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Evaluation of the potential of kartogenin encapsulated poly(L-lactic acid-co-caprolactone)/collagen nanofibers for tracheal cartilage regeneration

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Abstract

Tracheal stenosis is one of major challenging issues in clinical medicine because of the poor intrinsic ability of tracheal cartilage for repair. Tissue engineering provides an alternative method for the treatment of tracheal defects by generating replacement tracheal structures. In this study, we fabricated coaxial electrospun fibers using poly(L-lactic acid-co-caprolactone) and collagen solution as shell fluid and kartogenin solution as core fluid. Scanning electron microscope and transmission electron microscope images demonstrated that nanofibers had uniform and smooth structure. The kartogenin released from the scaffolds in a sustained and stable manner for about 2 months. The bioactivity of released kartogenin was evaluated by its effect on maintain the synthesis of type II collagen and glycosaminoglycans by chondrocytes. The proliferation and morphology analyses of mesenchymal stems cells derived from bone marrow of rabbits indicated the good biocompatibility of the fabricated nanofibrous scaffold. Meanwhile, the chondrogenic differentiation of bone marrow mesenchymal stem cells cultured on core-shell nanofibrous scaffold was evaluated by real-time polymerase chain reaction. The results suggested that the core-shell nanofibrous scaffold with kartogenin could promote the chondrogenic differentiation ability of bone marrow mesenchymal stem cells. Overall, the core-shell nanofibrous scaffold could be an effective delivery system for kartogenin and served as a promising tissue engineered scaffold for tracheal cartilage regeneration.

Keywords

Nanofibers, kartogenin, tracheal cartilage regeneration, bone marrow mesenchymal stem cells, poly(L-lactic acid-co-caprolactone)/collagen, drug delivery, coaxial electrospinning

Introduction

The trachea, a hollow cylindrical organ, is composed of cartilages, muscles, connective tissues, and mucous membranes. Severe injuries to the trachea, such as cancer, trauma, or inflammation can cause tracheal stenosis, which seriously affect normal physiological activities. Based on the above mentioned content, many different methods are used to repair tracheal defects, such as direct anastomosis, autografts, allografts, and prosthetic materials.[1–5] However, there is still no satisfactory solution to this problem. When a tracheal segment longer than 6 cm, anastomosis produce serious postoperative complications.[6] Similarly, allografts are accompanied by lifelong immunosuppressant therapy, which cause severe inflammatory reactions. But tissue engineering might be able to solve this problem, which can self-repair, regenerate, and remodel.[7] Vacanti et al. first reported regeneration of tracheal cartilage using tissue engineering techniques.[8]

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Currently, electrospinning is one of the most practical technologies for the fabrication of tissue engineering scaffolds, because the nanoscale fibers of electrospinning scaffolds can mimic the structure of native extracellular matrix (ECM).[9] The nanoscale fibers are easy to obtain through a simple and versatile electrospinning device. In the course of the fabrication of the fiber, a series of drugs, such as antibiotics[10] and proteins,[11] can be encapsulated in the electrospun mats. Blended electrospinning and coaxial electrospinning are the two main drug delivery methods. Drugs disperse in all parts of fibers prepared by bending electrospinning make a fast and burst release, but drugs loaded in the core of coaxial nanofibers produce a slow and sustained release. Moreover, coaxial fibers can protect bioactive drugs from degeneration.

Growth factors are one of the three key components in tissue engineering.[12] Studies have shown that growth factors such as transforming growth factor betas (TGF-β) can induce chondrogenic differentiation,[13] but proteinaceous growth factors have disadvantages of high cost and poor stability.[14] Therefore, researchers have been focusing on discovering some natural or synthesized small molecules that promote stem cells into chondrocytes.[15] Such as kartogenin (KGN), a heterocyclic molecule, has been discovered to effectively induce chondrogenesis of mesenchymal stem cells (MSCs) using an imaged-based high-throughput screen.[16] KGN functions by binding actin filaments A, and then perturbing the interactions between filamin A and the transcriptions factor core-binding factor β (CBFβ), which increase the amount of CBFβ-RUNX1 dimer, and finally activating the genes that promote stem cells differentiation into chondrocytes.[17]

C-shaped hyaline cartilage, an important part of the trachea, provides mechanical strength and prevent the collapse of the airway, which cause serious postoperative complications.[17] Thus, during the process of cartilages repair, biodegradable tissue engineering scaffolds need to give temporary support to the airway to prevent it from collapsing. P(LLA-CL), a nontoxic and biodegradable synthetic co-polymer, has been approved by US FDA, which have excellent mechanical strength and be widely used in tissue engineering.[18,19] But it lacks bioactivities provided by natural materials. Collagen, the main element of the extracellular matrix, has been reported to induce attachment and proliferation of cells.[20] Therefore, the study added collagen to compensate for the biocompatibility of polymer P(LLA-CL).

In this study, the aim of this work was to develop a coaxial electrospun fiber scaffold containing a small molecule compound KGN, which may promote the differentiation of MSCs derived from bone marrow of rabbits (BMSCs) into chondrocytes for tracheal cartilage repair. To further investigate the feasibility of this scaffold, its properties and functions were studied comprehensively.

Materials and methods

Materials

P(LLA-CL) with a molar ratio of 75% L-lactide was purchased from Dai gang Biomaterial Co., Ltd. (Jinan China). Collagen type I (molecular weight $1–3 \times 10^5$ Da) was purchased from Shandong International Biotechnology Park Co., Ltd. (Yantai, China). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was purchased from Co., Ltd. (Shanghai, China). Kartogenin (KGN) was purchased from Biogems. BMSCs and chondrocytes were provided by Shanghai Children's Medical Center (Shanghai, China). All chemicals were of analytical grade and were used without further purification.

