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Gas-foaming three-dimensional electrospun nanofiber scaffold improved three-dimensional cartilage regeneration

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Abstract

Repairing cartilage defect is always an intractable problem in joint surgery field. Tissue engineering, in the industry, is universally considered as a decent solution for overcoming this challenge. Especially the three-dimensional (3D) scaffolds play a significant role in cartilage repair. Thereinto, the electrospinning has become a very attractive method for the preparation of scaffolds. In recent years, however, these scaffolds are limited in terms of their three-dimensional (3D) applications due to their two-dimensional (2D) structure and pore size which are smaller than a cartilage cellular diameter and thus limit the cellular migration in these structures. To address this issue, this study will present an promising post electrospinning approach that can transform two-dimensional scaffolds into three-dimensional scaffolds via the way of insitu gas foaming within the pores of the nanofiber membranes as the driving force. Our previous study reported that agelatin/polycaprolactone (GT:PCL) ratio of 7:3 might be suitable for the cartilage regeneration [Zheng R, et al/The influence of Gelatin/PCL ratio and 3D construct shape of electrospun membranes on cartilage regeneration. Biomaterials 2014;35:152-164]. Therefore, in the present experiment, we chose the above ratio (GT:PCL = 7:3) to realize two types of scaffolds (2D and 3D scaffolds) transition via the gas-foaming technique and investigated whether the three-dimensional structure was more conducive to cartilage regeneration than 2D. The experiment results have revealed that 3D scaffolds can achieve a larger pore size, higher porosity and higher biocompatibility than 2D scaffolds. In addition, both scaffolds which were implanted with chondrocytes all had formed mature cartilage-like tissues after 8 weeks of culturing in rabbits, and the 3D scaffold formed a three-dimensional structure, whereas the 2D scaffold only formed a thin layer of cartilage. As the macroscopic and histological results showed after 12 weeks postoperation, in the 2D scaffold group, the defect was full of fibrillar connective tissue, and as shown by HE staining, obviously there is no staining with Saf-O/FG and toluidine blue on the surface of repaired site. On the contrary, in the 3D scaffold group, homogeneous and mature cartilaginous tissue were found in the defect area. The defect was filled with numerous new chondrocytes, and the histological staining revealed a large amount of regenerated cartilage tissue which was perfectly integrated with normal cartilage tissue. The results distinctly indicated that the 3D scaffold led to better cartilage repair effects than the 2D scaffold. Generally speaking, the current study demonstrated that a gas-foaming three-dimensional electrospun nanofiber scaffold would be a potential platform for cartilage regeneration and might provide a potential treatment option for repairing articular cartilage defects.
1. Introduction

The poor regeneration ability of cartilage is considered as one of the momentous reasons in repairing cartilage defects [1, 2]. Tissue engineering is widely regarded as an effective solution to the challenge [3, 4]. Three-dimensional scaffolds make a big difference to the cartilage repair. The ideal scaffold should have a customizable 3D shape, appropriate pore size and good mechanical properties [5, 6]. Therefore, fabricating a suitable 3D scaffold for cartilage regeneration is crucial anyway.

Electrospinning is a convenient and universal method of producing nanofibers with controllable diameters that can form a network mimicking the native extracellular matrix (ECM) [7–13]. Thus, possessing the advantages of low cost, controllable pore size, and high porosity, electrospinning has been a commonly used method for preparing cartilage tissue engineering scaffolds [14, 15]. The conventional electrospinning process uses a static plate collector placed below a charged nozzle which contains a polymer solution at a certain distance. However, one of the main disadvantages of traditional two-dimensional electrospinning nanofiber membranes is the small pore size due to the close accumulation of nanofiber layers (The pore diameter nanofibers prepared by electrostatic spinning usually range from 100 nm to 1 μm). The size of chondrocytes is generally within 100–150 μm), which impedes cell adhesion and formation of three-dimensional tissue structure [16].

Compared with 2D scaffolds, 3D scaffolds have a better three-dimensional structure and can achieve larger area in defect repair. 2D scaffolds can only achieve tissue regeneration on a single plane, while 3D scaffolds can achieve tissue regeneration of various shapes, of which the consequence is more in line with clinical requirements. Our previous experiment realized 3D tissue regeneration by the following methods: Firstly, complete the cartilage regeneration of multiple 2D electrospinning scaffolds, and then, stack multiple 2D cartilage together to achieve 3D regeneration. This structure is similar to a sandwich, so gaps between layers and the structure are unstable [17]. In this experiment, to solve the problem, the gas-foaming technology was applied to realize the three-dimensional structure of the electrospinning scaffold forming.

