5′-TGGAACTTGCTTGACTATCGA-3′
ALP	(f) 5′-GCATGGCCAAGAAGACATCC-3′
	(r) 5′-CCTCGGGTTTCCACGTCTC-3′
GAPCH	(f) 5′-CCCTGTTGCTGTAGCCGTA-3′
	(r) 5′-CCGGTGCTGAGTATGTCG-3′

2.7 Statistics

Statistical analysis was carried out using ANOVA followed by a Bonferroni correction. The significance level was set to $P < 0.05$.

2.8 In vivo pilot study

In vivo tissue responses to the electrospun membranes were examined in the subcutaneous tissue of rats for 1 week as a

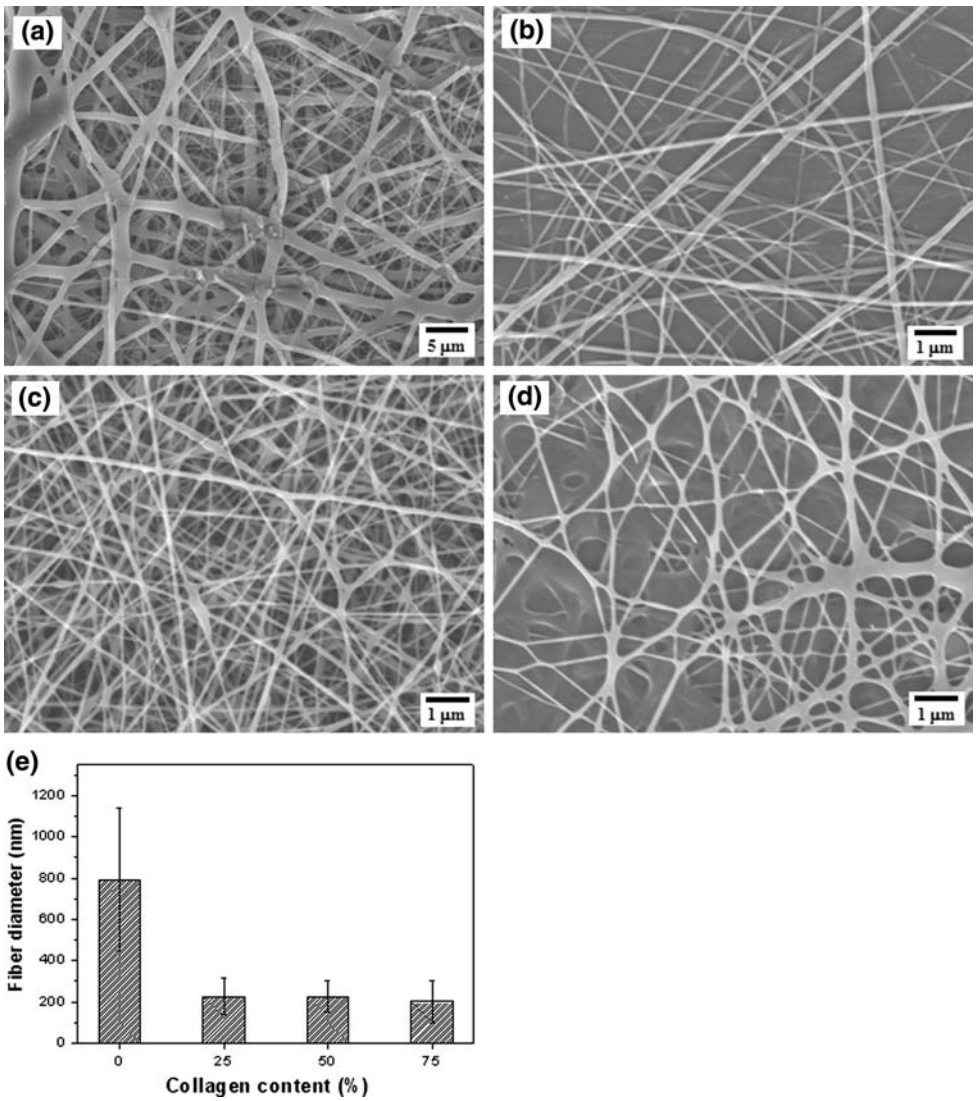

Fig. 1 **a–d** SEM morphology of the electrospun nanofibers obtained with different compositions: **a** pure PCL and **b–d** addition of collagen at **b** 25%, **c** 50%, and **d** 75%. **e** diameters of the nanofibers, as measured from the electron micrographs on arbitrarily selected 100 nanofiber sections of each composition (data are represented as mean ± SD)

pilot study. The animals (BALB/c mice) were anesthetized by intramuscular injection of ketamine hydrochloride/xylazine. After removing the hair on the back with electric clippers, the surgical site was scrubbed with 70% alcohol. A skin incision was then made and was large enough to accommodate the test samples. Pockets were formed by blunt dissection. The nanofibrous membranes (size of 10 mm × 10 mm) were gently placed into each pocket, and the skins were closed by overlapping suture. At 1 week after implantation, animals were sacrificed and the whole graft was removed from dorsal subcutaneous tissue. The samples were fixed in 4% paraformaldehyde overnight and embedded in paraffin. The paraffin blocks were cut into 4 μm sections and stained with hematoxylin & eosin for optical microscopic examination.

