

# PLGA/COL I Composite Scaffold Printed by a Low-temperature Deposition Manufacturing Technique for Application to Cartilage Tissue Engineering

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## Research article

**Keywords:** low-temperature deposition manufacturing, PLGA, collagen, hydrophilic modification, cartilage tissue engineering

**Posted Date:** June 22nd, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-34222/v1>

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# Abstract

## Background

Composite scaffolds of poly(lactic-co-glycolic acid) (PLGA) and PLGA/COL I were developed by a low-temperature deposition manufacturing (LDM) technique using three-dimensional printing technology. Their physical properties were tested, and the scaffolds were then used as cell culture platforms to prepare an ideal scaffold for cartilage tissue engineering.

## Methods

The LDM technique was used to fabricate PLGA and PLGA/COL I composite scaffolds. The macrostructure, micromorphology, porosity, hydrophobicity, mechanical properties, and chemical structure of these scaffolds were examined. Primary chondrocytes were isolated and identified, second-passage cells were seeded onto the two scaffolds, and the adhesion and proliferation of the cells were determined.

## Results

Both the PLGA and PLGA/COL I scaffolds prepared by LDM displayed a regular three-dimensional structure with high porosity. The PLGA scaffold had better mechanical properties than the PLGA/COL I scaffold, while the latter had significantly higher hydrophilicity than the former. The PLGA/COL I scaffold cultured with chondrocytes exhibited a higher adhesion rate and proliferation rate than the PLGA/COL I scaffold.

## Conclusion

The novel PLGA/COL I composite scaffold printed by the LDM technique exhibited favourable biocompatibility and biomechanical characteristics and could be a good candidate for cartilage tissue engineering.

## Background

Cartilage is known for its poor regeneration ability, and great efforts have been made to repair cartilage injury [1]. As one of the three cores used for cartilage tissue engineering, a scaffold could act as a temporary extracellular matrix (ECM) and supporting microenvironment to enhance seed cell adhesion, proliferation and differentiation [2]. A scaffold could provide space and support for physiological activities such as proliferation, differentiation, nutrient exchange, metabolism, and secretion of ECM *in vitro* and *in vivo* [3]. A successful biomaterial scaffold should have good biocompatibility and biodegradability, an appropriate degradation rate, good mechanical properties, plasticity, sufficient

surface activity, and a porous three-dimensional structure [4]. An increasing number of studies are being conducted on biomaterial scaffolds, and currently, according to the different biomaterials used, biomaterial scaffolds can be categorized according to those using natural polymers, artificially synthesized polymers, and composite materials [5]. Composite materials include natural composite materials, artificial materials, and artificial natural composite materials, which could complement each other [6]. Fabricating composite materials with advantageous properties is the focus of the current study.

Poly(lactic-co-glycolic acid) (PLGA), as an elastic, synthetic, macromolecule biomaterial with good biocompatibility, mechanical properties and biodegradability and a controllable degradation rate, is expected to be an ideal material for scaffold construction in cartilage tissue engineering [7]. As an extrusion/ejection-based method, the low-temperature deposition manufacturing (LDM) technique is a markedly superior preparation method compared to other traditional scaffold construction and rapid prototyping technologies [8]. The scaffold model and printing parameters, which can be specific to a patient's defect if necessary, were computationally designed. Cell adhesion and proliferation on the scaffolds could be enhanced by changing the hydrophilicity of the scaffolds using sodium hydroxide (NaOH) and compositing with collagen [9]. The cartilage scaffolds with macro- and micropores were individually printed to the specified design parameters [8].

In this study, a PLGA/type I collagen (COL I) composite was prepared using the LDM technique by compositing PLGA with COL I, which further helped to overcome the shortcomings of the PLGA scaffold, including its lack of hydrophilicity and low cell adhesion rate. By taking advantage of both materials, the PLGA/COL I scaffold is expected to serve as an ideal biomaterial for cartilage tissue engineering.

## Methods

### Preparation and modification of the PLGA scaffold

All the procedure in this experiment was shown in **Fig.1**. PLGA (specification 50/50, IV: 2.5 dl/g, Daigang Biomaterial Co., Ltd. Jinan, China) was dissolved in 1,4-dioxane (10 wt%, Lingfeng Chemistry Ltd. Shanghai, China) and thoroughly stirred to form a homogenized solution. The molding parameters of the LDM System (Tissue Form II, Tsinghua University, China) were selected as follows: the dimensions were  $2.4 \times 2.4 \times 2.4 \text{ cm}^3$ , the molding temperature was  $-25 \sim -35^\circ\text{C}$ , the nozzle diameter was 0.4 mm, the spinning pitch was 1.0 mm, the scanning speed was 15~30 mm/s, and the nozzle speed was 1.0~2.0 mm/s. By using computer-aided design combined with a low-temperature extrusion forming process, PLGA was moulded, solidified and stacked in a low-temperature molding chamber. The scaffolds underwent gas-solid phase separation to obtain three-dimensional porous PLGA scaffolds after freeze-drying in a freeze dryer (LYO-2, Biocool Ltd. Beijing, China) for 48 h.

Scaffolds were placed in a 0.2% NaOH (Sinopharm Chemical Reagent Co., Ltd, Beijing, China) solution in alcohol for 10 min, soaked in distilled water, rinsed, and dried to obtain alkali-treated PLGA (aPLGA) scaffolds. The aPLGA scaffolds were soaked in 0.5% collagen-acetate solution (0.5 g COL I; purchased

from Migrang Biotechnology Co., Ltd. Sichuan, China and dissolved in 100 mL 0.3% acetate) for 4 h, soaked and rinsed with distilled water to remove the excess collagen solution on the scaffold, and dried to obtain the PLGA/COL I composite scaffold.

The PLGA scaffold and the PLGA/COL I composite scaffold were immersed in 75% alcohol to sterilize them for 60 min, dried and then sealed tightly prior to further use.

### **General structure and SEM observation of scaffolds**

The PLGA scaffold and the PLGA/COL I composite scaffold were sprayed with gold on their surfaces using a sputter machine and then scanned by scanning electron microscopy (SEM, S-450, Hitachi, Ltd. Japan). The cross section and outer and inner sides of the scaffolds were scanned and observed. Image J software (National Institutes of Health, Bethesda, MD, USA) was used to measure the size of the primary aperture and secondary aperture of the section, and the average values were calculated.

### **Evaluation of scaffold porosity**

The porosity of the scaffold was evaluated by the liquid substitution method. Ethanol can penetrate into the pores between the scaffold fibres without causing expansion or contraction of the scaffold, so it can be used as the substitution fluid for measurement. First, the PLGA scaffold and the PLGA/COL I composite scaffold were cut into cuboids ( $n=5$ ) of the same size; the volume of the cuboidal scaffold was measured as  $V_1$ , and then the scaffold was placed in a measuring cylinder containing a specific volume ( $V_2$ ) of ethanol. The measuring cylinder was placed in a vacuum pump, and a vacuum was applied until no air bubbles were emitted from the scaffolds. The volume of the cylinder was measured as  $V_3$ . The porosity of the scaffold was calculated as  $\rho (\%) = (V_1 + V_2 - V_3)/V_1$ .

