Analysis and Demonstration of a Scaffold Finite Element Model for Cartilage Tissue Engineering

Kai Sun, Ruixin Li, Hui Li,* Meng Fan, and Hao Li

ABSTRACT: A three-dimensional finite element model of articular cartilage was established to explore the mechanical behavior of the repaired area under physiological compression loading. A circular vertical material and a circular inclined stacking material were fabricated, and a 3D printing method was employed for producing three-dimensional solids. The physical and biomechanical properties and biocompatibility were tested. The presence of cells in the circular vertically stacked scaffold significantly promoted the matrix deposition and the cell viability compared with that in the circular inclined scaffolds. The scaffolds made of the circular vertical material and the circular inclined stacking material scaffolds exhibited better overall performance.

INTRODUCTION

Cartilage cells and their extracellular matrix are the main components of cartilage. Cartilage contains no blood vessels, nerves, or lymphatic drainage and gains access to main nutrients from the synovial fluid.1 The cartilage structure determines its poor capacity for regeneration and self-repair. Because of trauma, inflammation, tumors, and other causes, cartilage injuries are extremely common in clinical settings. Current treatment options are also unable to restore native cartilage; thus, clinical needs have motivated cartilage tissue engineering. Cartilage tissue engineering involves addressing many problems, including the method of cell seeding, the choice of scaffold, the cartilage complex, and the repair of cartilage defects in the body. Current tissue engineering research has not yet constructed a suitable artificial cartilage complex. The failure of tissue engineering in repairing cartilage defects is related to poor integration of the cartilage complex in situ cartilage repair interface because the shape and size of the complex do not form an appropriate functional interface with the damaged area, and hyaline differentiation of the chondrocytes is stagnated. The size of the composite is directly related to the scaffold. As a carrier of cells cultured in vitro, scaffolds can be shaped according to the shape of the defective cartilage to determine whether the cartilage complex can adapt well to the recipient area and eventually repair the cartilage defect.2–4 The uncertainties of the repair results are related to the mechanical behavior of the repair area. The shape, depth, and load characteristics of the defect repair change the mechanical environment of the repair area to varying degrees. Therefore, the appropriate mechanical properties of artificial cartilage can be explored by studying the above parameters.

The methods for preparing a tissue engineering scaffold must produce a good overall effect, and the structure should be suitable for cell growth and attachment to promote nutrient transport.5–10 Additionally, the pore size and porosity were optimal for preventing cell loss from the scaffold and were in accordance with the size of the cartilage cells, among other criteria. The porosity properties of natural cartilage require more than 90% porosity. A better porosity and water absorption expansion rate of the scaffold encourage the ability to absorb the

Received: September 12, 2020
Accepted: November 24, 2020
Published: December 7, 2020
tested. The physical properties tested was significantly higher for the circular inclined scaffold than those evaluated for the circular inclined scaffold (P < 0.05).

**Biomechanical Properties.** The circular vertical and circular inclined stacking scaffolds were flexible, with ductility and viscoelasticity. The test used the elastic modulus as the detection index. The stress–strain curves show that both scaffolds exhibited viscoelastic behavior (Figure 6a,b).

**Cell Viability and Microstructures.** Cell viability and proliferation were evaluated by a thiazolyl blue tetrazolium bromide (MTT) assay, which is a quantitative colorimetric assay (Figure 7a). Purple crystals can be formed by metabolically active cells, which can be detected at 520 nm by spectrophotometry. Thus, the growth and proliferation of active cells can be tested indirectly using this method. As shown in Figure 4a, at time intervals of 1, 3, 5, 7, 9, 11, and 13 days, the optical density (OD) values from the assay of the circular vertical scaffold were significantly higher than that obtained by the circular inclined scaffold (P < 0.05), indicating that cells were proliferating quickly during the culture period. The morphology and viability of the scaffolds were then examined by an optical microscope (Figure 8a).
of bone marrow stem cells (BMSCs) seeded in the scaffolds were further analyzed (Figure 7b,c). As marked by the arrows shown, the cells seeded in the scaffold attached well. After a 14 day culture, the BMSCs could proliferate and deposit an extracellular matrix in intervals of the scaffold. The presence of BMSCs in the circular vertical scaffold significantly promoted the matrix deposition and the cell viability of the BMSCs than those in the circular inclined scaffold. From the scanning electron microscopy (SEM) images, for the circular inclined scaffold, large pore voids existed, but the interior of the circular vertical scaffold was covered with the cell and extracellular matrix.