3 Results and discussion

3.1 Nanofibers and characteristics

Figure 1 shows the SEM morphology of the electrospun nanofibers with different compositions. Electrospinning into a nanofiber form was possible at all compositions, showing the formation of a nonwoven fibrous sheet without the generation of beads. In pure PCL, some fibers appeared to have excessively large sizes of approximately a few micrometers (Fig. 1a). The addition of collagen into the PCL at 25 and 50 wt% were shown to preserve the electrospinnability, while producing nanofibers with a more uniform size (Fig. 1b, c). A higher level of collagen addition (75 wt%) appeared to reduce the spinnability (Fig. 1d). The diameter of the electrospun fibers was measured, as shown in Fig. 1e. Compared with the pure PCL fiber with a mean size of ~800 nm, all the collagen-added fibers showed significantly reduced sizes of ~200 nm. The large scattering in the PCL sample was due to the formation of microfibers, as observed in the SEM image. Electrospinning of 10 ml of each solution produced a membrane with a thickness of ~50–100 μm. Based on the electro-spinnability and the nanofiber morphology, PCL-50%collagen (PCol50) was chosen as the composition for further tests in comparison with pure PCL (P100).

The water affinity of the nanofibrous matrix was examined by means of water uptake capacity and contact angle. As shown in Fig. 2a, the weight change of the P100 and PCol50 samples was monitored by immersion in PBS for various periods up to 24 h and measuring the weight of the water-absorbed sample. Initially (within an hour), PCol50 showed a dramatic increase in weight, which was driven by the uptake of water, reaching the maximum value at 6 h (~900%), with a slight decrease at 24 h (~800%). However, the PCL nanofiber initially showed only a small

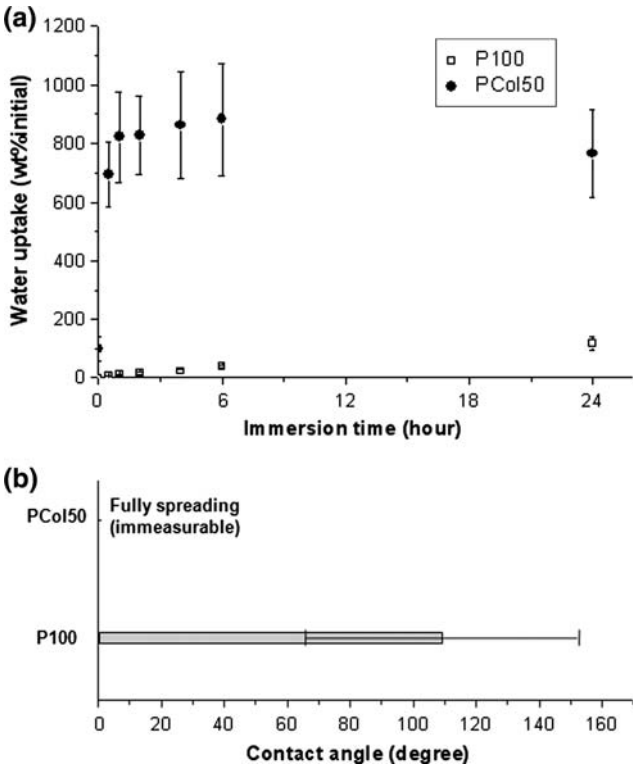

Fig. 2 **a** water uptake capacity of the electrospun nanofibrous web of the pure PCL (P100) and PCL-50%collagen (PCol50) during immersion up to 24 h. **b** contact angle measurements on the nanofibrous web, showing that the water droplet had spread completely on PCol50 while forming an angle of ~110° on pure PCL

change (less than 50%), with a gradual increase up to 24 h (~100%). As shown in Fig. 2b, the contact angle on the P100 sample was about 110°, indicating a high degree of hydrophobicity. However, the water in contact with PCol50 nanofiber permeated rapidly and spread totally, making it impossible to measure the contact angle. Based on the results, the addition of collagen into PCL proved to significantly enhance the water affinity and absorption of the nanofiber matrix.