For the past years, vast reports on the preparation of 3D scaffolds have been proposed, such as multilayering electrospinning [18], electrospinning followed by various posttreatments [19, 20], liquid-assisted collection [21], template-assisted collection [22], and porogen-added electrospinning. However, the high cost, complicated production process and long fabrication periods made the application of these methods failure. To avoid the adverse impact above, the gas-foaming method attracted our attention doubtlessly. The gas-foaming process can utilize the nucleation and the growth of gas bubbles that are generated in situ either via a chemical reaction or the addition of inert gases to the polymer phase in diverse physical environments [23–25]. However, by far no study has reported whether gas-foaming three-dimensional electrospun nanofiber scaffolds could be used on cartilage engineering field. Herein, gelatin and polycaprolactone were used as raw materials to prepare 2D scaffolds, and then adopting gas foaming technique to obtain 3D scaffolds transition.

Our previous study demonstrated that it was viable to generate 3D cartilage by using electrospun nanofibrous membranes composed of GT and PCL (GT:PCL = 7:3) [17]. Thus, two different kinds of electrospun scaffolds (2D and 3D) were prepared with the same ratio above. The mechanical properties, cell adherence rate, and biocompatibility of scaffolds were analyzed to evaluate whether the 3D scaffolds would be better than 2D scaffolds in effect. In the end, the 3D scaffold showed high biocompatibility in vivo and enhanced the repair of articular cartilage defects in rabbits. The schematic diagram of the experimental design is shown in figure 1.

2. Materials and methods

2.1. Animals

New Zealand Jackrabbit (2 months old) were purchased from Shanghai Jiagan Biotechnology Co.Ltd. All of the animal feeding, rearing, as well as the research procedures, were approved by the Animal Care and Experiment Committee of Weifang Medical University (Shandong, China). The number of the testing rabbits in each group was controlled at 30.

2.2. Preparation of the 2DGT/PCL scaffold

GT/PCL (the weight ratio of GT:PCL = 70:30,) membranes were prepared by electrospinning. In brief, GT and PCL with a weight ratio of 70:30 were dissolved in hexafluoropropanol to prepare 12% W/V solution, which were stirred magnetically for 24 h. The solution was then placed in a 10-ml syringe with a 20G stainless steel needle and dispersed by a syringe pump at a rate of 0.4 ml h−1 at 45%–55% humidity and 21 °C–22 °C. A high-voltage power supply (TXR1020N30–30, Tesla, Dalian, China) applied a 9 V DC electrospinning voltage was conducted to the needle. The distance between the tip of the syringe and the collector was controlled at 8 cm. The
electrospun membranes were collected on the aluminium foil mounted on the surface of the adjustable laboratory jack. The electrospun GT/PCL membrane was dried in vacuum oven at room temperature for one week so as to remove the residual solvent [26]. In order to improve the mechanical properties of scaffolds, they were subjected to a desiccator containing glutaraldehyde vapor for 6 h. Moreover, the crosslinked scaffolds were soaked in glutamic acid solution to remove residual glutaraldehyde. On top of it, the wet mats were put into the refrigerator at $-80^\circ C$ for 24 h, eventually transferred to a vacuum freeze dryer for 72 h to obtain the crosslinked and dried 2DNFS.

### 2.3. Preparation of the 3D GT/PCL scaffold

The dried 2D scaffolds were immersed into a 1M NaBH₄ aqueous solution. NaBH₄ reacts with water to generate hydrogen as follows: $\text{NaBH}_4 + 2\text{H}_2\text{O} \rightarrow \text{NaBO}_2 + 4\text{H}_2$. Taking advantage of this reaction, hydrogen bubbles would be able to penetrate into the internal pore space of the scaffolds. Of which the thickness and volume expansion could verify if the performance of this process worked. Current knowledge about the mechanism of the transformation from 2D mats to 3D scaffolds makes us aware that the driving force for this transformation is attributed to the in situ gas foaming within the pores of the nanofiber membranes. The surface pores of the membrane enhance the absorption capacity of the NaBH₄ solution by capillary forces, resulting in a diffusion of the solution from the outer dry layer to the core. Then, hydrogen ($H_2$) bubbles are generated in situ and rearrange the fibers into a three-dimensional structure by exerting pressure on the surrounding fibers. In addition, during vacuum drying, gas bubbles, trapped in the pores, escape, which can further expand the thickness and volume of the scaffold. After 1.5 h, the expanded scaffolds were undocked and washed with deionized water three times to obliterate residual NaBH₄. Subsequently, the expanded scaffolds would be kept in a freezer at $-80^\circ C$ for 24 h and finally transferred to a vacuum freeze-dryer for 72 h to complete the dried 3D scaffolds transition.

