Microstructure design and degradation performance in vitro of three-dimensional printed bioscaffold for bone tissue engineering

Hongyu Jin¹, Yue Zhuo¹, Yang Sun², Hongya Fu¹ and Zhenyu Han¹

Abstract
In bone tissue engineering, three-dimensional printed biological scaffolds play an important role in the development of bone regeneration. The ideal scaffolds should have the ability to match the bone degradation rate and osteogenic ability. This article optimizes the unit cell model of the microstructure including spherical pore, gyroid, and topology to explore degradation performance of scaffolds. Boolean operation of array microstructure unit cells and selected part of a computer-aided design (CAD) femur model are adopted to create a reconstructed scaffold model. Polylactic acid/b–tricalcium phosphate/hydroxyapatite scaffolds with spherical pore, gyroid, and topology-optimized structures are manufactured by three-dimensional printing utilizing the composition of bio-ink including polylactic acid, β-tricalcium phosphate, and hydroxyapatite. After degradation of the scaffolds in vitro for several days, the mechanical properties are analyzed to study the effects of different microstructures on the degradation properties. The results show that the gyroid scaffolds with favorable degradability still maintain excellent mechanical properties after degradation. Mechanical properties of the scaffolds with topology-optimized structure and spherical pore microstructure scaffolds have a significant decrease after degradation.

Keywords
Bone tissue engineering, biological scaffolds, three-dimensional printing, degradation rate, microstructure design

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Introduction
Reconstruction of sophisticated bone defects continues to have significant challenges in patients with insufficient bone dimensions. Although autogenous bone grafts harvested from a healthy region of the patient are still ordinarily considered the gold standard for enhancing bone repair, their use is restricted in clinical practice due to graft resorption rates, high donor site morbidity, and circumscribed bone availability.¹ There is an urgent need for a method of improving new bone formation and reducing the amount of autologous bone grafts. Recently, additive manufacturing (AM), which includes three-dimensional (3D) printing, stereolithography (SLA), fused deposition modeling (FDM), and selective laser sintering (SLS), has received developing attention in medical devices and tissue

¹School of Mechatronics Engineering, Harbin Institute of Technology, Harbin, China
²College of Basic Medicine, Heilongjiang University of Chinese Medicine, Harbin, China

Corresponding authors:
Yang Sun, College of Basic Medicine, Heilongjiang University of Chinese Medicine, No. 24, He-ping Road, Harbin 150040, China.
Email: yangsun66@sina.com
Zhenyu Han, School of Mechatronics Engineering, Harbin Institute of Technology, No. 92, West Da-zhi Street, Harbin 150001, China.
Email: hanzy@hit.edu.cn
In addition, bone tissue engineering (BTE) including 3D printed bioscaffold technology has become a promising approach to bone repair and reconstruction. Parry et al. created a biocompatible polymer scaffold with 3D printing technology, capable of sustaining vascularization and tissue ingrowth. Cox et al. presented a systematic characterization of bone tissue scaffolds fabricated via 3D printing from hydroxyapatite (HA) and poly(vinyl)alcohol (PVOH) composite powders.

Biocompatibility, mechanical properties, and biodegradation are crucial to tissue engineering scaffolds. Abert et al. used three different components in the composite material to avoid accelerated degradation and pronounced volumetric swelling. Many studies have been focused on the biomaterials, bioactivity, porosity, mechanical properties, cell proliferation, and protein differentiation of the 3D printed scaffolds. Domingos et al. investigated the influence of nano-hydroxyapatite and micro-hydroxyapatite (nHA and mHA, respectively) particles on the in vitro biomechanical performance of polycaprolactone/hydroxyapatite (HA) scaffolds. An ideal scaffold utilized for bone regeneration, which should be biocompatible and biodegradable, and have adequate physical and mechanical properties, should mimic characteristic features of natural extracellular matrix (ECM) and synchronize the speed of scaffold degradation and new bone growth. Drost et al. explored a new treatment strategy for urinary incontinence. Human bone marrow mesenchymal stem cells (MSCs) of the first in vitro passage were exposed to 5-azacytidine (AZA) to induce myogenic differentiation. Expression of stem cell surface antigens and intracellular α-actin was examined by flow cytometry at the end of each passage and compared to that of native MSC cultured in parallel. Yan et al. attempted to fabricate alginate/bacterial cellulose nanocrystals (BCNs)–chitosan–gelatin composite scaffold by the combined method involving the incorporation of BCNs in alginate matrix. The porous polylactic acid (PLA)-based combination scaffolds had been reasonably designed to achieve proper mechanical properties and beneficial biological activity. Egan et al. focused on conducting experiments to mechanically characterize lattice structures to measure properties that inform an integrated design, manufacturing, and experiment framework. Yang et al. examined endothelial cell adhesion, morphology, and viability on bare and titanium nitride (TiN)-coated nickel titanium (NiTi) alloys and chitosan film first and then identified the type and amount of serum proteins adsorbed on the three surfaces by proteomic technology. Lee et al. provided an in-depth discussion of the current methodologies used to regulate the surface chemistry of calcium phosphates (CaP) and their subsequent effects in regard to protein adsorption and delivery, as well as cell/material interactions. Although biological scaffolds of different materials have been prepared based on above references, it is also significant to study how the degradation of scaffolds with different microstructures influences biomechanical property for tissue engineering scaffolds.