**DISCUSSION**

Throughout the existing research results, a three-dimensional finite element model based on cartilage was established to explore the mechanical behavior of the repair area under the physiological compression load in order to reflect the shape and stacking way of the scaffold and to explore the influence of different repair shape and stacking way scaffold parameters on the mechanical behavior of the repair area, so as to provide a reference for the subsequent clinical repair research of cartilage defects. The results showed that the vertically stacked circular and rectangular scaffold have an overall shape rule, with strict symmetry about the X and Y axes. The circular vertical scaffold had significantly higher values for density, porosity, water absorption expansion rate, and compressive Young’s modulus tested than the circular inclined scaffold, with the exception of soluble loss rate. The stress–strain curves show that both the circular vertical scaffold and circular inclined scaffold exhibited viscoelastic behavior.

In cartilage tissue engineering, physiological stimuli are necessary, and the appropriate mechanical environment can overcome the traditional culture conditions of cartilage cells. Cells in the scaffold can meet nutritional needs and reduce the growth and death of cells in the center of the scaffold because of malnutrition or a poor metabolism. Koh et al.11 found that the rate of proliferation and production of a matrix of chondrocytes increased significantly after artificial cartilage experienced mechanical loading in vitro. In a study on the construction of tissue engineered cartilage in vitro, Mauck12 inoculated chondrocytes in alginate block sine dynamic pressure and found that the glycosaminoglycan (GAG) and collagen content was significantly higher than that in the nonloading group after 4 weeks of loading. Therefore, mechanical factors are critical for cell growth, proliferation, and differentiation. Furthermore, the larger and more distributed pores and porosity are conducive to the infiltration of nutrients and metabolic waste discharge. The OD values from the assay of the circular vertical scaffold were significantly higher than that obtained by the circular inclined scaffold. The presence of cells in the circular vertical scaffold significantly promoted the matrix deposition and the cell viability of the BMSCs than those in the circular inclined scaffold. All these prior results showed that analysis and demonstration of the scaffold finite element model for cartilage tissue engineering did not destroy the biological activity of the raw scaffold and that good compatibility of the scaffold could promote the proliferation and differentiation of chondrocytes. The effects of different types of the scaffold finite element model and parameters of the artificial cartilage scaffold were discussed. Finite element model analysis can be used to repair this kind of irregular defects, where actually its shape properties are difficult to meet the clinical requirements. The whole microstructure of

![Figure 2](https://dx.doi.org/10.1021/acsomega.0c04378)
the cartilage defect area is basically realized in this study, but the finite element model to achieve complete biomechanical anisotropy needs further study. In the next step, we will evaluate its effect on repairing cartilage defects in vivo.

■ CONCLUSIONS
In this study, the circular scaffold is preferable compared to the scaffold with a rectangular shape; vertical stacking was conducive to repairing cartilage tissue compared with inclined stacking, but the strain distribution of the circular inclined stacking scaffold can basically be satisfied and chosen. Compared with the circular inclined scaffold using the 3D printing method, the circular vertical scaffold exhibited a better overall performance.

■ MATERIALS AND METHODS
Preparation of the Scaffold. In summary, silkworm silk was boiled, dissolved, centrifuged, dialyzed, concentrated, and obtained in an 8 wt % silk fibroin solution. Fresh bovine tendon was stripped, soaked in Tris buffer, dialyzed, and obtained in a 2.5 wt % slurry of type II collagen. The silk fibroin solution was mixed with a slurry of type II collagen. Thus, SF/C scaffolds were fabricated using the 3D printing method. The printing conditions were set at a 10 mm/s deposition rate, 0.3 mm slice thickness, −20 °C forming platform temperature, 0.09 mm/min extrusion rate, and 0.36 mm nozzle tip size.

Establishment of the Model and Parameters.
(1) Scaffolds were cut, divided into circular and rectangular shapes, stacked vertically and inclined, and arranged into three-dimensional solids, where the size and stacking diagram is shown below (Figure 1).