The degradation behavior of the nanofibers was observed by incubating in PBS for up to 8 weeks (56 days). The decrease in weight of the nanofibers was measured by immersion in PBS for various periods and measuring the weight of the dried samples, as shown in Fig. 3a. The PCol50 sample showed a gradual weight reduction for up to ~28 days (~38% total), with a further slight change up to 56 days (~42% total). However, the P100 sample showed very little change in weight for up to 56 days (within ~5%). Results clearly show the PCol50 nanofiber has a higher rate of degradation compared with P100, particularly during the relatively early period (about 14 days). This difference was attributed to the existence of collagen, which would be dissolved as a major component particularly at the initial period. The remaining PCL is

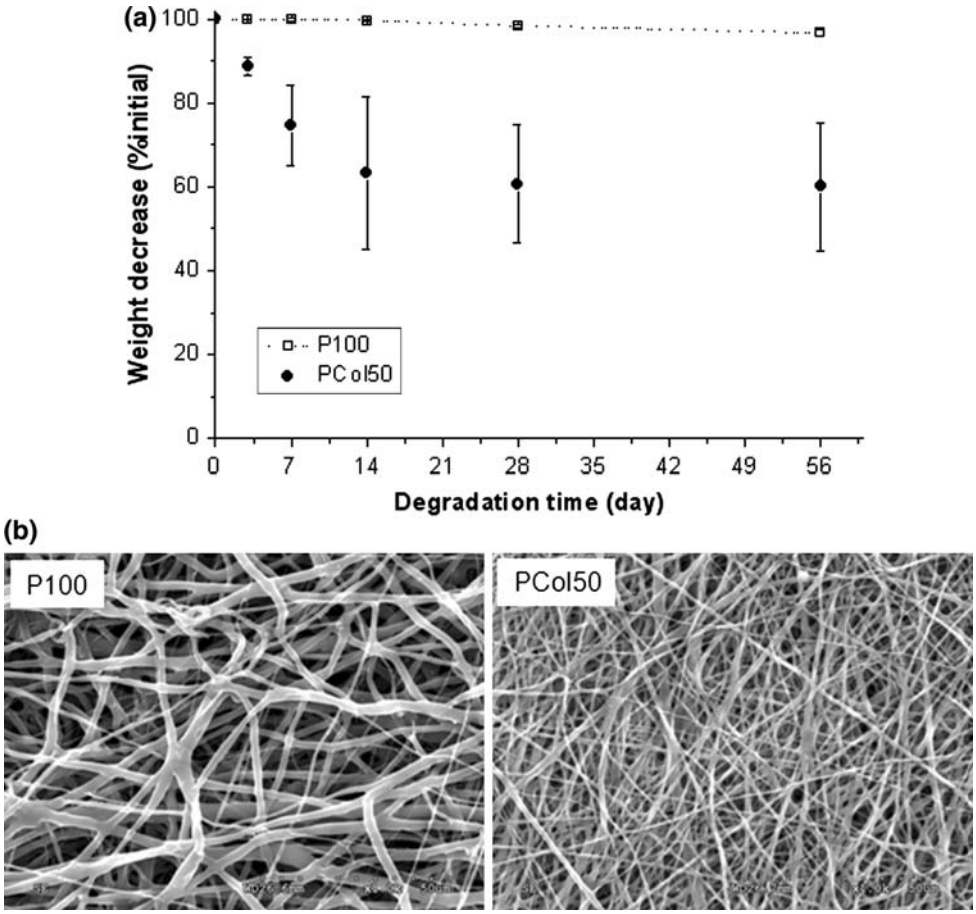

Fig. 3 Degradation tests of the electrospun nanofibrous webs of pure PCL (P100) and PCL-50% collagen (PCol50): **a** weight change during immersion in PBS for up to 8 weeks, and **b** morphological change observed by SEM after degradation for 8 weeks