Fabrication of nanofibrous scaffolds

The process of coaxial electrospinning was performed according to previous studies with some modifications.[21] The core solution was obtained by dissolving KGN in a mixture solvent of DMSO/HFIP (with the volume ratio of 10/90) at a concentration of 2.5 mg ml^{-1}. The sheath solution was prepared by dissolving P(LLA-CL) and collagen in HFIP with mass ratio of 75/25 at a concentration of 12%w/v. During coaxial electrospinning process, the solution of P(LLA-CL)&Collagen and KGN solution were delivered to the coaxial outer and inner needles, respectively. The applied high voltage and the electrospinning distance were set at 12 kV and 12 cm. The shell solution was injected at a flow rate of 1.2 ml h^{-1} and the core solution at 0.2 ml h^{-1}. The formed nanofibers were named KGN@PC and collected by a grounded rotating plate covered with aluminum foil. Then the obtained nanofibers were cross-linked in 25% GTA aqueous solution for 30 min.

The same polymer solution of P(LLA-CL)&Collagen was fabricated by traditional electrospinning technique as the control samples. Similarly, the blend P(LLA-CL)&Collagen nanofibrous scaffolds were named PC and cross-linked with GTA vapor mentioned above. The resultant scaffolds were kept at a vacuum drying oven before further characterization.

Characterizations of nanofibrous scaffolds

The surface morphology of the nanofibrous scaffolds were observed under a Digital Vacuum scanning electron microscope (SEM; Phenom XL, Phenom, Netherlands) operated at an acceleration voltage of 10 kV. Diameters of fibers were measured with image visualization software Image J (National Institutes of

Health, USA) and more than 100 counts were performed for each scaffold. To verify the core-shell structure of nanofibers, the fibers were collected on carbon-coated Cu grids and observed by transmission electron microscope (TEM, Hitachi H-800, Japan).

The porosity of the membranes was tested via the ethanol infiltration method.[22] A slice of the membrane was immersed in the ethanol; the volume of ethanol in the measuring cylinder before and after the nanofiber membrane immersion was set as V_1 and V_2, respectively. After 15 min, the membrane was removed from the ethanol, and the remaining volume was marked as V_3; the porosity of the tested membranes was calculated according to equation (1):

$$\text{Porosity } (\%) = \left(\frac{V_1 - V_3}{V_2 - V_3}\right) \times 100\% \quad (1)$$

The water contact angles (WCA) for the nanofibrous scaffolds were applied to study the surface hydrophilicity by a contact angle analyzer (OCA40, Dataphysics, Germany).

The mechanical properties of nanofibrous scaffolds were performed by a universal materials tester (H5K-S, Hounsfield, UK) at an ambient temperature of 25°C and humidity of 65%. The size of samples for this test was $30 \times 10\,mm^2$. A cross-head speed of 10 mm min^{-1} was applied for all specimens during the process.

KGN release profile from KGN@PC nanofibrous scaffolds

The KGN@PC nanofibrous scaffolds (weight of 35 mg) were immersed in 1.8 ml of phosphate-buffered saline (PBS, pH 7.4) and placed in a shaking incubator (150 rpm) at 37°C. For the detection of release kinetics of KGN from KGN@PC nanofibrous scaffolds, total volume of PBS was collected after centrifugation and replaced by the same volume of PBS at each sampling time. The cumulative release profiles were evaluated via HPLC. The samples were analyzed on a Waters C-18 reverse phase column ($4.6 \times 250\,mm^2$, 5 μM) with a mobile phase of acetonitrile/water (v/v, 35/65) plus 0.1% formic acid. The flow rate was set at 1 mL/min and absorbance of CPT was monitored at 274 nm.[23]

The activity of released KGN

Studies have shown that KGN at 100 μM have no toxicity in chondrocytes and maintain chondrocyte phenotype.[17] Thus, the activity of KGN released from scaffold was tested by evaluating its effect on maintain the synthesis of type II collagen (COL2) and glycosaminoglycan (GAG) by chondrocyte. The rat chondrocytes were cultured in DMED/F12. When the confluence was about 90%, the cells were trypsinized and seed into 24-well plates. After culturing for 1 day, the cells were divided into two groups: Group I was cultured with DMEM/F12 supplemented with released solution of KGN (release of 2 week); Group II was only cultured with DMEM/F12. Both of the two groups were cultured for 7 days. Ultimately, the COL2 and GAGs synthesized by chondrocytes were analyzed by immunofluorescence and histological staining, respectively. The images of immunofluorescence and histological staining were randomly divided into four sections for quantitative analysis by Images-Pro Plus 6.0 software.

Cell proliferation and morphology on nanofibrous scaffolds

BMSCs were cultured in α-MEM with 5% Helios UltraGRO^{TM}, 2 U mL^{-1} heparin and 1% pen/strep, under 5% (v/v) CO^2 at 37°C and 95% air humidity. For cell seeding, nanofibrous scaffolds were sterilized under the alcohol steam for 48 h and washed with PBS 3 times. The BMSCs were seeded on to round nanofibrous scaffolds (15 mm in diameter) and tissue culture polystyrene (TCP) as a control group in 24 well plates at a density of 2×10^4 cells cm^{-2}.

To investigate cell proliferation on scaffolds and TCP, cell counting Kit-8 (CCK-8) assay was conducted after culturing for 1, 4, and 7 days to evaluate the number of viable cells. Briefly, the cells on scaffolds and TCP were incubated with 360 μL pure α-MEM and 40 μL CCK-8 solution for 1 h in incubator at 37°C. Subsequently, 100 μL of the solution was pipetted in to 96-well plate and measured the absorbance by a microplate reader (Multiskan MK3, Thermo, USA) under 450 nm. Meanwhile, the viability of cells was determined using Live & Dead cell viability assays after 4 days of culture.