### **Evaluation of the scaffold hydrophilicity**

The PLGA and PLGA/COL I composite scaffolds were cut into cubes ( $n=4$ ) of  $0.9 \times 0.9 \times 0.9 \text{ cm}^3$ , and the mass of the cube was weighed as  $W_1$ . The scaffold was immersed in distilled water for 3 d until the water had been fully absorbed. Filter paper was used to absorb the surface moisture, and the mass of the scaffold was weighed as  $W_2$ . The hydrophilicity of the scaffold was determined as hydrophilicity (%) =  $(W_2 - W_1)/W_2 \times 100\%$ .

### **Mechanical testing of the scaffold**

The compressive strength and elastic modulus of the scaffold were measured using the Universal Testing System (Instron, Massachusetts, United States). The scaffold was cut into  $0.9 \times 0.9 \times 0.9 \text{ cm}^3$  cubes ( $n=4$ ), and the compressive strength of the PLGA and PLGA/COL I composite scaffolds was measured with a load of 100 N and a loading speed of 1 mm/min. The elastic modulus of the scaffold was calculated.

### **Fourier transform infrared spectroscopy (FTIR)**

The PLGA scaffold, COL I, and the PLGA/COL I composite scaffold were detected by FTIR, and the changes in the chemical group absorption peaks were recorded.

### **Isolation, culturing and identification of rabbit chondrocytes**

The animal experiment was carried out in accordance with the relevant guidelines and regulations and was approved by the Medical Ethics Committee of the First Affiliated Hospital of Shenzhen University (grant number: LL2015028). Six-week-old New Zealand White Rabbits (Guangdong Animal Experiment Center, Guangzhou, China) (n=5) were used for chondrocyte isolation. All rabbits were euthanized by air embolism. The knee joint articular cartilage was stripped in aseptic conditions. After rinsing and cutting it into small pieces, the cartilage was placed in 5 ml collagenase solution and shaken in an oscillator at a constant temperature for 6-8 h. The cell suspension was obtained after filtration with a 100 µm cell strainer and centrifugation at 1500 r/min for 10 min (Centrifuge 5804/5804 R, Eppendorf, Germany). The supernatant was discarded, and the cells were resuspended by pipetting and adding them into the chondrocyte medium solution. Cells were seeded into a culture dish and placed in a 37°C cell culture incubator (Heracell 150i, Thermo Fisher Scientific, United States) with 5% CO<sub>2</sub>. The culture medium (DMEM/F12 with 10% FBS, all purchased from Gibco, United States) was changed every 2 to 3 d, and subculturing was performed at 90% confluency, as determined under a microscope.

The second-passage (P2) chondrocytes were subjected to toluidine blue staining (Sigma-Aldrich, Missouri, United States) to identify chondrocytes. The second and fifth passages (P5) underwent immunofluorescence staining for the detection of COL I and type II collagen (COL II).

### **RNA isolation and RT-qPCR**

Total RNA was extracted from cartilage tissues using TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA). For real-time quantitative polymerase chain reaction (RT-qPCR) analysis, RNA was reverse-transcribed into cDNA using iScript Reverse Transcription Supermix (Bio-Rad, Hercules, CA). qPCR was carried out using SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad) and the CFX Connect™ Real-Time PCR Detection System (Bio-Rad). 18S rRNA was used as an internal reference for each sample. Primer sequences are listed in **Table 1**. Target gene mRNA expression was normalized to 18S rRNA expression and is shown as the fold change.

### **Combinational culture of rabbit chondrocytes and scaffolds and cell adhesion and proliferation assays**

The PLGA scaffold and the PLGA/COL I composite scaffold were cut into disc-shaped scaffolds with a diameter of 10.2 mm and a thickness of 1 mm, sterilized, dried, and placed into a 48-well culture plate. The P2 chondrocytes from rabbits were adjusted to a density of 5×10<sup>4</sup>/ml using a cell counting analyser. One millilitre of cell suspension was inoculated onto the disk-shaped PLGA and PLGA/COL I composite scaffolds in a 48-well culture plate with an appropriate amount of chondrocyte culture medium. The plate was placed in a 37°C cell culture incubator with 5% CO<sub>2</sub>.

After the combinational culture of the cells and the scaffolds for 1, 3, 5, 7, and 10 d, the medium was discarded, 500  $\mu\text{L}$  culture medium and 100  $\mu\text{L}$  of the MTT solution (Life Technologies, Shanghai, China) were added to each well, and the plate was cultured in a 37°C incubator with 5%  $\text{CO}_2$  for 4 h. The solvent on the surface of the scaffold was discarded, and 200  $\mu\text{L}$  dimethyl sulfoxide (DMSO) was added to each well. The plate was shaken on a shaker for 10-20 min to completely dissolve the crystals, 100  $\mu\text{L}$  of the solution from each well was transferred to a 96-well plate, and cell proliferation was measured by a microplate reader by determining the optical density (OD) at 490 nm.

### Statistical analysis

Data are presented as the mean  $\pm$  SD. The software SPSS 16.0 (Chicago, IL, USA) was applied for statistical analysis and management. One-way analysis of variance (ANOVA) and the SNK-q and Dunnett's T3 tests were applied for comparisons of the means of multiple samples and the determination of the heterogeneity of variance. Any difference for which  $p < 0.05$  was considered statistically significant.

## Results

### Macroscopic observation

Macroscopic observation of both the PLGA and PLGA/COL I scaffolds revealed a three-dimensional porous structure with a regular and orderly grid-shaped pore distribution. The scaffolds were flexible and elastic and could be produced in different sizes, which was convenient for characterization and further application (**Fig. 2**).

### SEM observation

SEM revealed that the surfaces of the PLGA scaffolds were covered with large circular pores of uniform diameter, and the printed wires around the pores formed a microporous structure (**Fig. 3A-D**). In the PLGA/COL I scaffold, collagen covered both its surface and interior. The surfaces of the macropores were smooth, and sharp edges were notably reduced, although the poor distribution of collagen at a few locations on the surface caused occlusion of the pores (**Fig. 3E-H**). In the PLGA group, the diameters of the macropores and micropores were  $533.65 \pm 82.78 \mu\text{m}$  and  $9.52 \pm 2.59 \mu\text{m}$ , respectively; in the PLGA/COL I group, the diameters were  $445.13 \pm 141.05 \mu\text{m}$  and  $6.95 \pm 1.47 \mu\text{m}$ , respectively. There were significant differences in the macropore and micropore sizes of the PLGA/COL I group compared to those of the PLGA and alkali treatment groups ( $p < 0.05$ ).

### Porosity of the scaffolds

The porosity of the PLGA group scaffold was  $86.73 \pm 1.81\%$ , while that of the PLGA/COL I group scaffold was  $84.68 \pm 1.66\%$ ; there was no significant difference between the two groups ( $p > 0.05$ , **Table 2**).

### Hydrophilicity of the scaffolds

The hydrophilicity of the PLGA/COL I scaffold group was significantly higher than that of the PLGA scaffold group ( $66.92\pm 2.30\%$  vs  $52.04\pm 2.69\%$ ,  $p<0.05$ , **Table 2**), indicating that the introduction of collagen significantly improved the hydrophilicity of the PLGA scaffold.