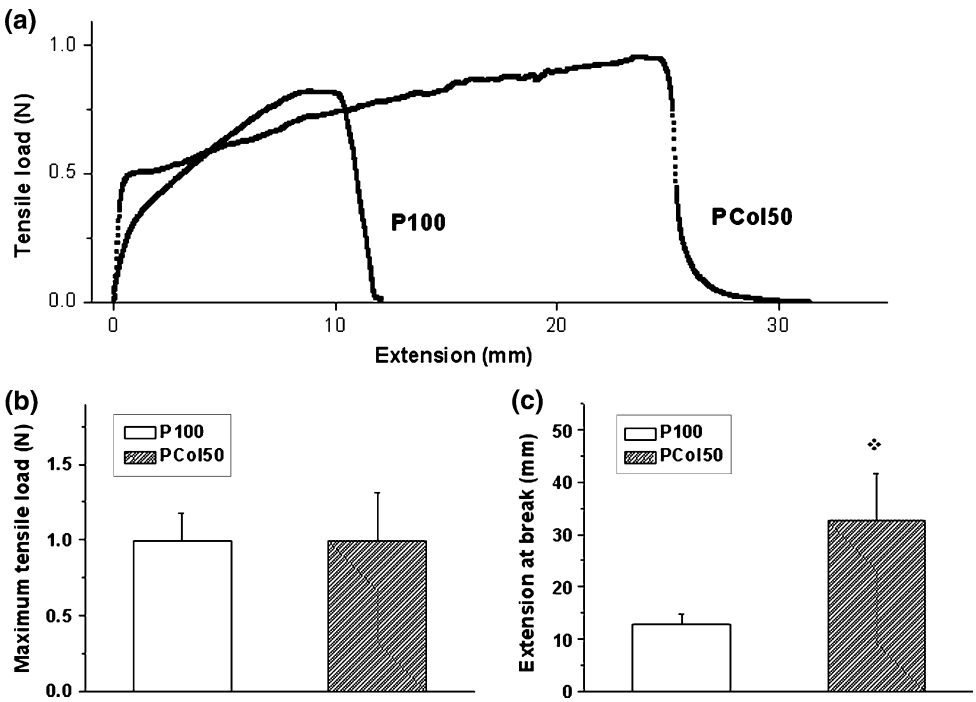

Fig. 4 Mechanical tests of the nanofibrous webs: **a** representative load-extension curves of the nanofibrous samples in response to a tensile loading, **b** measurement of the maximum tensile load, and **c** extension at break. The tensile load was applied to the nanofibrous web prepared with a dimension of ~ 3 mm width $\times$ 20 mm gauge length. The maximum load applied before failure and the extension at break were measured on ten different specimens and averaged. The data comparison between the groups on $n = 10$ by ANOVA showed a significant difference ($^*P < 0.05$) in extension at break while there was no difference in the maximum tensile load

considered to degrade slowly over a longer time. Although the collagen in the PCol50 dominated the initial degradation, the dissolved sample showed an almost identical morphology to that of the initial as-spun sample, as shown in Fig. 3b, and this is deemed that the collagen was mixed homogeneously with PCL in the nanofiber.

Figure 4 shows the tensile mechanical properties of the nanofibrous web. The load-displacement curves of P100 and PCol50, as shown in Fig. 4a, revealed a dramatic difference in the elongation behavior between the samples while showing similar trends in the initial resistance and maximum load recorded. As also shown in Fig. 4b and c, the elongation rate was significantly higher in the PCol50 nanofiber (~2.8-fold increase) but the maximum load was similar. It was deduced that the added collagen made the nanofiber more effective in the absorption of energy against an applied load through the higher degree of elongation. Interestingly, when the applied load was withdrawn the elongated PCol50 nanofiber recovered its initial shape. As a further work it would be meaningful to examine the responses under dynamic loading conditions as well as the properties related to the shape recovery and visco-elasticity of the collagen–PCL nanofibrous web.