(2) Scaffold parameters: elastic modulus $E = 5.0$ MPa and Poisson’s ratio $\nu = 0.3$

Model and Mesh Generation. The model was established and analyzed using ANSYS12.0 (USA ANSYS 12.0). Solid95 software (USA Dassault Systemes) was used for the mesh generation, and the process is shown in the following chart (Figure 2a–d).

Boundary Condition and Load Application. The boundary condition was set to the bottom constraint at all degrees of freedom (level $Z = 0$), and the top pressure was applied.
applied with a uniform load of 10 kPa (Z = 10 mm) (the concentrated load for the circular scaffold was equal to approximately 1 N and that for the rectangular scaffold was equal to approximately 0.1 N).6,8

Displacement. The displacement reflects the change in the spatial position of the model under the load in the finite element calculation. The experimental output was the displacement component of the model along the X, Y, and Z axes.

Strain and Stress. Strain is a measure of the relative deformation of the dimensionless parameters and can reflect the local deformation; the larger the strain value of a node is (absolute value), the more deformation there is at that point. Because the scaffold is a linear elastic scaffold, the stress is directly proportional to the strain (σ = Eε).

Table 1. Axial Displacement Maximum Statistical

| material            | UX max/μm | UY max/μm | UZ max/μm |
|---------------------|-----------|-----------|-----------|
| circular vertical   | 6.50      | 6.50      | 19.78     |
| circular inclined   | 7.07      | 20.86     | 38.94     |
| rectangular vertical| 0.94      | 1.22      | 19.79     |
| rectangular inclined| 3.88      | 138.97    | 92.49     |

Table 2. Von Mises Strain Distribution

| material          | <1000 με (%) | 1000–3000 με (%) | >3000 με (%) |
|-------------------|--------------|------------------|-------------|
| circular vertical | 1.4          | 96.1             | 2.5         |
| circular inclined | 24.3         | 68.5             | 7.2         |
| rectangular vertical | 0.2      | 99.0             | 0.8         |
| rectangular inclined | 16.5     | 51.5             | 32.0        |

Table 3. Von Mises Stress Distribution

| material          | <5 KPa (%) | 5–10 KPa (%) | 10–20 KPa (%) | >20 KPa (%) |
|-------------------|------------|--------------|---------------|-------------|
| circular vertical | 1.4        | 62.2         | 35.6          | 0.8         |
| circular inclined | 24.6       | 38.3         | 34.5          | 2.7         |
| rectangular vertical | 0.2      | 59.5         | 40.2          | 0.1         |
| rectangular inclined | 16.9     | 23.9         | 38.7          | 20.6        |
Quantitative Analysis. The calculation for the X, Y, and Z directions of the displacement and the local strain of the extreme value and distribution of the data were gathered statistics. In this experiment, the distribution data for the axial displacement of...
the circular and rectangular scaffold in the vertical and inclined stacking cases as well as the local von Mises stress/strain distribution are shown in result.

Scaffold Physical Performance. According to the statistical results, a circular vertical scaffold and a circular inclined stacking scaffold were fabricated with silk fibroin/collagen using the 3D printing method. The scaffold was dried, measured at 105 °C, and labeled as M1 (g). In a centrifuge tube with a bath ratio of 1:100, deionized water was added, preheated for 30 min in a 37 °C thermostatic oscillator, filtered, and dried again, and the residual weight was measured and labeled as M2. Thus, the soluble loss rate in hot water was calculated as (% = (M2 − M1)/M1 × 100%).

The tube was selected with a scale and anhydrous ethanol was added with a volume of V1; the scaffold sample was immersed in it for 5 min and degassed under negative pressure until there no bubble escaped from the scaffold, and the ethanol volume recorded at this time was V2; the scaffold sample was taken out, and the remaining ethanol volume was denoted as V3. The porosity rate was calculated as (% = (V1 − V3)/(V2 − V3) × 100%.

The densities of fibroin and collagen were D1 and D2, respectively, and the mass fractions of silk fibroin and collagen in the blends were W1 and W2, respectively, and the density of the scaffold was measured and calculated according to the following formula: 1/D = W1/D1 + W2/D2. The scaffold surface was sprayed, and the pore size and microstructure were evaluated using an SEM.