### **Mechanical properties of the scaffolds**

The stress-strain curves of the scaffolds under compression are shown in **Fig. 4A**. The compressive Young's modulus of the PLGA and PLGA/COL I scaffolds was  $33.82\pm 10.99$  MPa and  $23.35\pm 5.81$  MPa, respectively, and a significant difference was found between these two scaffolds ( $p<0.05$ , **Fig. 4B**).

The stress-strain curves of the scaffolds under tensile stress are shown in **Fig. 4C**. The tensile Young's modulus of the PLGA and PLGA/COL I scaffolds was  $39.45\pm 7.95$  MPa and  $24.48\pm 4.90$  MPa, respectively, and a significant difference was found between these two scaffolds ( $p<0.05$ , **Fig. 4D**).

The maximum elongation in the PLGA and PLGA/COL I scaffold groups was  $33.16\pm 3.10\%$  and  $61.05\pm 9.57\%$ , respectively, and a significant difference ( $p<0.05$ , **Fig. 4E**) was found, indicating that the maximum elongation of the composite scaffold was significantly improved.

### **Infrared spectra of the scaffolds**

As shown in **Fig. 4F**, for the PLGA scaffold, the characteristic absorption band at  $1754\text{ cm}^{-1}$ , which is attributed to the stretching vibration of the C=O groups, indicates that the scaffold contains ester bonds. The infrared spectra of COL show that COL has strong absorption peaks at  $1550\text{ cm}^{-1}$  and  $1650\text{ cm}^{-1}$ , which are the absorption peaks of amide I and amide II [10]. These characteristic absorption peaks can be seen in the infrared spectra of the PLGA/COL I scaffold, but the absorption peaks of amide I and amide II were notably weaker, which could be related to the low concentration of the collagen solution and the heterogeneous composition of the collagen and the scaffold.

### **Chondrocyte culture**

Primary chondrocytes were isolated, and P2 chondrocytes were identified (**Fig. 5, 6**). P2 chondrocytes were round and polygonal, and a heterochromatic matrix was seen around the chondrocytes after toluidine blue staining. Blue-purple heterochromatic granules were observed within the chondrocytes, while some heterochromatic granules were also found around the chondrocytes, indicating the secretion of extracellular matrix, including glycosaminoglycan.

### **Immunofluorescence staining**

The nuclei of the chondrocytes were blue, COL I was red, and COL II was green, indicating that COL I and COL II were expressed in P2 and P5 of chondrocytes (**Fig. 7**). In each subsequent culture generation, the expression of COL II decreased gradually, while that of COL I increased gradually, which was caused by the dedifferentiation of the chondrocytes.

## Detection of gene expression in chondrocytes of different passages by RT-qPCR

The expression of Col I increased gradually after each passage, while the expression of Col II, Sox 9 and aggrecan tended to gradually decrease (**Fig. 8**). The secretion of chondrocyte matrix was maintained at a high level in P2 chondrocytes but began to decrease in the third passage, and this became increasingly significant with each subsequent passage, proving that dedifferentiation of the *in vitro* chondrocyte monolayer culture occurred gradually. Since chondrocytes maintained a good cell phenotype prior to P2, they could be used for the composite culture of cells and scaffolds.

## Cell adhesion rate

The cell adhesion rate of the PLGA group was  $45.62 \pm 1.75\%$  and that of the PLGA/COL I group was  $53.69 \pm 2.74\%$ , showing the significant difference between the two groups ( $p < 0.05$ , **Table 2**). This indicates that with the addition of collagen, the cell adhesion rate and biocompatibility of the PLGA scaffold increased.

## Cell proliferation

The cell adhesion rate in the PLGA/COL I group was higher than that in the PLGA group within 24 h after seeding, suggesting that the scaffolds modified with NaOH and COL I were more conducive to cell adhesion (**Fig. 9**). The cells in the PLGA/COL I group grew linearly from day 3 to 7 post seeding, and the cell growth rate in the PLGA/COL I group was significantly higher than that in the PLGA group. The growth rate of cells decreased 7 d after inoculation, but the growth rate of the PLGA/COL I group remained higher than that of the PLGA group. A significant difference in cell proliferation between the two groups was observed ( $p < 0.05$ ).

## Discussion

In this study, a three-dimensional porous PLGA scaffold was successfully prepared using LDM technology. The printed size was smaller than the specified design size, and some of the large pores were blocked; it was believed that this was due to elastic retraction, which occurred after sublimation in 1,4-dioxane during freeze-drying. The hydrophilicity of the PLGA scaffold was increased through NaOH treatment, and the surface of the PLGA scaffold detected via SEM became rough with an increase in small depressions. There was no significant change in pore size between the two groups. However, the mechanical properties of the modified group were slightly weakened compared with those of the unmodified group, as shown by a decrease in Young's modulus, but there was no significant difference at 70% compression.

Then, a PLGA/COL I scaffold was prepared by compositing PLGA with a COL I solution. SEM images showed that the surface of the scaffold was covered with collagen, which was smooth with a decrease in rough edges. FTIR results indicated that the characteristic absorption peaks of PLGA and collagen were present in the composite scaffold, but the absorption peak of collagen was significantly reduced.

Compared with those of the first two groups, the pore and micropore sizes of the composite scaffold were reduced, which caused by a low collagen concentration and poor collagen coverage. Clogging of the collagen scaffolds also occurred, which was likely caused by inadequate rinsing of the collagen. There was no significant difference in the porosity of the PLGA and PLGA/COL I scaffolds, but the hydrophilicity of the PLGA/COL I scaffold was higher than that of the PLGA scaffold. Moreover, cellular experiments showed that the PLGA/COL I scaffold was more conducive to cell adhesion and proliferation than the PLGA scaffold. The elastic modulus of the PLGA scaffold was higher than that of the PLGA/COL I scaffold, possibly due to hydrolysis and corrosion after NaOH treatment. However, there was no significant difference in compressive strength between the two scaffolds at 70% compression. The reason for this may be the fact that both scaffold groups had passed the elastic yielding stage (where the ability to resist denaturation is lost) and were in the strengthening stage, in which this ability is restored. It has previously been reported that scaffolds with pore sizes of 70–120  $\mu\text{m}$  are more suitable for chondrocyte growth [11]; however, the pore sizes of the scaffolds manufactured in this study were markedly larger, indicating that the design and formation parameters of our scaffolds need to be further improved. The mechanical test results show that with the addition of collagen, the compressive and tensile Young's moduli decreased. The tensile Young's modulus of the composite scaffold was closer to that of human cartilage, which has been suggested by recent studies to range from 2–25 MPa [12]. Meanwhile, the addition of collagen markedly improved the elasticity of the scaffold; the Young's modulus decreased slightly, making it more suitable for cartilage tissue engineering as the elasticity increased.