According to the deficiencies in the above references, this research investigated the effect of different microstructures on the degradation performance and mechanical properties of 3D printed PLA/β-TCP (tricalcium phosphate)/HA scaffolds with reconstituted unit cells of spherical pore, gyroid, and topology structure. In order to study the mechanical properties and degradation characteristics of the scaffold model, phosphate-buffered saline (PBS) was selected as the degradation solution for in vitro degradation experiments. The concentration of various ionic compositions in this solution is close to that of human body fluid, the experimental conditions were closer to the degradation in vivo. However, due to the long cycle of in vitro degradation experiments, it usually takes several months for many polymer and calcium phosphate materials to change. To observe the results in a short time, an in vitro accelerated degradation experiment was carried out at a temperature of 70°C. Furthermore, scanning electron microscopy (SEM), mass analysis, and mechanical testing were implemented to characterize the scaffolds.

Materials and methods

Microstructure design of scaffolds

This research established three microstructures of 3D printed scaffolds, which consist of spherical pore, gyroid, and topology structure, to explore the relevance between the micro-architecture of the scaffolds and degradation property. The scaffolds with size of 10 × 10 × 10 mm were printed for mechanical testing. Through the uniaxial tensile test of blended materials (PLA:β-TCP:HA with ratio of 2:1:1), Young’s modulus was determined to be 213.3 MPa and Poisson’s ratio was 0.3. The porosities of all scaffolds were set at 70%. The size of all reconstituted unit cells was fixed as 2.4 mm.

1. Spherical pore microstructure

Reconstituted unit cell of spherical pore was obtained by boolean calculation through cube and sphere. The objective of optimization was to achieve the best initial mechanical performance of the scaffold at constant porosity.
Design variables, objective functions, and constraints were established according to the following equations

\[
\begin{align*}
\text{Find } & \quad x_1, x_2, \\
\text{Min } & \quad f(x_1, x_2), \\
g(x_1, x_2) & \geq \lambda
\end{align*}
\]

where \(x_1\) is the spherical pore radius and \(x_2\) is the circular pore diameter. \(f(x_1, x_2)\) calculates the maximum deformation and the maximum equivalent stress; \(g(x_1, x_2) \geq \lambda\) indicates the variation of model volume and design variables at different porosities.

The size of reconstituted unit cell with spherical pore microstructure was optimized utilizing ANSYS Workbench (ANSYS, Inc. Canonsburg, PA, USA). Response surface charts obtained a more intuitive response of the two design variables to the output variables, as shown in Figure 1. By optimizing the minimum values of the maximum deformation and the maximum equivalent stress, the spherical pore radius was 1.3429 mm and the circular pore diameter was 1.1097 mm at the porosity of 70%.

2. Gyroid microstructure

Implicit surface modeling can describe the characteristics of scaffolds by using single mathematical equations. Triply periodic minimal surfaces (TPMSs) can promote cell migration and maintain high structure stiffness due to its open structure simultaneously. Here, the scaffold models were established by gyroid, which were usually used in scaffold design as a kind of TPMSs.

The gyroid surfaces were built using the following equation

\[
\cos(x)\sin(y) + \cos(y)\sin(z) + \cos(z)\sin(x) + 0.55 = 0
\]

The study implemented the visualization of the gyroid model and the OBJ file through K3DSurf software (Abderrahman Taha, open source), as shown in Figure 2. The OBJ file was imported into Rhinoceros software (CAD International, Avalon, NSW, Australia) to divide the surface into physical and spatial domains for creating the gyroid unit cell microstructure.