Biomechanical Properties. The dynamic mechanical properties of the scaffolds were used to represent the mechanical behavior under wet conditions and cyclic loading. The scaffold was similar to the physiological environment in vivo under 0.01

### Table 4. Properties of the Circular Vertical and Circular Inclined Scaffold

| scaffold                  | density (g/mL) | porosity (%)  | water absorption expansion rate (%) | compressive Young’s modulus (kPa) | soluble loss rate (%) |
|---------------------------|----------------|---------------|-------------------------------------|-----------------------------------|-----------------------|
| circular vertical scaffold| 0.28 ± 0.03    | 97.5 ± 4.5    | 1331.8 ± 166.5                      | 28.6 ± 6.5                        | 0                     |
| circular inclined scaffold| 0.16 ± 0.01    | 66.4 ± 7.1    | 476.8 ± 180.5                       | 17.6 ± 7.1                        | 3.85 ± 1.33           |

$P < 0.05$ $< 0.05$ $< 0.05$ $< 0.05$ $< 0.05$

**Figure 6.** (a) Stress–strain curves suggesting that circular vertical scaffolds exhibited viscoelastic behavior (green color corresponds to compress stress, and red color corresponds to compress strain). (b) Stress–strain curves suggesting that circular inclined scaffolds exhibited viscoelastic behavior (green color corresponds to compress stress, and red color corresponds to compress strain).
MPBS, and the test conditions were set using a universal testing machine (Instron 5865) with a saw tooth waveform of 0.5 Hz, a preload of 0.1 N, an increment of 50%, and a speed of 100.0%/minute. Finally, the stress−strain curve was drawn; thus, the Young modulus of the scaffold was obtained.

**Cell Viability and Microstructure.** The scaffolds were each seeded with 100 μL of BMSC suspension containing $3 \times 10^5$ cells. The MTT assay was used to assess the cell viability. The concept of the assay is based on the metabolic activity of the live cells. After 14 days in culture, the scaffolds with attached cells were observed using a scanning electron microscope.

---

**AUTHOR INFORMATION**

**Corresponding Author**

Hui Li − Tianjin Medical University General Hospital, Tianjin 300192, China; Email: lihuiortholivea@sina.cn

**Authors**

Kai Sun − Department of Orthopaedics, Tianjin First Central Hospital, School of Medicine, Nankai University, Tianjin 300192, China; orcid.org/0000-0002-9432-8386

Ruixin Li − Institute of Medical Equipment, Academy of Military and Medical Sciences, Tianjin 300000, China

Meng Fan − Department of Orthopaedics, Tianjin First Central Hospital, School of Medicine, Nankai University, Tianjin 300192, China

Hao Li − Institute of Medical Equipment, Academy of Military and Medical Sciences, Tianjin 300000, China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c04378

---

**Author Contributions**

K.S. carried out the studies and drafted the manuscript. R.L., H.L., M.F., and H.L. participated in the design of the study and performed the statistical analysis. H.L. conceived the study and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

**Funding**

This work was funded by grants from the Tianjin First Center Hospital Foundation of China (2019CM12).

**Notes**

The authors declare no competing financial interest. The study was approved by the institutional review board of Tianjin First Center Hospital, Tianjin Medical University General Hospital and Institute of Medical Equipment, Academy of Military and Medical Sciences, and all authors and patients have read and approved the final manuscript. All authors have read and approved the final manuscript. All data generated or analyzed during this study are included in this published article.

---

**ACKNOWLEDGMENTS**

The authors would like to thank Tianjin First Center Hospital, Tianjin Medical University General Hospital and Institute of Medical Equipment, Academy of Military and Medical Sciences for providing the database.

**ABBREVIATIONS**

BMSC, bone marrow stem cell; 3D, three dimensional; MTT, thiazolyl blue tetrazolium bromide; OD, optical density; SEM, scanning electron microscopy; GAG, glycosaminoglycan; CVSM, circular vertical stacking material
REFERENCES