Glycosaminoglycan is an alkaline glycol polymer that becomes heterochromatic when it is reacted with the alkaline dye toluidine blue [13]. In our study, blue-purple heterochromatic granules were observed in chondrocytes, and small amounts of heterochromatic granules were observed around the chondrocytes, indicating that glycosaminoglycan and other extracellular matrix components were secreted by the chondrocytes. The collagen immunofluorescence analysis results confirmed that COL I and COL II existed in the P2 and P5 chondrocytes; the toluidine blue staining results and the surgical region from which the cells were obtained also proved that the cultured cells were chondrocytes. The dedifferentiation phenomenon appeared in the *in vitro* monolayer culture of chondrocytes [14, 15], and with the increase in the passage and culture time, the chondrocytes lost their original phenotype and gradually changed in morphology from primarily polygonal and circular cells into fibroblast-like spindles. This is consistent with the observation that the chondrocyte morphology changed from polygonal and round in the primary first and second passages to long and spindle-shaped in the third, fourth and fifth passages. Along with the changes in shape, several cell characteristics also changed; the expression of COL II, proteoglycan and the SOX9 gene decreased, while the expression of COL I increased. The above results were also confirmed by RT-qPCR. Therefore, chondrocytes used in clinical cartilage tissue engineering should be replated before P2 of chondrocyte culture, when possible, as chondrocytes still maintain a good phenotype at this stage.

The chondrocyte adhesion assay showed that cells adhered more to the PLGA/COL I scaffold than to the PLGA scaffold. The MTT assay showed that cells grew better on the collagen composite scaffold and

indicated that the organic solvent 1,4-dioxane, which was used in the fabrication process, was completely washed away with no obvious remaining cytotoxicity in the scaffold. The biocompatibility of the composite scaffold was greatly improved and was more conducive to the adhesion and proliferation of chondrocytes.

However, the limitations of this study also need to be acknowledged. First, the pore size of the printed scaffolds was relatively large, the scaffolds shrunk, and some of the macropores were blocked. It is necessary to improve the model of stratification and the printing design parameters and to adjust the ratio of the two materials used. Second, the degradation time of PLGA has been reported in the literature to be relatively long, and the detection of its degradation needs to be improved [16]. Third, the degradation products of PLGA can result in immunogenicity, as they are acidic and possibly cause inflammation. This should be addressed to minimize any potential irritation of tissues. Last but not least, an animal experiment involving the repair of a cartilage defect with a cell-scaffold composite needs to be conducted in our future works.

## Conclusion

The novel PLGA/COL I composite scaffold printed by the LDM technique exhibited favourable biocompatibility and biomechanical characteristics and could be a good candidate for cartilage tissue engineering.

## Abbreviations

ANOVA: One-way analysis of variance; aPLGA:alkali-treated PLGA; COL I:type I collagen; COL II:type II collagen; DAPI:4',6-diamidino-2-phenylindole; ECM:extracellular matrix; FTIR:Fourier transform infrared spectroscopy; LDM:low-temperature deposition manufacturing; NaOH:sodium hydroxide; OD:optical density; P2:second passage; P5:fifth passage; PLGA:poly(l-lactide-co-ε-caprolactone); RT-qPCR:real time quantitative polymerase chain reaction; SEM:scanning electron microscopy.

## Declarations

### Ethics approval and consent to participate

All the animal related experiments were approved by the Institutional Animal Ethics Committee of the Shenzhen Second People's Hospital, Shenzhen, Guangdong Province, China.

### Consent for publication

Not applicable.

### Availability of data and materials

All data generated or analysed during this study are included in this published article.

## Competing interests

The authors declare that they have no competing interests.

## Funding

This study was supported by the National Natural Science Foundation of China (81672234, 81902558, 81902303), Guangdong Basic and Applied Basic Research Foundation (2020A151501048), Shenzhen Science and Technology Project (201606018, GJHZ20180416164801042, JCYJ20180305124912336), and Clinical Research Project of Shenzhen Second People's Hospital (20173357201814). The funding body was involved in reagents and animals purchase, data collection and analysis, and interpretation of data by supporting with salary for the time needed. They were not involved in the design or writing the manuscript.

## Authors' Contributions

LQP designed the study, performed the experiments, and performed data collection and analyses. YH performed the experiments and performed data collection and analyses. WX performed language editing. WMZ performed data collection. WL performed data collection. YH performed data collection. LJJ performed data collection. ZHD designed the study and participated in data analyses and manuscript preparation. WFX performed data analyses and manuscript preparation. DPW contributed to study design. All authors have read and approved the manuscript.

## Acknowledgements

Not applicable.

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## Tables

**Table 1** Primer information

Genes	Primer	Sequence (5'-3')
18s	Forward primer	CGTCTGCCCTATCAACTTTTCG
	Reverse primer	CGTTTCTCAGGCTCCCTCT
COL I	Forward primer	GCGGTGGTTACGACTTTGGTT
	Reverse primer	AGTGAGGAGGGTCTCAATCTG
COL II	Forward primer	CAGGCAGAGGCAGGAAACTAAC
	Reverse primer	CAGAGGTGTTTGACACGGAGTAG
Aggrecan	Forward primer	ATGGCTTCCACCAGTGCG
	Reverse primer	CGGATGCCGTAGGTTCTCA
SOX9	Forward primer	GTACCCGCACCTGCACAAC
	Reverse primer	TCCGCCTCCTCCACGAAG

**Table 2** Porosity and hydrophilicity of scaffolds and cell adhesion rate in the PLGA and PLGA/COL I groups.

Indexes	Groups	Samples					Mean	P
		1	2	3	4	5		
Scaffold porosity (%)	PLGA	85.65	85.75	87.47	89.58	85.19	86.73±1.81	>0.05
	PLGA/COL I	84.32	83.64	85.97	86.75	82.73	84.68±1.66	
Scaffold hydrophilicity (%)	PLGA	48.19	54.40	53.18	52.38	NA	52.04±2.69	<0.05
	PLGA/COL I	63.82	67.07	67.39	69.39	NA	66.92±2.30	
Cell adhesion rate (%)	PLGA	45.54	43.25	47.38	46.32	NA	45.62±1.75	<0.05
	PLGA/COL I	50.24	52.84	55.26	56.42	NA	53.69±2.74	