To observe the cells morphology, BMSCs on scaffolds were fixed with 4% paraformaldehyde for 30 min at 4°C after culturing for 4 and 7 days. Thereafter, the scaffolds with cells were dehydrated with gradient ethanol and dried under blowing overnight. Then, the samples were sputter-coated with gold and observed under SEM.

The chondrogenic differentiation of BMSCs on nanofibrous scaffolds

To detect the chondrogenic differentiation of BMSCs cultured on different scaffolds, the gene expression of hyaline cartilage-specific markers such as Sox9 and COL2 were examined using real time-quantitative polymerase chain reaction (RT-qPCR) after culturing 14 and 21 days. Briefly, the RNA was isolated and

converted to cDNA using reverse transcriptase (Applied Systems, Foster City, CA, USA). Real-time polymerase chain reaction (PCR) was performed on an Applied Biosystems 7300 using Taqman primers and probes specific for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and other relative genes. Sequences of the primers used are listed in Table 1. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH as the reference gene.

Statistical analysis

All the data were obtained at least in triplicate and the values were expressed as means ± standard deviation (SD). Statistical analyses were performed by the one-way analysis of variance (ANOVA) using Origin 8.5 (OriginLab Inc., USA). The statistical difference was considered statistically significant at $P < 0.05$.

Results and discussion

Characterizations of nanofibrous scaffolds

KGN@PC scaffold was fabricated by coaxial electrospinning, which allows for electrospinning shell solution and core solution in two separate setups and the fabrication process was shown in Figure 1. PC scaffold was fabricated by traditional electrospinning. The morphology of the fabricated KGN@PC and PC nanofibrous scaffolds were observed by SEM, which is shown in Figure 2. The nanofibers were uniform, smooth, and bead free with interconnected pore. The average diameter of KGN@PC nanofibers (654.31 ± 144.04 nm) was bigger than PC nanofibers (423.91 ± 137.16 nm), indicating the encapsulation of KGN. The porosity of KGN@PC nanofibers (73.7 ± 5.8%) was higher than PC nanofibers (64.5 ± 3.8%). The core-shell structure of single fiber was seen clearly by

Table 1. Sequence of primers used for RT-PCR.

Gene	Forward primer	Reverse primer
GAPDH	5′-TGGCGCTGAGTACGTCGTG-3′	5′-ATGGCATGGACTGTGGTCAT-3′
Sox9	5′-CCCTTCAACCTCCCACACTAC-3′	5′-TCCTCAAGGTCGAGTGAGCTG-3′
COL2	5′-ACGGCGGCTTCCACTTCAGC-3′	5′-TTGCCGGCTGCTTCGTCCAG-3′

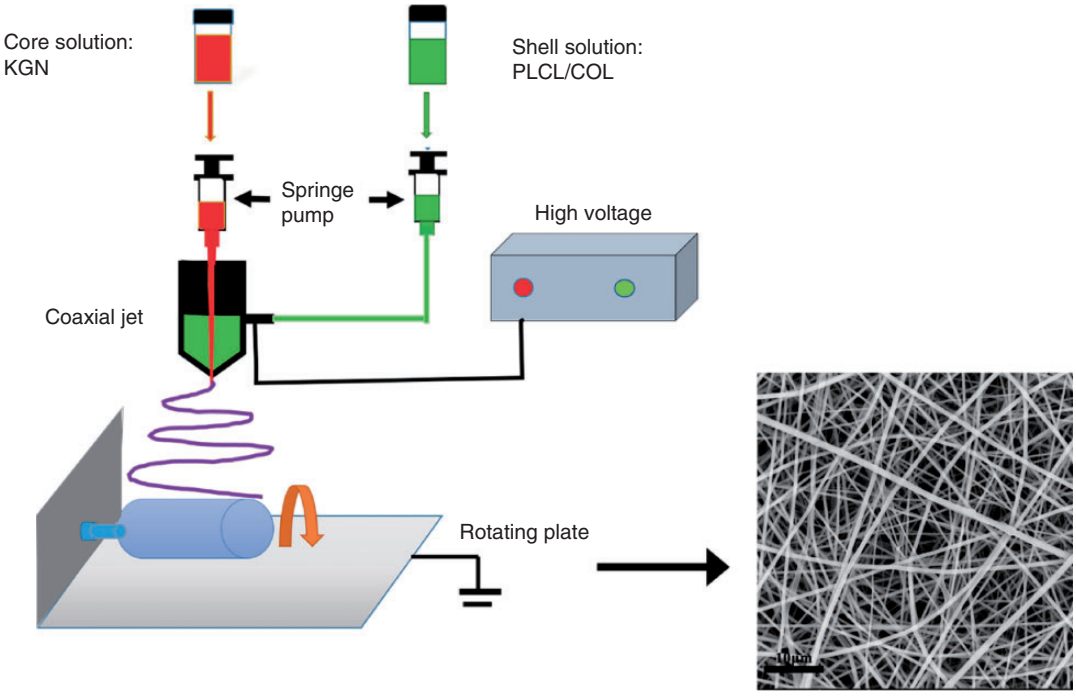

Figure 1. Schematic illustration of the fabrication process of KGN@PC nanofibrous scaffold.