(1) Desjardins, M. R.; Hurtig, M. B. Cartilage healing: A review with emphasis on the equine model. Can. Vet. J. 1990, 31, 565–572.
(2) Bhardwaj, N.; Kundu, S. C. Chondrogenic differentiation of rat MSCs on porous scaffolds of silk fibroin/chitosan blends. Biomater 2012, 33, 2848–2857.
(3) Maia, F. R.; Carvalho, M. R.; Oliveira, J. M.; Reis, R. L. Tissue Engineering Strategies for Osteochondral Repair. Adv. Exp. Med. Biol. 2018, 1059, 353–371.
(4) Qasim, M.; Dong, S. C.; Lee, N. Y. Advancements and frontiers in nano-based 3D and 4D scaffolds for bone and cartilage tissue engineering. Int. J. Nanomed. 2019, 14, 4333–4351.
(5) Janarthanan, G.; Kim, I. G.; Chung, E. J.; Noh, I. Comparative studies on thin polycapro- lactone-tricalcium phosphate composite scaffolds and its interaction with mesenchymal stem cells. Biomater. Res. 2019, 23, 1.
(6) Sun, K.; Li, R.; Li, H.; Dong, L.; Jiang, W. Comparison of three-dimensional printing for fabricating silk fibroin-blended scaffolds. Int. J. Polym. Mater. 2018, 8, 480–486.
(7) Karadjian, M.; Essers, C.; Tsitlakidis, S.; Bruno, R.; Moghaddam, A.; Boccaccini, A. R.; Westhauser, F. Biological Properties of Calcium Phosphate Bioactive Glass Composite Bone Substitutes. Int. J. Mol. Sci. 2019, 20, 305.
(8) Sun, K.; Li, R.; Jiang, W.; Sun, Y.; Li, H. Comparison of three-dimensional printing and vacuum freeze-dried techniques for fabricating composite scaffolds. Biochem. and Biophy. Res.Commu. 2016, 477, 1085–1091.
(9) Fenn, S. L.; Oldinski, R. A. Visible light crosslinking of methacrylated hyaluronan hydrogels for injectable tissue repair. J. Biomed. Mater. Res. B Appl. Biomater. 2016, 104, 1229–1236.
(10) Zhou, Y.; Liang, K.; Zhao, S.; Zhang, C.; Li, J.; Yang, H.; Liu, X.; Yin, X.; Chen, D.; Xu, W.; Xiao, P. Photopolymerized maleilated chitosan/methacrylated silk fibroin/nanocomposite hydrogels as potential scaffolds for cartilage tissue engineering. Int. J. Biol. Macromol. 2018, 108, 383–390.
(11) Koh, R. H.; Jin, Y.; Kang, B. J.; Hwang, N. S. Chondrogenically primed tonsil-derived mesenchymal stem cells encapsulated in riboflavin-induced photocrosslinking collagen-hyaluronic acid hydrogel for meniscus tissue repairs. Acta Biomater. 2017, 33, 318–328.
(12) Morrison, R. J.; Nasser, H. B.; Kaslan, K. N.; Zopf, D. A.; Milner, D. J.; Flanagan, C. L.; Wheeler, M. B.; Green, G. E.; Hollister, S. J. Co-culture of adipose-derived stem cells and chondrocytes on three-dimensionally printed bioscaffolds for craniofacial cartilage engineering. Laryngoscope 2018, 128, E251–E257.
(13) Rasheed, T.; Bilal, M.; Zhou, Y.; Raza, A.; Shah, S. Z. H.; Iqbal, H. M. N. Physiochemical characteristics and bone/cartilage tissue engineering potentials of protein-based macromolecules- A review. Int. J. Biol. Macromol. 2019, 121, 13.
(14) Yamasaki, A.; Kunitomi, Y.; Murata, D.; Sunaga, T.; Kuramoto, T.; Sogawa, T.; Misumi, K. Osteochondral regeneration using constructs of mesenchymal stem cells made by bio three-dimensional printing in mini-pigs. J. Orthop. Surg. Res. 2019, 37, 1398–1408.
(15) Paul, A.; Manoharan, V.; Kral, D.; Assmann, A.; Uquillas, A.; Shin, S. R.; Hasan, A.; Hussain, M. A.; Memic, A.; Gaharwar, A. K.; Khademhosseini, A. Nanoengineered biomimetic hydrogels for guiding human stem cell osteogenesis in three dimensional microenvironments. J. Mater. Chem. B 2016, 4, 3544–3554.
(16) Roseti, L.; Cavallo, C.; Desando, G.; Parisi, V.; Petretta, M.; Bartolotti, I.; Grigolo, B. Three-Dimensional Bioprinting of Cartilage by the Use of Stem Cells: A Strategy to Improve Regeneration. Material 2018, 11, 1749.