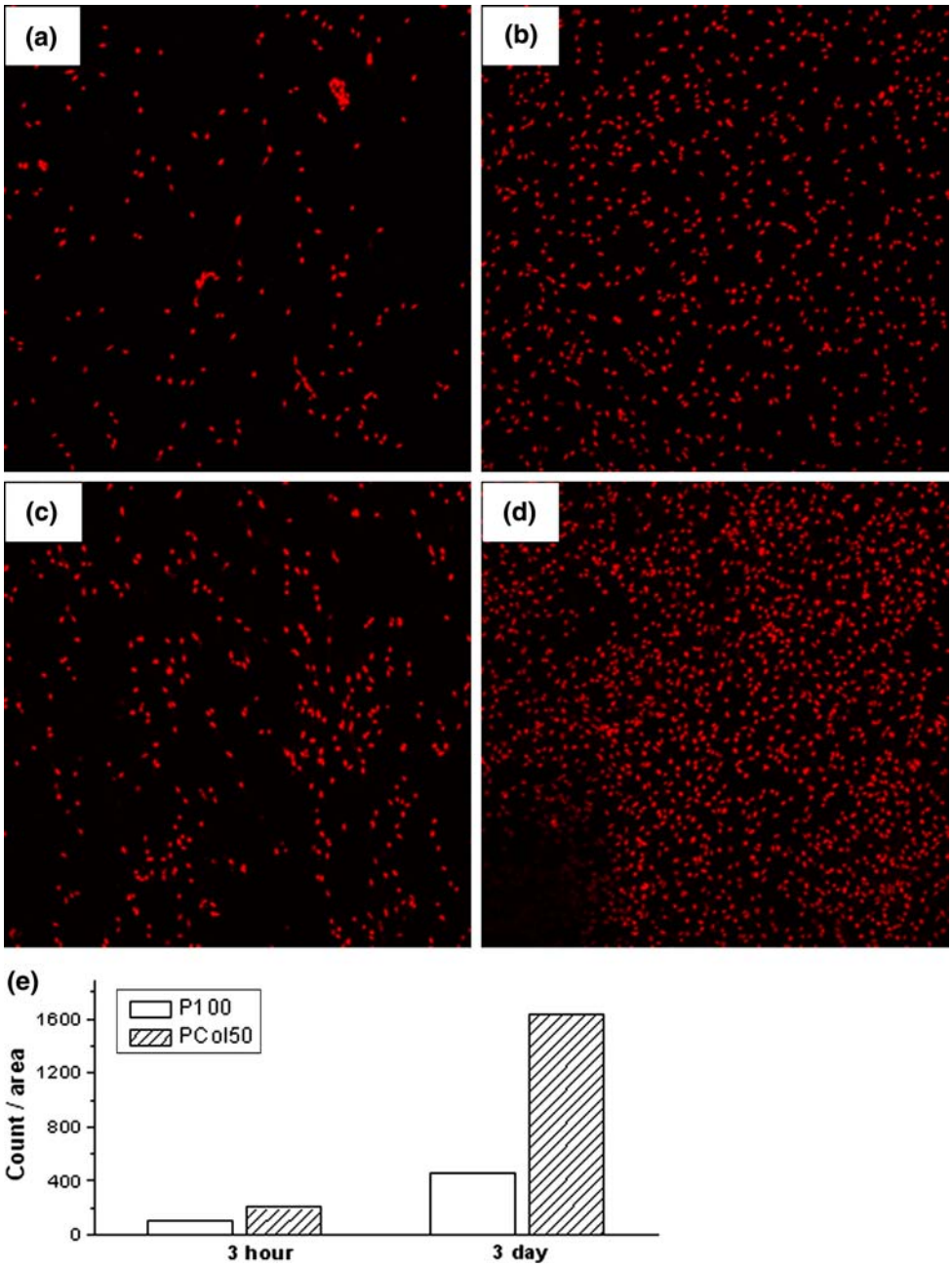

Fig. 5 Confocal z-line reconstructed images with Propidium Iodide (PI) performed 3 h (**a**, **c**) and 3 d (**b** ,**d**) after seeding of MC3T3-E1 on P100 (**a**, **b**) and PCol50 (**c**, **d**). The cell nuclei were shown in red (magnification: ×100). **e** Counts of stained nuclei, showing approximately 2-fold and 3-fold higher level in PCol50 than in P100 at 3 h and 3 days, respectively

Overall, the addition of collagen to PCL improves the water affinity significantly, accelerates degradation and improves the mechanical elongation of the nanofibrous matrix. In further experiments, the in vitro biological responses of the nanofibers were investigated using osteoblastic cells.

3.2 Tissue cell responses

The cellular responses of the collagen–PCL nanofibrous web were addressed in terms of the cell adhesion and growth and the expression of bone-associated genes. Figure 5 shows the presence of cells on the P100 (Fig. 5a, b) and PCol50 (Fig. 5c, d), as revealed by the nuclei staining and CLSM construction after culturing the cells for 3 h (Fig. 5a, c) and 3 days (Fig. 5b, d). The initially adherent cells for 3 h were more abundant on the PCol50 (Fig. 5c) than on the P100 (Fig. 5a). With further culturing for 3 days, the number of cells increased for both cases and a larger number of cells populated on the PCol50 (Fig. 5d) with respect to P100 (Fig. 5b). The counts of the stained nuclei were presented in Fig. 5e, clearly showing up-regulation in the PCol50 by approximately 2- and 3-fold at 3 h and 3 days, respectively, when compared with P100.

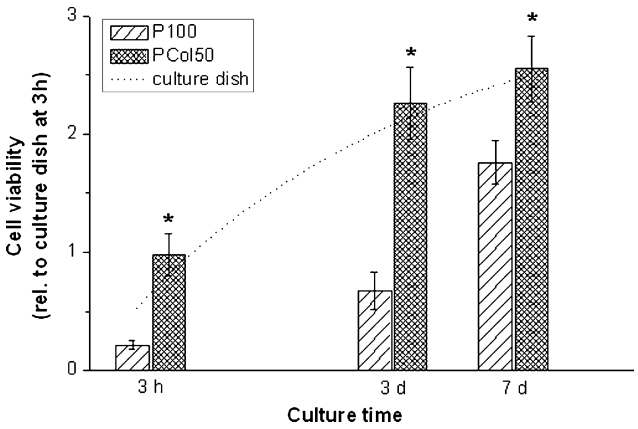

Fig. 6 Cell viability on the nanofibrous substrates, as assessed by the MTS method. A statistically significant difference was observed between P100 and PCol50 (*$P < 0.05$ by ANOVA)

Figure 6 shows the cell viability, as measured by the mitochondrial dehydrogenase activity using an MTS method at 3 h and 3- and 7-days. Data showed that the cells on the PCol50 grew to a significantly higher level than on the P100 ($P < 0.05$) at all culturing periods.