## Figures

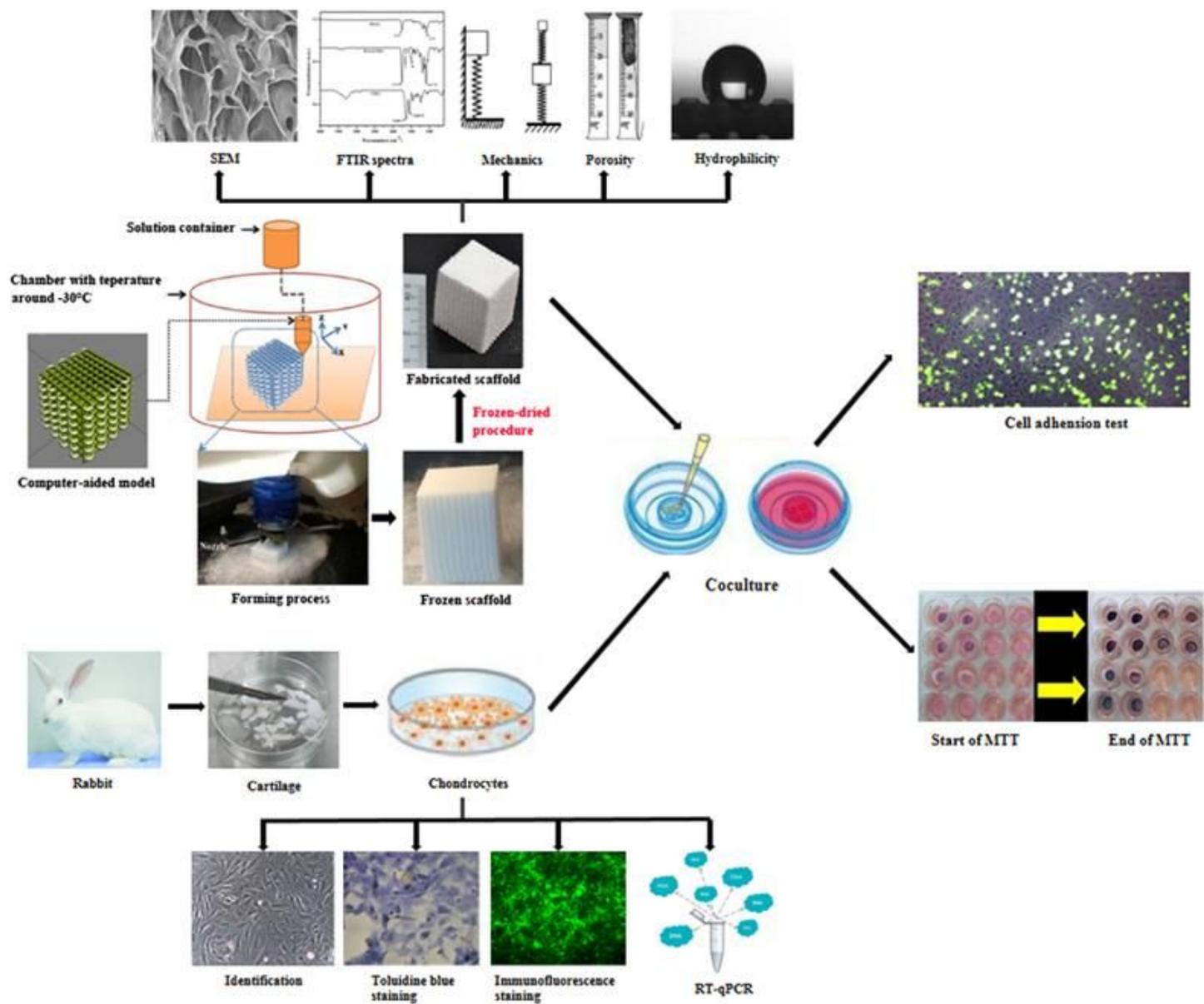


Figure 1

Graphical abstract.

PLGA

PLGA/COL I

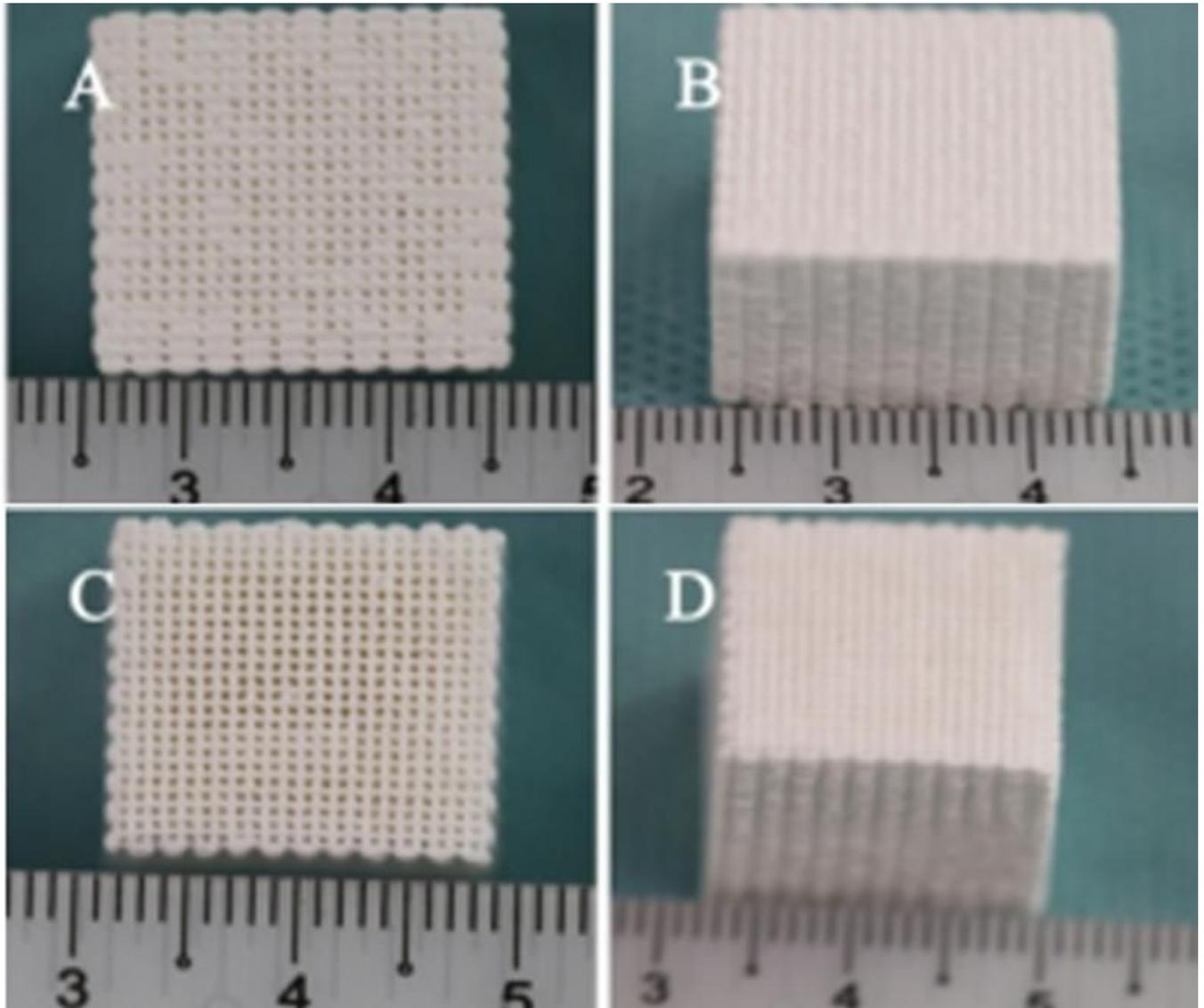


Figure 2

Macroscopic observation of PLGA (A, B) and PLGA/COL I scaffolds (C, D).