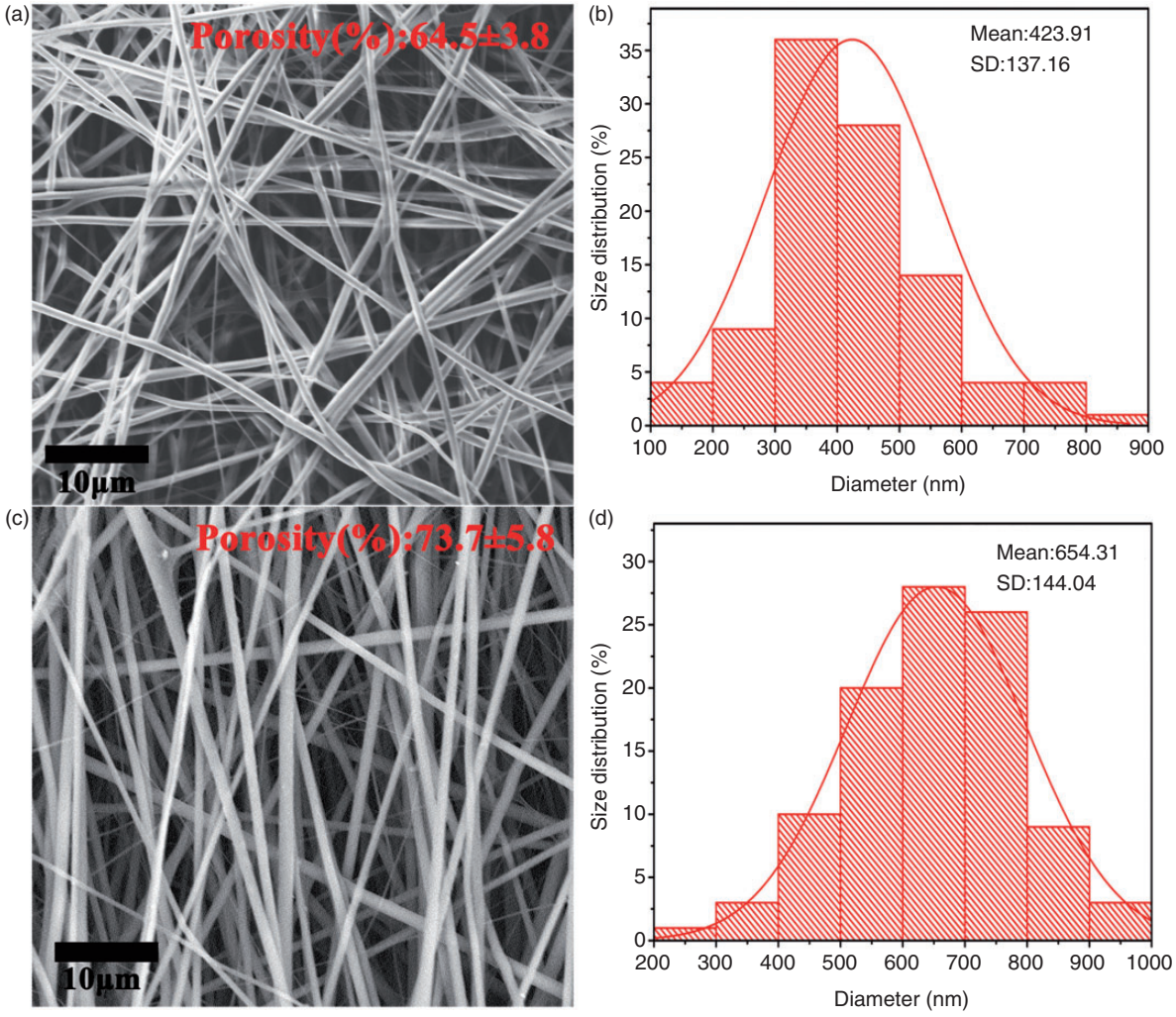

Figure 2. SEM image, porosity and diameter distribution histogram of PC nanofibers mats (a) and (b), KGN@PC nanofibers mats (c) and (d).

TEM (Figure 3). Figure 3 shows the core-shell structure of nanofiber with P(LLA-CL)&Collagen in the shell and KGN in the core.

For tissue-engineered tracheae, the mechanical strength of the scaffold is very important in preventing the collapse of the airway, which would cause serious postoperative complications.[24,25] Tensile properties of the scaffolds were investigated by tensile measurement shown in Figure 4(a), and the stress–strain curves were shown in Table 2. Tensile strength, Young's modulus and elongation at break of KGN@PC scaffold all decreased because of loading KGN. The value of the tensile strength of KGN@PC scaffold at break decreased from 6.39 ± 0.46 MPa (PC scaffold) to 4.23 ± 0.37 MPa, but it was very close to human tracheal cartilage (4.6 ± 1.7 MPa) under normal physiological condition.[26] The value of the Young's modulus of KGN@PC dropped from 174.78 ± 12.96 MPa to 125.93 ± 9.63 MPa. The value of the elongation at break of KGN@PC nanofibers dropped from $69.95 \pm 8.87\%$ to $46.95 \pm 5.16\%$. It can be explained that KGN solution as the core of KGN@PC nanofibers had no tensile strength, which decreased the tensile strength and rigidity of PL(LA-CL) nanofibers.

For tissue engineering scaffold, the hydrophilic property is very important in promoting adhesion and migration of cells.[27] Collagen, a natural polymeric material, could improve the hydrophilic property of PL(LA-CL), which is a hydrophobic polymeric material. The WCA of PC and KGN@PC scaffold were both decreased with the extension of time (Figure 4(b)). The value of the contact angle of PC nanofibers decreased from 126.25° to 68.96° in 135 s, while KGN@PC nanofibers decreased from 121.94° to 50.14° in 87 s. In 60 s, the value of contact angle of PC scaffold was 122.21° (hydrophobicity), while KGN@PC is 89.46° (hydrophily). It could be explained that the addition of KGN increased the diameter of the nanofibers, which

produced larger pore size and then improve the hydrophilicity of the nanofibers. In addition, HFIP, the core solvent of coaxial electrospinning nanofibers, can be dissolved with water at any ratio and could also affect the hydrophilicity of the fiber membrane.