The collagen in the PCL composition clearly demonstrated its favorable effect on the initial adhesion and further growth behavior of cells. In addition to its role in the dramatic increase in the hydrophilicity, which facilitating the rapid supply of a culturing medium within the nanofibrous network as well as the adsorption of serum proteins, collagen, as one of the adhesion molecules, might affect the cell adhesion and further mitosis during the culturing period.

The tissue cell compatibility of the collagen–PCL membrane was confirmed by the in vivo pilot study. Figure 7 presents the tissue response to the membranes subcutaneously implanted in mouse at 1 week following operation. Results showed no significant inflammatory reaction being noticed on both types of membranes, confirming good tissue

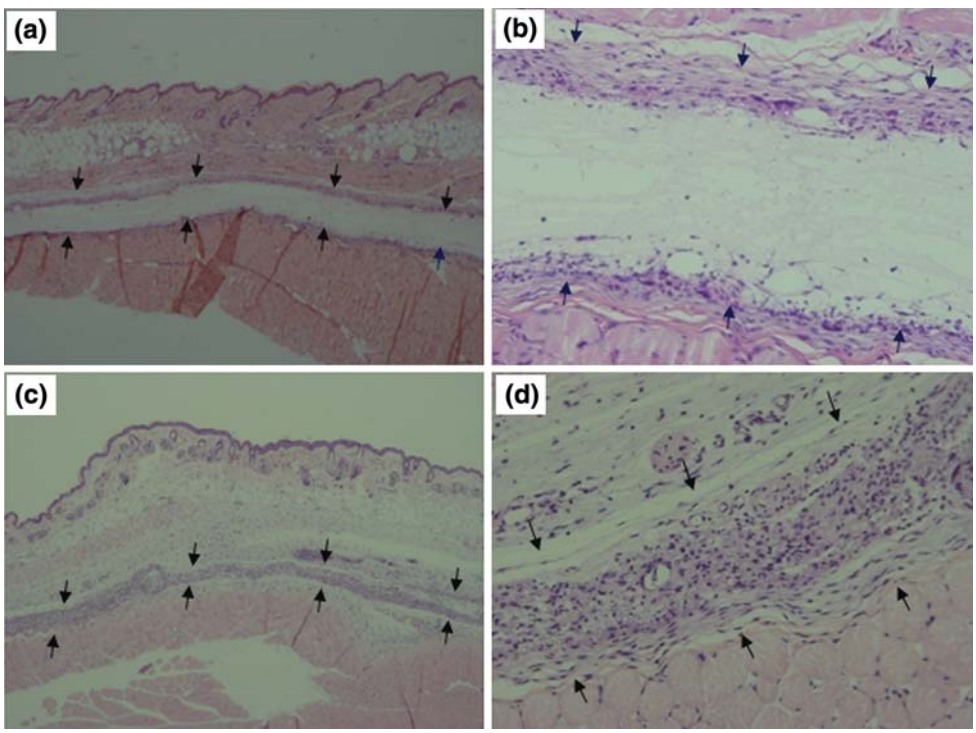

Fig. 7 In vivo tissue reaction to the nanofibrous mesh subcutaneously implanted in mouse at 1 week after operation, and samples were stained with hematoxyline & eosin: (**a**, **b**) P100 and (**c**, **d**) PCol50 at low (×40, (**a**, **c**)) and high magnification (×200 (**b**, **d**)). Magnified images showing the tissue cells penetrated well through the nanofibrous web of PCL-collagen (**d**), whilst such cell penetration was significantly limited in the PCL nanofiber (**b**). Arrows indicate marginal region of the membrane in contact with tissue

cell affinity. Of special note was the tissue cells penetrated well through into the nanofibrous structure of the collagen–PCL membrane, which was not observed in the case of pure PCL. It is considered that the excellent hydrophilicity of the collagen–PCL membrane would facilitate the cell migration into the nanofibrous structure, and this fact supports the usefulness of the collagen–PCL nanofibrous membrane as a tissue engineering scaffold.

In Fig. 8, the intracellular expression of the bone-associated genes including collagen type I, osteopontin, and alkaline phosphatase, on the nanofibrous webs was presented, as quantified by a real-time PCR. Data showed the significant up-regulation of those genes on the PCol50, particularly at day 1 and 3. Both the collagen type I and osteopontin were up-regulated more at day 1 by a factor of ~4, while ALP showed a larger increase at day 3, as much as ~9-fold. This shows that the initial gene expression was stimulated better on the collagen-containing nanofiber than on the PCL nanofiber. It is possible that the significantly enhanced cellular adhesion also favors the gene expression through the intracellular signal transduction, and the collagen itself, which is easily absorbable during the culturing period, should favorably affect the cellular expression of the bone-associated genes [21, 22].