3. Topology-optimized microstructure

Topology optimization as a mathematical method can optimize the material distribution to obtain desired structure according to prescribed constraints. A unit cell with three circular pores was selected as the original model, and the size of the unit scaffold was also fixed.

This research defined the objective function as strain energy, the constrain function as the volume, and controlled the porosity by volume. Topology optimization based on the principle of minimum strain energy was performed using ABAQUS software (Abaqus Inc. Palo Alto, CA, USA), as shown in Figure 3. As a result, reconstructed unit cell based on topological optimization was obtained.

Preparation of scaffolds

Digital representation (CAD model) of porous scaffold was established by 3D reconstruction of an average adult human femur, which converts computed tomography (CT) images to stereostucture by Mimics (Materialise Inc., Leuven, Belgium) and Geomagic Studio (Geomagic Inc., North Carolina, USA). Boolean operation of array reconstructed unit cells and selected part of the CAD femur model was adopted to create reconstructed scaffold model. As shown in Figure 4, a reconstruction model with spherical pore structure was introduced.
Regarding material composition, HA, β-TCP, and PLA are generally utilized materials for orthopedic applications. HA is the considerable inorganic component of natural bone, and it had been used widely in BTE based on its good bioactivity. Moreover, PLA is an artificial polymer approved by the US Food and Drug Administration (FDA) to be extensively applied in the biomedical field.

The bio-inks for 3D printing of the scaffolds are composed of PLA, β-TCP, and HA. PLA is purchased from Dongguan ChengXin Materials Company (Dong Guan, China). HA was purchased from DeKe Technology Company (Beijing, China). β-TCP was

![Figure 2. Gyroid surface structures: (a) Gyroid surface of single cycle. (b) Gyroid surface of multiple cycles.](image)

![Figure 3. Process of topology optimization. (a) Initial model. (b) 6th topology optimization model. (c) 12th topology optimization model. (d) 18th topology optimization model. (e) 31st topology optimization model. (f) Reconstructed unit cell based on topological optimization.](image)
purchased from MaiKun Chemical Company (Shanghai, China). Diethylene dioxide (1,4-dioxane), as a good solvent, was purchased from Changchun SanBang Medicines Company (Changchun, China).

The composite materials can be obtained with PLA:β-TCP:HA ratio of 2:1:1. PLA was dissolved in 1,4-dioxane at a concentration of 12% (w/v) while heating at 80°C for 1.5 h assisted with a stirrer. Then, the HA and β-TCP powders were added into the solution and stirred for 1.5 h at 80°C. The composite slurry can get appropriate viscosity by rotary evaporation for 4 h through constant testing.

Three-dimensional bio-printer (Regenovo Biotechnology Corp.) was then used to fabricate all PLA/β-TCP/HA scaffolds based on a layer-by-layer manufacturing principle. The printing speed was set at 0.20 mm/min by applying air pressure of 0.25 MPa under −5°C. The printing needle had an inner diameter of 610 μm. An overview of the designed and built architectures with spherical pore, gyroid, and topology was given in Figure 5.

**Degradation model of scaffolds**

To establish the degradation model, a mathematical expression that can indicate the degradation rate and other factors is needed. Due to many factors affecting the degradation of materials, there is not a recognized degradation model. The present degradation models can be roughly divided into three categories: phenomenon model, probability model, and empirical model. Phenomenal models are models based on diffusion, dissolution, and reactions that can be applied to a wide range of polymer types. The probability model calculates the degradation probability according to the degradation time by assuming that the degradation of bone biological scaffolds is a random process. The empirical model is mainly based on a large number of experimental data to establish the regression equation, easy to determine the importance of certain factors.

PLA was used as the main material in this article. As the hydrolysis of PLA was dominated by bulk degradation, the degradation model was selected. First, the change of water content is simulated by Fick’s second law

$$\frac{dc}{dt} = \alpha \Delta^2 c$$

where \(\alpha\) is the diffusion coefficient, \(c\) is the water content, and \(\Delta\) the is gradient operator.

The change of molecular weight is caused by hydrolysis and is related to water content

$$W(t) = -\beta c$$
where $W(t)$ is the molecular weight and $\beta$ is the disappearance rate constant.

The change of elastic modulus is considered to have a linear relationship with molecular weight

$$E_s(W(t)) = E_{s0} \frac{W(t)}{W_0}$$

(5)

where $E_{s0}$ is the initial elastic modulus and $W_0$ the is initial average molecular weight.