### 2.4. Mechanical analysis

The mechanical properties of 2D and 3D scaffolds were measured by uniaxial material testing machine. The Young’s Modulus of the scaffolds was analyzed and the stress–strain curve was drawn [27].

### 2.5. Scanning electron microscopy (SEM)

The 2D and 3D scaffolds were examined by SEM (JEOL-6380 IV, Japan).

### 2.6. Hydrophilicity evaluation

Deionized water drops were added to the surfaces of the two scaffolds respectively (2D and 3D), and the contact angles would be measured by a video goniometer after 4 seconds upon the water drops touched the surfaces of the scaffolds [27, 28].
2.7. Isolation and culture of chondrocytes
The auricular cartilage was cut into about 1.0 mm³ piece each and treated in 0.15% type II collagenase (Gibco) in Dulbecco’s modified Eagle’s medium (DMEM, Gibco) at 37 °C for 8 h. The chondrocytes were cultured in DMEM supplemented with 10% FBS (Gibco) and 1% penicillin-streptomycin (Gibco) at 37 °C with 5% CO₂ [29].

2.8. Cell proliferation assay
After 1, 4 and 7 days of culturing separately, the proliferation of the cells on the scaffold was evaluated by the Live & Dead Cell Viability Assay (Invitrogen Inc., USA), and the scaffold was examined by confocal microscopy (Nikon, JP) [30]. Viable cells were analyzed by using Cell Counting Kit-8 (CCK-8; Dojindo, Japan) complying with the manufacturer’s instructions. The optical density (OD) was measured at 450 nm, and the mean value derived from five wells could be calculated then.

2.9. Adherence rate
After 24 h of cultivation, gently rinsed the chondrocyte-scaffold structure with PBS, mean while, counted the number of cells (N) in the rinse solution and petri dish. The computational formula of the cell adhesion rate of the scaffold is as follow: (total number of cells - N) / total number of cells × 100% [31].

2.10. Preparation of chondrocyte-scaffold constructs
The 2D and 3D GT/PCL scaffolds were placed in different petri dishes with 5 ml cell suspensions of 100 × 10⁶ cells ml⁻¹ added to both scaffolds. After resting in the petri dishes for one hour, DMEM medium containing 10% fetal bovine serum was slowly added severally, and made sure that the scaffolds were cultured at 37 °C and 5% CO₂. Two weeks later, one group was cultured in vitro for another 2 weeks and the other group was subcutaneously implanted into rabbits for 8 weeks.

2.11. Analysis of subcutaneous cartilage regeneration
The New Zealand Jackrabbit were anesthetized with 10% chloral hydrate (4 ml kg⁻¹), and then separated the subcutaneous tissue, following the next step of laying the chondrocyte-scaffold structure under the skin, at last closed the incision. Samples would be taken 8 weeks later after surgery [32].

2.12. Articular cartilage repair in rabbits
The New Zealand Jackrabbits weighing about 2.5 kg were randomly selected to be tested in the experiment. The 2D and 3D chondrocyte-scaffold structures (diameter: 3.5 mm, depth: 4 mm) were prepared by above steps. A trephine would be used to drill cartilage defects (diameter: 3.5 mm, depth: 4 mm) at the knee joint. Rabbits were randomly allotted into two groups (N = 10): 2D scaffold group and 3D scaffold group. All testing rabbits would be killed for the purpose of the experiment 12 weeks later after operation to collect specimens. The specimens of HE staining, Safranin-O/Fast Green staining (SaF-O/FG), immunohistochemical staining and toluidine blue staining were performed on the knee joint. Finally the histological analysis of the cartilage specimens was performed [32].

2.13. Histological and immunohistochemical analyses
The samples of regenerated cartilage were also stained histologically and immunohistochemically conforming to above procedures, and conducted histological stain analysis. Histological staining included HE staining, Safranin-O/Fast Green staining (SaF-O/FG), and toluidine blue staining. Type II collagen was identified by IHC.

2.14. Biomechanical analyses
The biomechanical properties of the regenerated cartilage were evaluated by analyzing Young’s modulus with a biomechanical analyzer (Instron-5542, Canton, Ma, USA) [33].