KGN release profile from KGN@PC nanofibrous scaffold

The KGN released from KGN@PC nanofibrous scaffold was shown in Figure 5. The release profile of KGN was mainly composed of two stages; an initial burst release until day 2, followed by a relatively slow release. During the 60-day study, 0.0573 ± 0.0056 mg of KGN was release from 39.343 ± 0.703 mg of the KGN@PC scaffold and the cumulative release rate up to 33.8%, which suggested that KGN was successfully loaded in the core of coaxial fibers. There was an initial burst release about 21% because some KGN located in the surface of nanofibers could easily release from

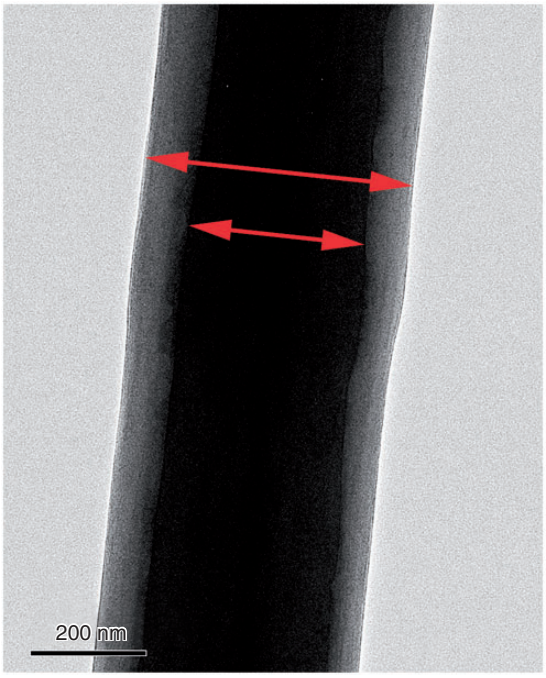

Figure 3. TEM micrograph of KGN@PC nanofiber.

Table 2. Tensile properties of PC and KGN@PC nanofibrous scaffolds.

Specimen	Average tensile strength (MPa)	Average elongation at break (%)	Young's modulus (MPa)
PC	6.39 ± 0.46	69.95 ± 8.87	174.78 ± 12.96
KGN@PC	4.23 ± 0.37	46.95 ± 5.16	125.93 ± 9.63

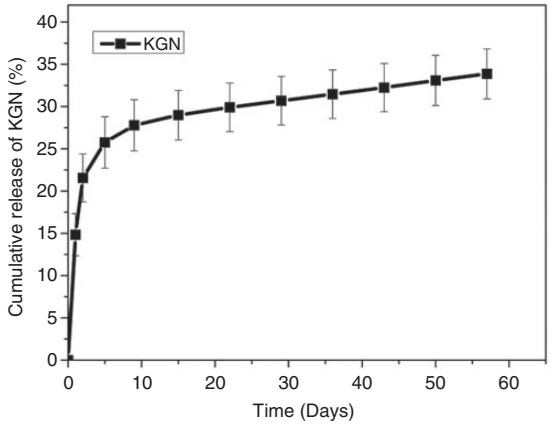

Figure 5. Cumulative release profile of KGN determined by HPLC.

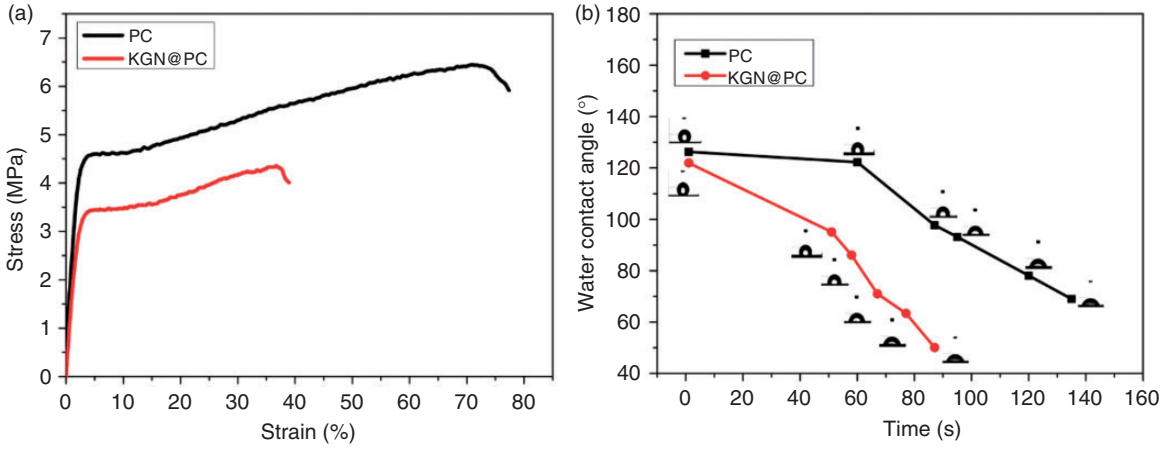

Figure 4. (a) Stress–strain curve of electrospun nanofibers and (b) the WCA of electrospun nanofibers.