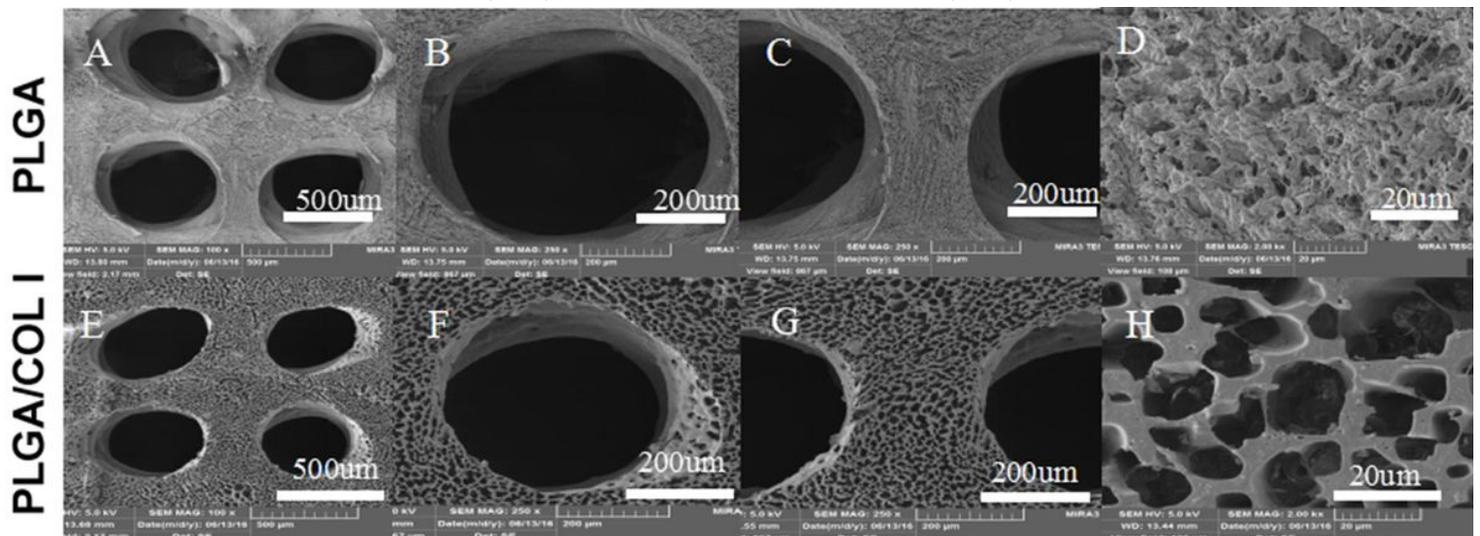


Figure 3

SEM observation of PLGA (A-D) and PLGA/COL I scaffolds (E-H).

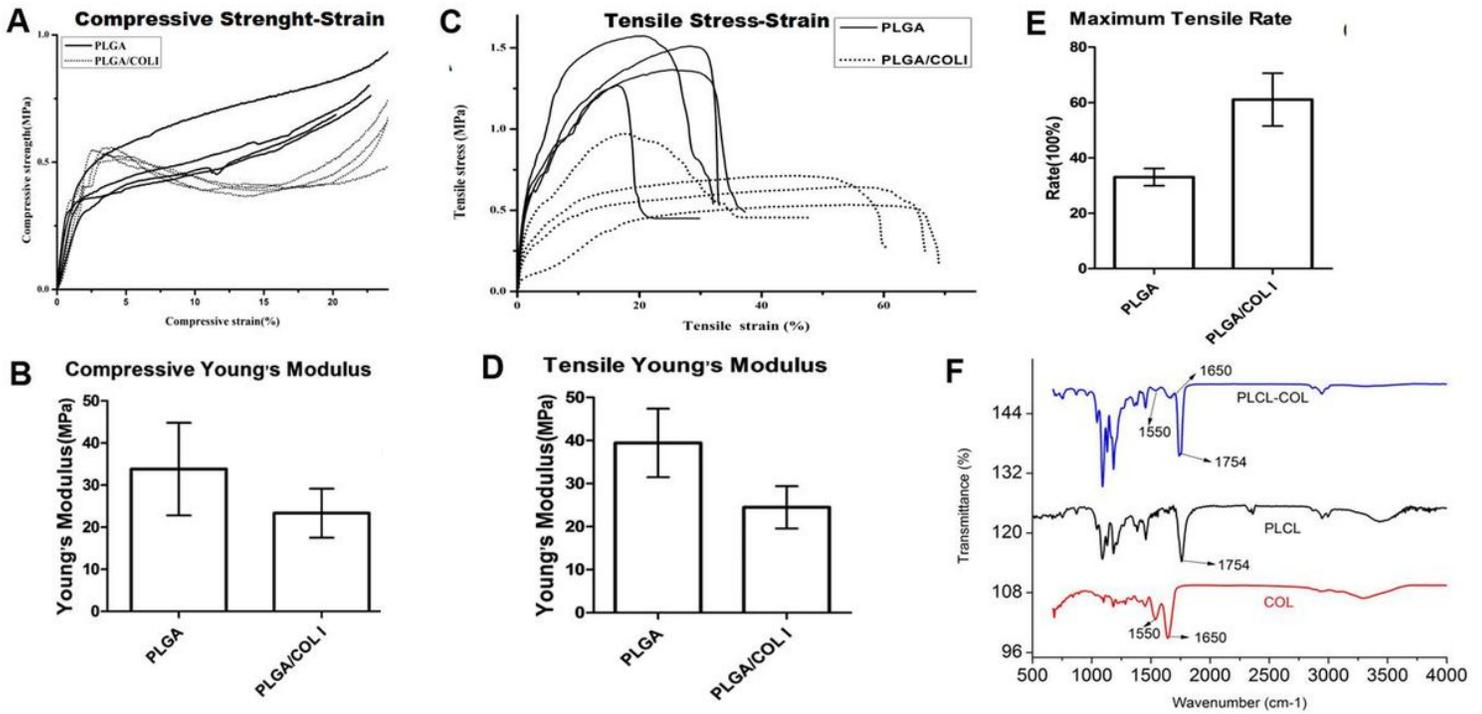


Figure 4

Mechanical property testing of PLGA and PLGA/COL I scaffolds. (A) Stress-strain curves of scaffolds under compression. (B) Young's modulus of scaffolds under compression. (C) Stress-strain curves of scaffolds under tensile stress. (D) Young's modulus of scaffolds under tensile stress. (E) Maximum elongation. (F) Infrared spectra of the scaffolds.