Although further assessments will be needed, such as the bone matrix synthesis and bone formation, the present in vitro study highlights the potential of adding collagen to the PCL nanofibers in terms of the cell viability and initial gene expression steps. These results, in conjunction with the physicochemical traits, suggest the effectiveness of the collagen–PCL nanofibrous matrix in the bone regeneration area.

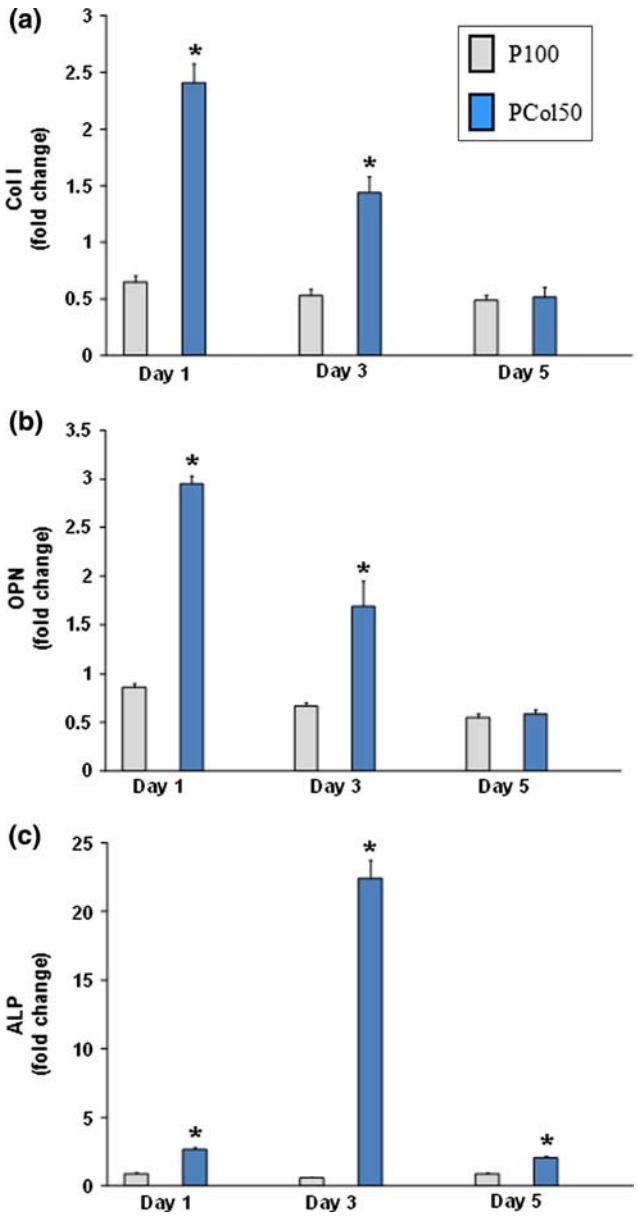

Fig. 8 Gene expression of the cells on the nanofibrous substrate at days 1, 3 and 5, as assessed by real-time RT-PCR: **a** collagen type I (Col I), **b** osteopontin (OPN), and **c** alkaline phosphatase (ALP). A statistically significant difference was observed ($*P < 0.05$ by ANOVA)

4 Concluding remarks

Collagen was added to the PCL composition to produce nanofibers through an electrospinning method. The addition of collagen significantly enhanced the physicochemical properties of the PCL nanofibers, such as the water affinity, degradation behavior and mechanical elongation. The collagen-added nanofiber showed better tissue cell responses, such as cell adhesion and growth in vitro and penetration in vivo. Moreover, the expression of the bone-associated genes, including collagen type I, osteopontin and alkaline phosphatase, were significantly up-regulated on the collagen-added nanofiber. Overall, the collage-added PCL nanofibers may have potential use as a cell supporting substrate in bone regeneration.

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References

1. Sill TJ, von Recum HA. Electrospinning: applications in drug delivery and tissue engineering. Biomaterials. 2008;29:1989–2006. doi:10.1016/j.biomaterials.2008.01.011.
2. Phanm QP, Sharma U, Mikos AG. Electrospinning of polymeric nanofibers for tissue engineering applications: a review. Tissue Eng. 2006;12(5):1197–211. doi:10.1089/ten.2006.12.1197.
3. Chen G, Sato T, Ohgushi H, Ushida T, Tateishi T, Tanaka J. Culturing of skin fibroblasts in a thin PLGA-collagen hybrid mesh. Biomaterials. 2005;26:2559–66. doi:10.1016/j.biomaterials.2004.07.034.