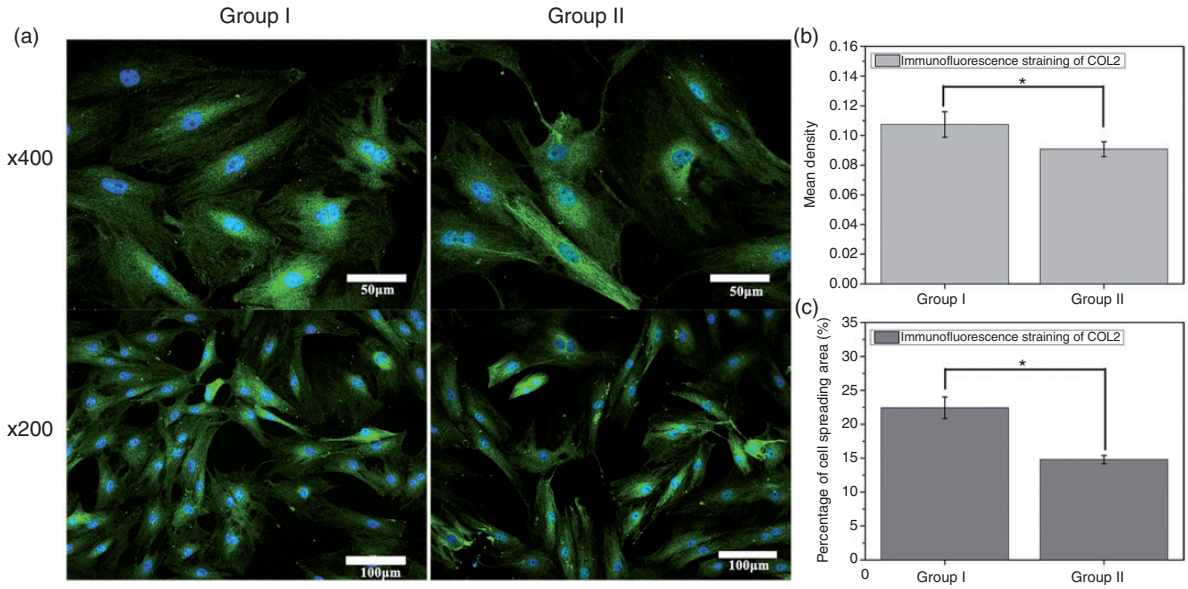

Figure 6. Quantitative and qualitative analysis of COL2 synthesized by chondrocytes cultured with different medium for 7 days. (a) Images of immunofluorescence; (b) fluorescence mean intensity of immunofluorescence images ($n = 4$, $^*P < 0.05$); (c) percentage of cells spreading area (%) of immunofluorescence images ($n = 4$, $^*P < 0.05$). Group I: cultured with DMEM/F12 supplemented with released solution of KGN; Group II: cultured with DMEM/F12. Green: COL 2; Blue: nucleus.

nanofibers to PBS buffer solution at the initial stage. Then there was a relatively slow release because KGN located in the core of nanofibers needed to firstly disperse to shell polymer and finally disperse to PBS in later stage.

The activity of released KGN

Previous research has shown that KGN is no toxicity to MSCs and chondrocytes at high concentrations (100 μM) and protect existing chondrocytes.[17] The expression of COL2 was tested by immunofluorescence staining (Figure 6(a)), where COL2 showed green and nucleus showed blue. As shown in Figure 6(a), COL2 (green) synthesized by chondrocytes showed better cell morphology (fuselage) and wider spreading area when chondrocytes were cultured in DMEM/F12 with KGN release solution (Group I). The results of quantitative analysis of immunofluorescence images were consistent with the above results. The mean density of COL2 immunofluorescence was obviously higher when chondrocytes were cultured in Group I (Figure 6(b)). Similarly, percentage of chondrocytes spreading area (%) was also obviously higher when chondrocytes were cultured in Group I (Figure 6(c)). The numbers of chondrocytes were no significant difference between cultured in Groups I and II.

Toluidine blue (blue) and safranin O (red) were both conducted to evaluate the expression of GAGs (Figure 7(a)). The result of safranin O showed that there was more red area cultured in Group I than Group II. The results of quantitative analysis of safranin O images were consistent with the above results. The result of percentage of chondrocytes spreading area (%) was obviously higher when chondrocytes were cultured in Group I (Figure 7(b)). The number of chondrocytes was no significant difference between cultured in Groups I and II (Figure 7(c)). The result of toluidine blue showed that the numbers of chondrocytes were no significant difference between cultured in Groups I and II, but GAGs have better spreading behavior (blue area) cultured in Group I. The results of quantitative analysis of toluidine blue images were consistent with the above results. Percentage of chondrocytes spreading area (%) (Figure 7(d)) and the number of chondrocytes (Figure 7(e)) were no significant difference between cultured in Groups I and II.

Thus the analysis of immunofluorescence straining and histological staining showed that the released KGN was not only no toxic to chondrocytes but also could effectively maintain the phenotype of cartilage, which indicated the activity of KGN released from KGN@PC scaffold.

Cell viability and morphology on nanofibrous scaffolds

In order to investigate the cell viability of nanofibrous scaffolds, BMSCs cultured on PC and KGN@PC were measured by CCK-8 assay for 1, 4, and 7 days. As shown in Figure 8(a), all samples were no significant difference for cell viability after 1 and 4 day of culture,