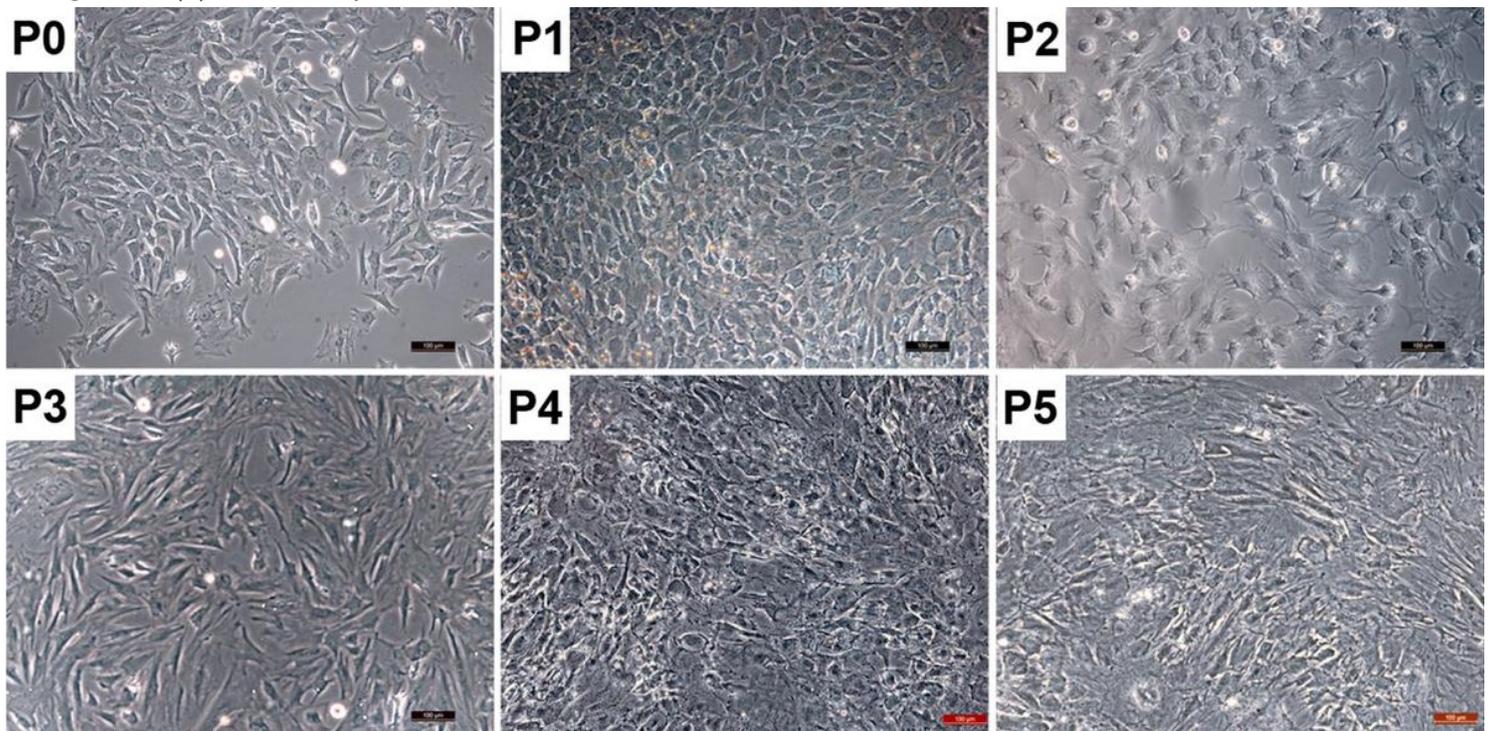


Figure 5

Chondrocyte cultures at different passages.

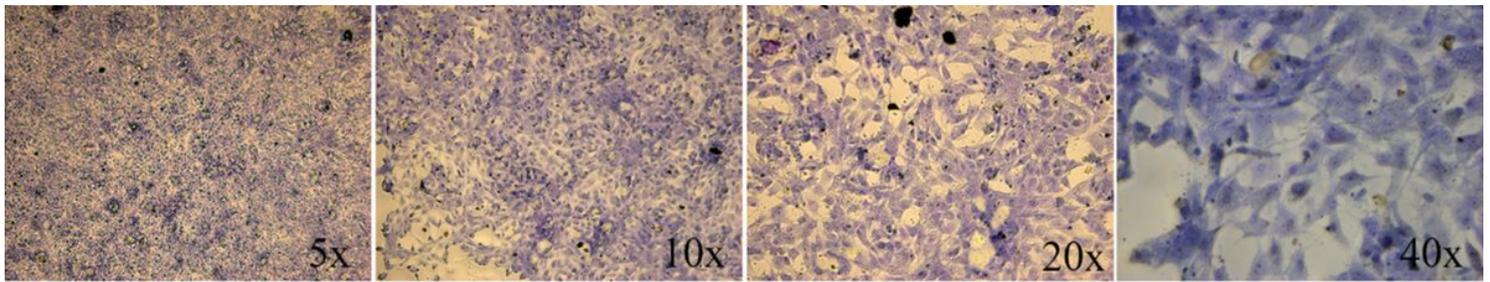


Figure 6

Toluidine blue staining of chondrocytes (P2).

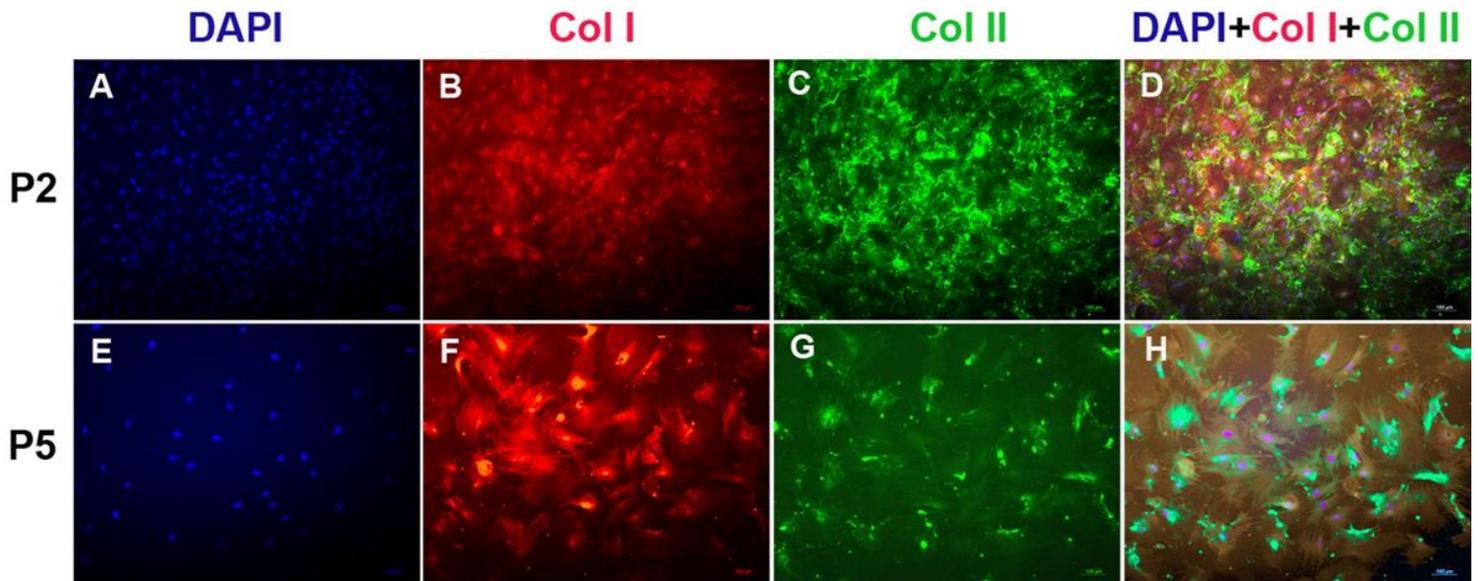
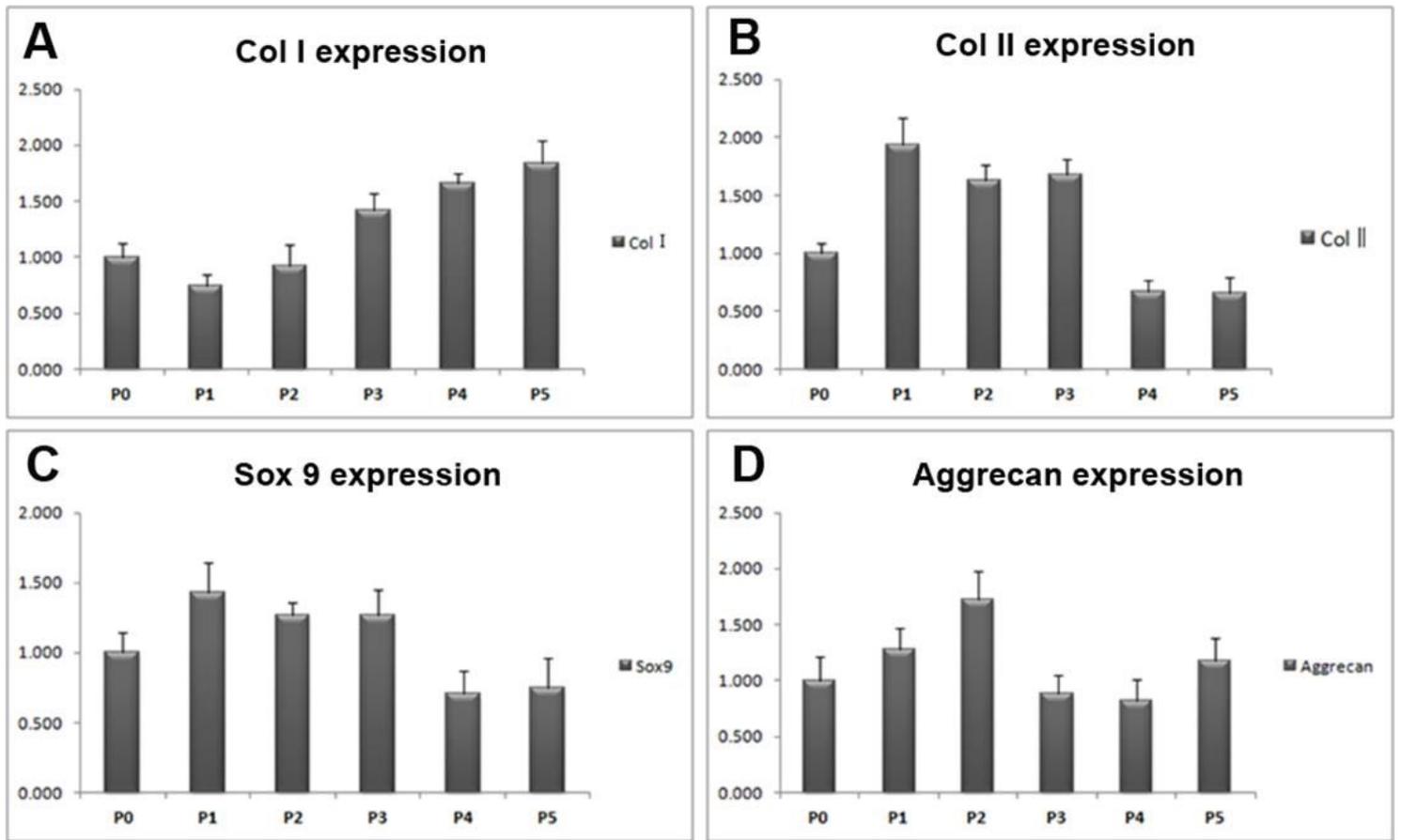