4. Yoshimoto H, Shin YM, Terai H, Vacanti JP. A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering. Biomaterials. 2003;24:2077–82. doi:10.1016/S0142-9612(02)00635-X.
5. Song JH, Kim HE, Kim HW. Production of electrospun gelatin nanofiber by water-based co-solvent approach. J Mater Sci Mater Med. 2008;19:95–102. doi:10.1007/s10856-007-3169-4.
6. Smith MJ, McClure MJ, Sell SA, Bernes CP, Walpoth BH, Simpson DG, et al. Suture-reinforced electrospun polydioxanone-elastin small-diameter tubes for use in vascular tissue engineering: a feasibility study. Acta Biomater. 2008;4:58–66. doi:10.1016/j.actbio.2007.08.001.
7. Park SH, Kim TI, Chung CP, Han SB, Yu JH, Lee SP, et al. Effect of hydroxyapatite-coated nanofibrous membrane on the responses of human periodontal ligament fibroblast. J Ceram Soc Jpn. 2008;116:31. doi:10.2109/jcersj2.116.31.
8. Song JH, Yoon BH, Kim HW, Kim HW. Bioactive and degradable hybridized nanofibers of gelatin-siloxane for bone regeneration. J Biomed Mater Res A. 2008;84A:875–84. doi:10.1002/jbm.a.31330.
9. Kim HW, Song JH, Kim HE. Nanofiber generation of gelatin-hydroxy-apatite biomimetics for guided tissue regeneration. Adv Funct Mater. 2005;15:1988–94. doi:10.1002/adfm.200500116.
10. Corey JM, Lin DY, Mycek KB, Chen Q, Samuel S, Feldman EL, et al. Aligned electrospun nanofibers specify the direction of dorsal root ganglia neurite growth. J Biomed Mater Res A. 2007;83A:636–45. doi:10.1002/jbm.a.31285.
11. Brocchini S, James K, Tangpasuthadol V, Kohn J. Structure-property correlations in a combinatorial library of degradable biomaterials. J Biomed Mater Res. 1998;42:66–75. doi:10.1002/(SICI)1097-4636(199810)42:1<66::AID-JBM9>3.0.CO;2-M.
12. Wool R. The science and engineering of polymer composite degradation. In: Scott G, Gilead D, editors. Degradable polymers and applications. London: Chapman and Hall; 1995. p. 135–52.
13. DanKers PYW, Harmsen MC, Brouwer LA, Van Luyn MJA, Meijer EW. A modular and supramolecular approach to bioactive scaffolds for tissue engineering. Nat Mater. 2005;4(7):568–74. doi:10.1038/nmat1418.
14. Reis RL, Roman S. Degradable systems in tissue engineering and regenerative medicine. London: CRC Press; 2005. p. 177–201.
15. Stevens MM, George JH. Exploring and engineering the cell surface interface. Science. 2005;310:1135–8. doi:10.1126/science.1106587.
16. Kimura Y. Biodegradable polymers. In: Tsuruta T, editor. Biomedical applications of polymeric materials. Boca Raton: CRC press; 1993. p. 163–89.
17. Kim TG, Lee DS, Park TG. Controlled protein release from electrospun biodegradable fiber mesh composed of poly(epsilon-caprolactone) and poly(ethylene oxide). Int J Pharm. 2007;338:276–83. doi:10.1016/j.ijpharm.2007.01.040.
18. He W, Ma ZW, Yong T, Teo WE, Ramakrishna S. Fabrication of Collagen-Coated Biodegradable Copolymer Nanofibers and its potential for endothelial cells growth. Biomaterials. 2005;26:7606–15.
19. Kim HW, Yu HS, Lee HH. Nanofibrous matrices of poly(lactic acid) and gelatin polymeric blends for the improvement of cellular responses. J Biomed Mater Res A. 2007;87A:25–32. doi:10.1002/jbm.a.31677.
20. Kleiman HK, Klebe RJ, Martin GR. Role of collagenous matrices in the adhesion and growth of cells. J Cell Biol. 1981;88:473–85. doi:10.1083/jcb.88.3.473.
21. Celic S, Katayama Y, Chilco PJ, Martin TJ, Findlay DM. Type I collagen influence on gene expression in UMR106-06 osteoblast-like cells is inhibited by genistein. J Endocrinol. 1998;158:377–88. doi:10.1677/joe.0.1580377.
22. Anselme K. Osteoblast adhesion on biomaterials. Biomaterials. 2000;21:667–81. doi:10.1016/S0142-9612(99)00242-2.