2.15. Biochemical analyses
All Specimens were handled in a Sigma-Aldrich solution at 65 °C Papain. The content of glycosaminoglycan sulfate (GAG) was determined by the Alcian blue method. As for the DNA content, it would be detected by the nucleic acid protein quantitative analyzer (Nanodrop2000). Repeat analysis three times for each sample. The content of total Collagen in each group was determined by hydroxyproline assay [34–36].

2.16. Statistical analysis
Quantitative data were analyzed by Student’s t-test, in which only p-Values <0.05 would be considered statistically significant. All values were expressed as mean ± standard deviation.
3. Results

3.1. Characteristic analysis of 2D and 3D scaffolds

3.1.1. Surface morphology characterization

The morphology of the 2D and 3D scaffolds was both examined by SEM (figures 2(A2), (A3), (B2), (B3)). As a result, the 2D scaffold was entirely composed of dense nanofiber layers with superficial tiny pores (figure 2(A3)). In contrast, the 3D scaffold exhibited larger pores with deeper connections, and the fibers were twisted to generate a wave-like structure (figure 2(B3)). The diameter of the obtained electrosprun fibres did not change after the gas-foaming. The cross-section images revealed that the 3D scaffold had a looser multilayer structure between the layers and a more certain degree of layer stacking upon expansion, along with a greater porosity and an increased thickness of the scaffold (figures 2(B2), (B3)). The 3D scaffold had a directional layered structure, and the gap width was in the range of 20 ~ 150 μm, which provided favorable conditions for cell diffusion and proliferation. The results demonstrated that the dense 2D scaffold was successfully converted into a 3D sponge-like scaffold with a looser multilayer structure and larger pores after the gas-foaming process and the expansion. The ratio was close to 1000% (figures 2(A1), (B1)).

3.1.2. Mechanical properties

Stress-strain curves of 2D and 3D scaffolds are shown in figure 3(A). It was evident that the tensile modulus of the 2D scaffold was lower than that of the 3D scaffold, which was as what we had expected because the layered architecture and the presence of pores within the scaffolds reduced the ability to support the stress and increased the porosity. Elongation at break of the 3D scaffold slightly increased compared to that of the 2D scaffold, which might be attributed to the higher porous (less dense) and looser structures of the 3D scaffold. Although the mechanical properties of 3D scaffold are not as good as 2D scaffold, (after the 2D and 3D chondrocyte-GT/PCL scaffold constructs were cultured for 2 weeks, and subcutaneously implanted into rabbits for 8 weeks), the 3D constructs still showed better mechanical properties than 2D constructs.

3.1.3. Pore diameter and porosity analysis

The pore diameters of the 2D and 3D scaffolds were measured by using a capillary flow porometer. As shown in figure 3(D), the maximum pore diameter of the 3D scaffold was approximately 3.0 μm, which was larger than the maximum pore diameter of the 2D scaffold of 1.5 μm. The pore diameter of the 2D scaffold got a scope of 0.7 μm to 0.9 μm, while that of the 3D scaffolds increased the values ranging from 1.0 μm to 1.7 μm (figure 3(C)). As shown in figure 3(B), the 3D scaffold showed a significant increase in porosity to 88.4% ± 6.8% compared to
the porosity of the 2D scaffold, which was 78.8% ± 3.4%. The above experimental results showed that the 2D scaffolds have been successfully transformed into 3D scaffolds through gas-foaming technology, and the pore size and porosity were significantly increased.

3.1.4. Contact angle
The hydrophilicities of the two scaffolds were analyzed by testing the water contact angles. Through the observation, at the 15th second, the 2D scaffold was still at an Angle of 2 degrees with the water, but the 3D scaffold could no longer see any Angles. The results apparently revealed that the hydrophilicity of the 3D scaffold was higher than that of the 2D scaffold (figure 4). This may be due to the looser multilayer structure as well as the larger pore size on the surface and higher porosity of the 3D scaffold.

3.2. Biocompatibility and adherence rate of porous FC scaffolds
To explore the effect of electrospinning residual solution on cartilage regeneration, the two scaffolds were colonized with chondrocytes as the method. Live-dead staining assay showed that chondrocytes survived and proliferated well from 1–7 days (figures 5(A1)–(A3), (B1)–(B3)), which was further substantiated by the cell viability assay (figure 5(D)), as both of these scaffolds had the same composition. Additionally, the adherence rates of 2D and 3D scaffold were 79% and 88% respectively, also the 3D scaffold showed a higher adherence rate than the 2D scaffold because of its looser multilayer structure and larger pore size (figure 5(C)).