Figure 7

The immunofluorescence staining results for collagen in P2 and P5 chondrocytes (10x). (A, E) Cell nuclei were stained with DAPI; (B, F) COL I staining; (C, G) COL II staining; (D, H) cell staining showing expression of collagen. DAPI, 4',6-diamidino-2-phenylindole.



**Figure 8**

The expression of COL I, COL II, Sox 9 and aggrecan as determined by RT-qPCR.

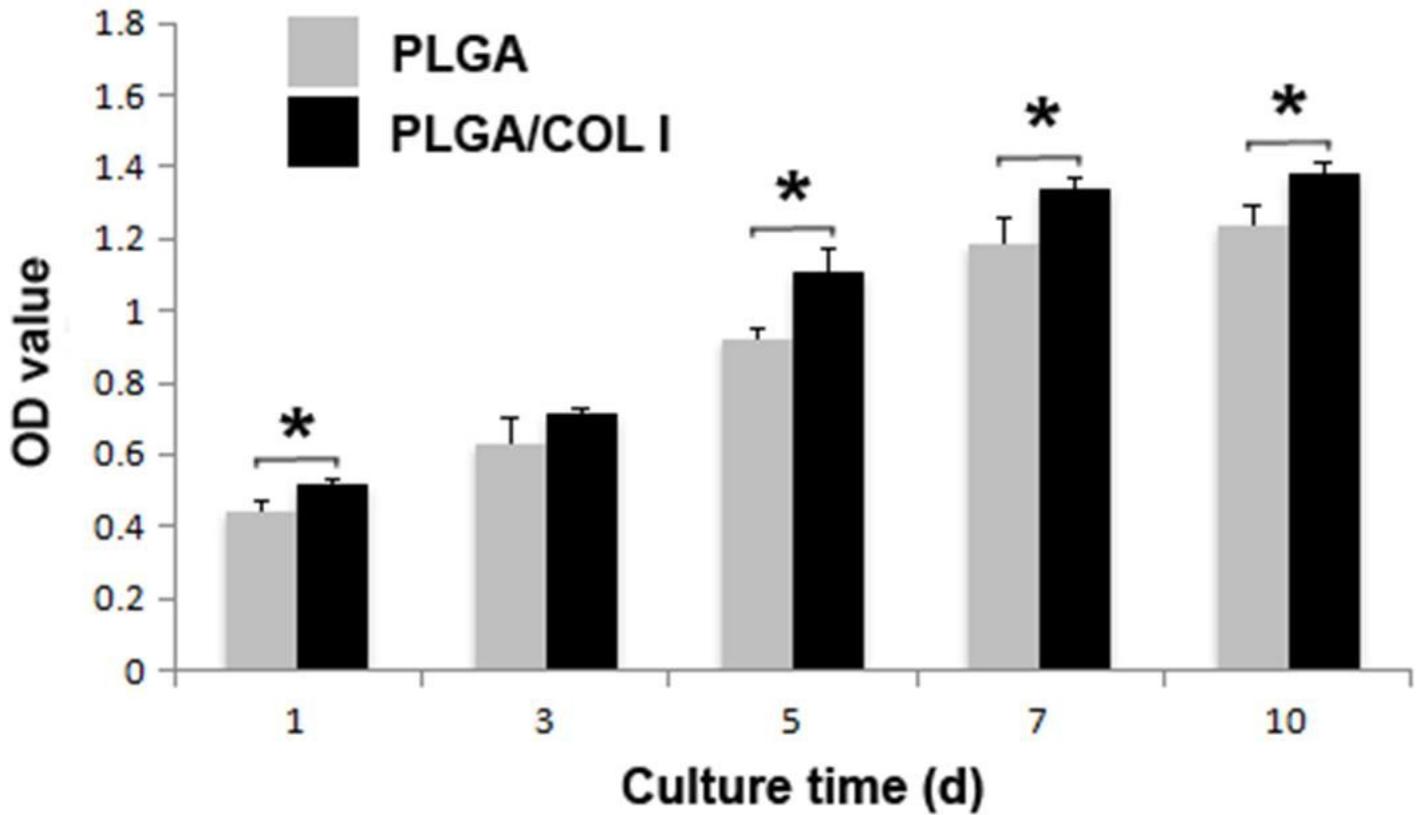


Figure 9

Cell proliferation in PLGA and PLGA/COL I scaffolds.

## Supplementary Files

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- [NC3RsARRIVEGuidelinesChecklistfillableZD.pdf](#)
- [SupplementaryfileLanguageEditingCertificate.pdf](#)