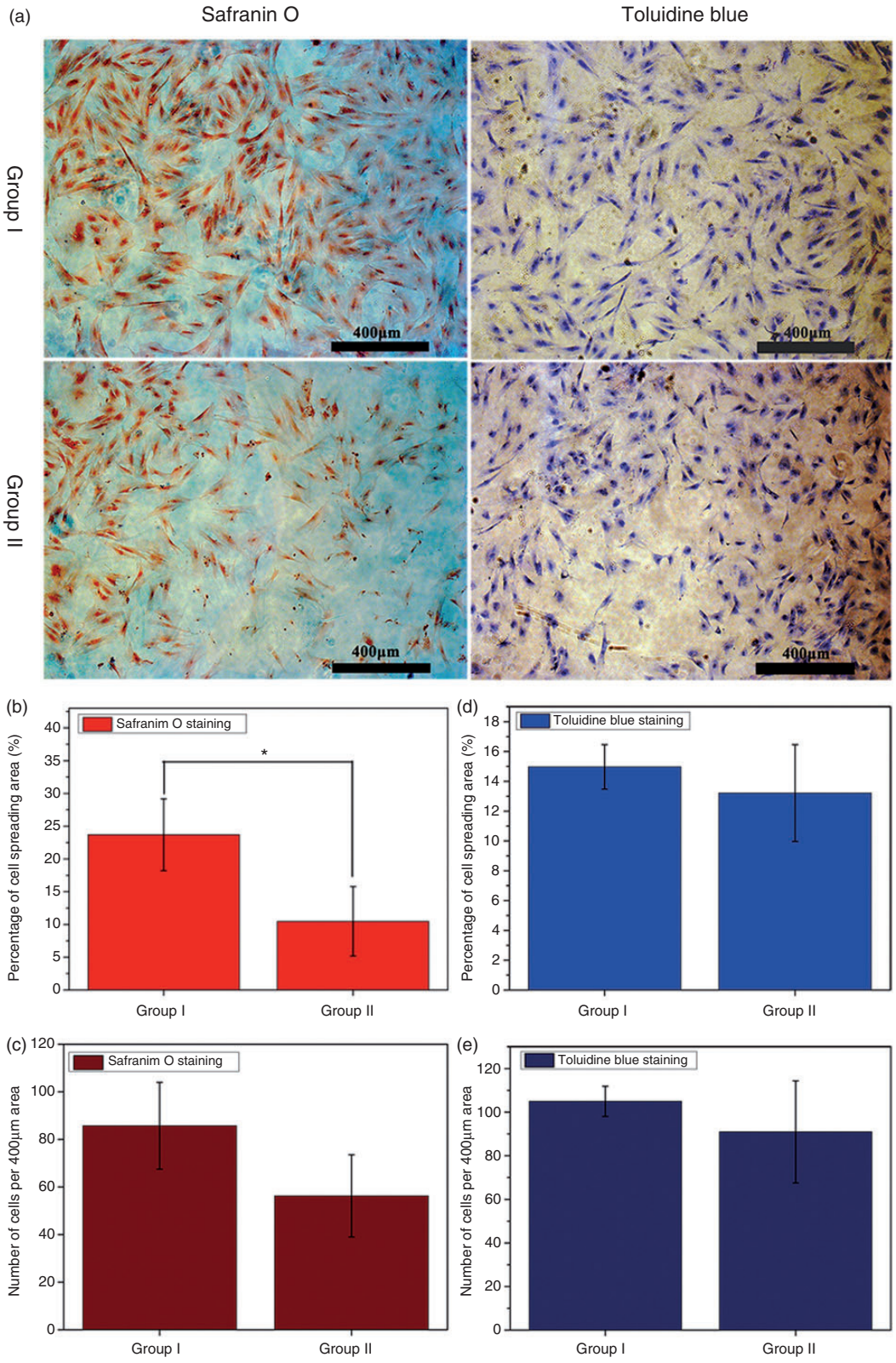

Figure 7. Quantitative and qualitative analysis of GAG synthesized by chondrocytes cultured with different medium for 7 days. (a) Images of histological staining; (b) percentage of cells spreading area (%) of safranin O images; (c) the number of cells of safranin O images; (d) percentage of cells spreading area (%) of toluidine blue images; (e) the number of cells of toluidine blue images. Group I: cultured with DMEM/F12 supplemented with released solution of KGN; Group II: cultured with DMEM/F12. Blue: toluidine blue; Red: Safranin O ($n = 4$, $^*P < 0.05$).

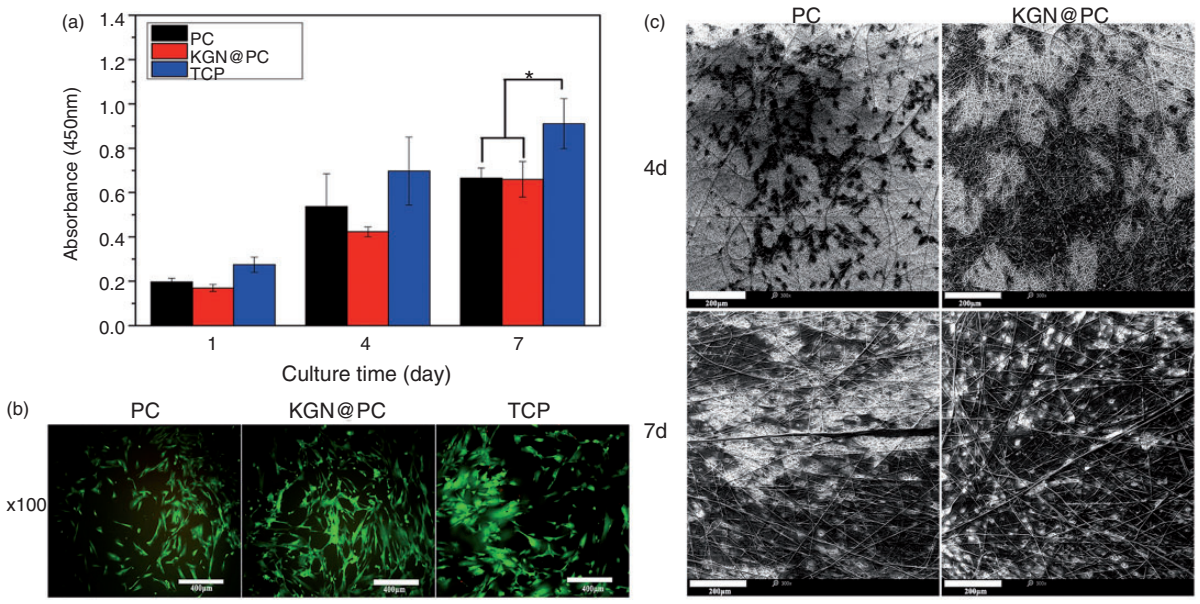

Figure 8. Cell viability assays and cell morphology on scaffolds. (a) CCK-8 assay of BMSCs proliferation ($n = 3$, $^*P < 0.05$). (b) Fluorescent images of BMSCs cultured on the KGN@PC scaffold, PC scaffold and TCP at day 4 (scale bar = 400 μm). Live and dead cells dyed green and red, respectively. (c) SEM images of BMSCs cultured on KGN@PC scaffold and PC scaffold after seeding for 4 and 7 days (scale bar = 200 μm).