3.3. In vivo cartilage regeneration
To evaluate whether the 3D scaffolds have the superiority for cartilage regeneration comparing to 2D scaffolds, the two scaffolds, precultured in vitro for 2 weeks, were subcutaneously implanted into the rabbits respectively. After 8 weeks of in vivo culturing, there was no changes in the structure and morphology of the 3D scaffolds, and mature cartilage tissue was formed, as observed in the gross view image in figure 6(B1). Histological analysis revealed that all samples showed cartilage specific extracellular matrix deposition and cartilage capsule structures, as observed by positive GAG and type II collagen staining (figures 6(B2)–(B4)). In contrast, the 2D scaffold maintained its shape and formed only one layer of cartilage-like tissue within 8 weeks after in vivo

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**Figure 3.** Mechanical analyses of stress-strain curves (A), quantitative analyses of porosity (B) and pore size distribution (C), (D) of 2D scaffold and 3D scaffold. The results showed that the 2D scaffolds were successfully transformed into 3D scaffolds through gas-foaming technology, and the pore size and porosity were significantly increased. *p < 0.05.
implantation (figure 6(A1)). Histological images showed a homogeneous cartilage-specific ECM deposition and a typical lacuna structure (figures 6(A2)–(A4)). Additionally, biomechanical and biochemical analysis revealed that Young’s modulus (figure 7(A)), GAG content (figure 7(D)), DNA content (figure 7(B)) and total collagen
In vivo engineered cartilage increased over time, and the 3D scaffold constructs within 8 weeks were even more comparable to native articular cartilage. The 2D scaffolds only formed a thin layer of cartilage around the material, and the regenerated cartilage was less numerous and less mature. In comparison, the 3D scaffolds can form a complete larger mature cartilage, which can be more basically filled with cartilage tissue, along with less residual material, and the cartilage specificity indexes were close to those of the natural cartilage. In view of that, the experimental results showed that the 3D scaffold has a more significant advantage in cartilage regeneration compared to the 2D scaffold.

3.4. In situ repair of articular defects in rabbits

In situ repair of articular cartilage defects in tested rabbits is the most direct experimental basis for predicting its clinical application. In this study, two kinds of scaffolds were both used to repair cartilage defects of rabbit knee to illustrate the advantages of 3D scaffolds. After 12 weeks of the operation, the cartilage defect was not completely repaired in the 2D scaffold group (figure 8(A1)). The cartilage defect was repaired well in the 3D scaffold group instead. (figure 8(B1)).

In the 2D scaffold group, the defect was clearly demarcated from the normal site, and was full of fibrillar connective tissue, as shown by HE staining (figure 8(A2)) which obviously no staining with Safranin-O/FG and toluidine blue appeared (figures 8(A3), (A4)) on the surface of repaired site. It indicated that no mature cartilage formation and no cartilage-specific ECM deposition occurred either. However, in the 3D scaffold group, homogeneous and mature cartilaginous tissue were found in the defect area (figure 8(B1)). The defect was filled
with numerous new chondrocytes, and histological staining that revealed a large amount of regenerated cartilage tissue was perfectly integrated with the normal cartilage tissue (figures 8(B2)–(B4)). Immunohistochemical analysis manifested that less deposition of type II collagen in the 2D scaffold group existed (figure 8(A5)). But a large amount of deposition was found in the type II collagen of 3D scaffold group (figure 8(B5)), and a large amount of regenerated cartilage tissue revealed. To sum up, the whole experiment confirmed that 3D scaffolds have greater advantages than 2D scaffolds in repairing rabbit articular cartilage defects.

Because the 2D group was mainly repaired by fibrous tissue, in contrast, the 3D group was mainly repaired by cartilage tissue, all the quantitative data (figure 9) including Young’s modulus, DNA content, total collagen content and GAG content showed that 3D group was much higher than 2D group.

4. Discussion

The repair of cartilage defects has been an intractable trouble in articular surgery filed. Lacking of blood vessels and nerves, the self-regeneration of cartilage is very feeble [37, 38]. Therefore, tissue engineering is naturally viewed as a better solution for overcoming this challenge. As one of the three elements of the tissue engineering, three-dimensional scaffolds play an irreplaceable role in cartilage repair.

Electrospinning has been employed as one of the common techniques for fabricating tissue engineering scaffolds nowadays because of its superiority, such as simple manufacturing devices, low costs, wide variety of spinnable materials and controllable process. Especially in tissue engineering, electrospinning is a simple, cost-effective technique, and a way to fabricate porous scaffolds that can simulate the structure of extracellular Matrix nanofiber membranes [39]. However, one of the major disadvantages of traditional two-dimensional (2D) electrospun nanofiber membranes is that the dense nanofiber layer results in a small pore size, which impedes cell infiltration and the three-dimensional tissue formation. Therefore, it is necessary to introduce a new method that can transform traditional two-dimensional electrospun nanofiber membranes into three-dimensional structures with looser structures and more appropriate pores. Compared with 2D scaffold, 3D scaffold can provide a more suitable platform for cell adhesion, cell proliferation and cell migration, which is more favorable for 3D tissue regeneration [40].

In this experiment, PCL and GT were used as raw materials to prepare 3D wave-shaped nanofiber scaffolds by gas foaming (figure 1). Our previous study reported that a ratio of 70:30 GT/PCL might be suitable for the
cartilage regeneration. Therefore, we used this ratio to prepare two different scaffolds (2D and 3D scaffolds), which were cultured with 5 ml of a chondrocyte suspension with \(100 \times 10^6\) cells ml\(^{-1}\). The two constructs were cultured in vivo for 8 weeks to repair articular cartilage defects and evaluate the superiority of 3D scaffolds. The experimental results showed that the 2D scaffold was entirely composed of dense nano fiber layers with superficial smaller pores. On the contrary, the 3D scaffold showed a looser multilayer structure with larger pores between the layers (figure 2). The 3D scaffold with higher pore size and porosity was more beneficial to cells growth, cell adhesion and proliferation, nutrient infiltration and ECM secretion. The result proved that the 3D scaffold showed a higher porosity and larger pore size than 2D scaffold (figures 3(B)–(D)). In addition, the hydrophilicity, cell adhesion rate and proliferation rate of the 3D group were higher than those of the 2D group (figure 5), because the 3D scaffold generated a looser multilayer structure with larger pores between the layers. The above observations suggested that, compared with the 2D scaffold, the 3D scaffold exhibited a more suitable pore structure, higher biocompatibility, and higher mechanical strength, which have more beneficial properties for cartilage regeneration.

After being cultured for 8 weeks, 3D scaffold-based tissue engineering cartilage formed typical cartilage lacunae and secreted a large amount of cartilage matrix. Notably, the neocartilage properties, including Young’s modulus, GAG content, DNA content and total collagen, were obviously comparable to those of native articular cartilage. The underlying cause is that the interconnected 3D porous structure provides an perfect microenvironment for chondrocytes to proliferate and secrete the cartilage ECM.

The feasibility of repairing articular cartilage defects on rabbits is an important experimental basis for clinical application of three-dimensional scaffold. During the experiment, 3D scaffold was successfully used to repair

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**Figure 8.** In situ cartilage repair in a rabbit articular defect model at 12 weeks postoperation. Gross images (A1, B1), and images of HE staining (A2, B2), toluidine blue staining (A3)–(B3), Sbf-O/FG staining (A4, B4), and type II collagen staining (A5, B5) of the repaired cartilage defects in 2D scaffold and 3D scaffold at 12 weeks postoperation. Green arrows indicate the border of native cartilage and defect areas.
articular cartilage defects of rabbits. After 12 weeks of implantation, homogeneous, stable mature cartilage tissue was formed and integrated well in situ cartilage (figures 8(B1–5)). The looser multilayer structure, larger pores, and the rate of higher cell adhesion and proliferation could be the main reason why the 3D scaffold showed a more ideal effect in cartilage repair than the 2D scaffold. Moreover, because of the interconnected internal and sponge-like structures, growth factors in the blood and mesenchymal stem cells in the bone marrow can penetrate into the scaffold and facilitate cartilage repair.

5. Conclusion

In the current study, we prepared a 3D scaffold with sponge-like structures that exhibited satisfactory physicochemical properties, including suitable pore structure, high biocompatibility, and high mechanical strength. In addition, based on the electropun nanofiber scaffold, we achieved satisfactory cartilage regeneration following subcutaneous implantation in rabbits and observed the process of cartilage repairing in situ. The experimental conclusions provide support for the use of gas-foaming three-dimensional electropun nanofiber scaffolds in future clinical applications.

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Data availability statement

The data generated and/or analysed during the current study are not publicly available for legal/ethical reasons but are available from the corresponding author on reasonable request.
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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