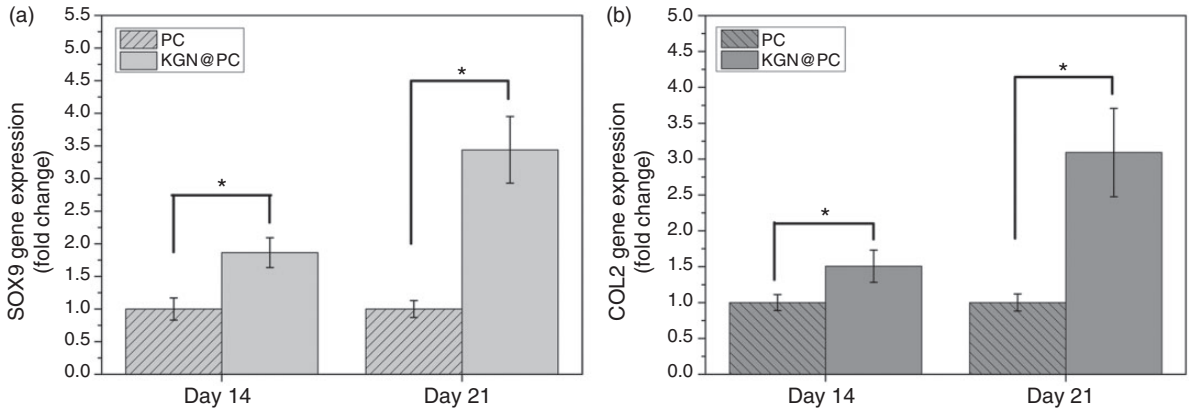

Figure 9. Analyses of chondrogenic differentiation for BMSCs on two different nanofibrous scaffolds. (a) RT-PCR analysis of Sox9 gene expression of BMSCs after culturing 14 and 21 days. (b) RT-PCR analysis of COL2 gene expression of BMSCs after culturing 14 and 21 days ($n = 3$, $^*P < 0.05$).

and the proliferation of cells increased slowly on PC and KGN@PC scaffolds compared with TCP, which indicating that the biocompatibility of PC and KGN@PC were slightly lower than TCP because of residual of glutaraldehyde after crosslinking collagen. The BMSCs were tested by live and dead assay, which could be found in Figure 8(b). It could be found that live cells (green) grew much more than dead cells (red). After 4 days of culture, the numbers of live cells cultured on these two scaffolds and TCP were very close and the cells all exhibited good morphology, which presented similar cell viability as the result of CCK-8 assay.

Cells adhesion and proliferation property of scaffolds were evaluated by SEM (Figure 8(c)). BMSCs were also cultured on PC and KGN@PC for 4 and 7 days. As shown in Figure 8(c), cells were able to adhere on the surface of scaffolds. Cells had contacted each other and formed a cell sheet after 7 days of culture. The results of cell viability and morphology all suggested that PC and KGN@PC possessed a good biocompatibility.

Chondrogenic differentiation of BMSCs on nanofibrous scaffolds

To study the effect of scaffolds on the chondrogenic differentiation, BMSCs were cultured on KGN@PC scaffold, with PC nanofibrous scaffold as control. The expression of cartilage-specific genes COL2 and SOX9 in PC and KGN@PC scaffolds was detected by RT-PCR. The level of expression of each target gene was normalized to GAPDH. As shown in Figure 9(a), more SOX9 genes were expressed in the KGN@PC scaffold than PC scaffold. In addition, the expression of SOX9 genes was higher in the KGN@PC scaffold after a 21-day incubation than a 14-day incubation. Figure 9(b) shows that more COL2 genes were expressed in the KGN@PC scaffold than PC scaffold. For time, the expression of COL2 genes was higher in the KGN@PC scaffold after a 21-day incubation than a 14-day incubation.

The results of RT-PCR analysis of SOX9 and COL2 was consistent with the previous study.[21] The previous study also fabricated a coaxial electrospun scaffold composed of PL(LA-CL)&COL (shell) and TGF-β3 (core) to induce chondrogenesis of MSCs. Thus the sustained release of KGN from KGN@PC nanofibers scaffold contributed to enhancing chondrogenesis.

Conclusions

In this study, core-shell nanofibrous scaffold encapsulated with KGN was fabricated by coaxial electrospinning technology. TEM micrograph of KGN@PC nanofiber indicated that the KGN was distributed in the core of the nanofibers. The KGN released from KGN@PC nanofibrous scaffold in a sustained and stable manner for about 2 months. BMSCs were able to attach and proliferate onto KGN@PC scaffold without observable negative effect on cellular performance. In addition, KGN released from nanofibers could maintain the phenotype of chondrocytes (synthesis of COL2 and GAG) and enhance the chondrogenic differentiation of BMSCs in vitro. Kartogenin encapsulated P(LLA-CL)/collagen nanofibers scaffold fabricated in our study could be served as tissue engineered scaffold for tracheal cartilage regeneration to inducing BMSCs differentiation.

Authors' Note

Haiyue Yin and Xiaomin He are co-first authors of the article.

Declaration of Conflicting Interests

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