Since the water molecules are diffused from the outer scaffold, the water content at the boundary is 1. According to its boundary conditions, the solution of Fick’s second law in the finite diffusion volume is

$$c = 1 - 4 \sum_{v=0}^{\infty} \sin \frac{(2v + 1)\pi h}{2v + 1} \cdot \exp \left[ -\left( \frac{(2v + 1)\pi h}{2} \right)^2 \alpha t \right]$$

(6)

where $h$ is the thickness of the scaffold and $t$ is the degradation time.

The average concentration of the commonly used volume in engineering is

$$\bar{c} - c_f = \frac{8}{\pi^2} \exp \left( -\frac{\alpha \pi^2 t}{h^2} \right)$$

(7)

where $\bar{c}$ is the average concentration, $c_0$ is the initial concentration, and $c_f$ is the final concentration.

The above equations can observe the real time change of elastic modulus of bone biological scaffolds in the degradation process so that further simulation analysis can be carried out.

**Mechanical testing of scaffolds**

The surface appearance of the scaffolds was observed by SEM (S-570; Hitachi, Japan) after the scaffolds were coated with gold by a sputter coater. The morphology and mechanical properties of the scaffolds were analyzed in the process of degradation. Compressive mechanical properties were analyzed by a mechanical tester (Instron 5569; Instron Corp., USA). Compressive force was applied in the Z direction of 3D printed scaffold with a 2.5-kN load cell and a cross-head speed of 1.5 mm/min.

**Scaffold degradation testing in vitro**

Ideal tissue engineering scaffold could gradually degrade when it was implanted into the human body. The scaffold rate of degradation in vivo should match with the growth rate of repair tissue. In vitro accelerated degradation experiments were performed to explore how scaffolds changed during degradation in a short time. PBS was chosen as the degradation liquid. The scaffolds are placed in a calorstat of 70°C after the scaffolds were put into the PBS. Due to the long cycle of in vitro degradation experiments, it usually takes several months for many polymer and calcium phosphate materials to change. To observe the mechanical properties of the scaffold in a short time, an in vitro accelerated degradation experiment was carried out at a temperature of 70°C based on the standard of accelerated degradation experiments. (70°C is the recommended temperature for accelerated degradation of PLA.) The timing method of accelerated degradation experiment is the same as that of non-accelerated degradation experiment, which is measured in days. In vitro accelerated degradation experiment is helpful to quickly observe how the mechanical properties of scaffolds change with degradation. But more accurate results still need to be obtained through in vivo degradation experiments to be studied in the future.

## Results and discussion

### Mechanical testing of scaffolds

The mechanical properties of the 3D printed scaffolds were shown in Figure 6. The results demonstrated that the mechanical properties (Young’s modulus and compressive stress) of the 3D printed scaffold with topology-optimized structure were better than the structures of gyroid and spherical pore before degradation. However, Young’s modulus reduction of the scaffolds with topology structure presented significantly higher than the scaffolds with spherical pore structure after degradation ($p \leq 0.001$). The Young’s modulus reduction of the scaffolds with gyroid structure had no significant influence after degradation for 3 and 6 days ($p \leq 0.05$). As the degradation time increases, the mechanical properties of the scaffolds with gyroid structure decline more slowly. Furthermore, according to the degradation model (equations (3)–(7)), the molecular weight $W(t)$ of the scaffold can be calculated.
from $1.6 \times 10^5$ to $0.4 \times 10^5$ in 6 days. The variation trend is consistent with the test results of elastic modulus.

**Microstructure analysis of scaffolds**

The cell adhesion on the surface of scaffolds is affected by protein adsorption. After the scaffolds are implanted in the body, the protein is quickly adsorbed on the surface. The adsorbed proteins act as ligands to bind receptors on cell membranes, leading to cell adhesion. The microstructure of the scaffold surface plays an important role in the regeneration of BTE.

SEM image analysis of 3D printed PLA/β-TCP/HA scaffolds with spherical pore, gyroid, and topology structures was performed before and after degradation, as shown in Figure 7. As the degradation time increases, the number of micropores on the surface of the scaffolds was enhancing. The number of micropores of the scaffolds was in the following order: gyroid, spherical pore, and topology structures (after degradation for 6 days). The micropore number of the scaffolds with gyroid structure was significantly higher than that of the scaffolds with spherical pore and topology structure. The scaffold with gyroid structure is more favorable for cell adsorption.

**Mass analysis of scaffolds**

In order to explore the mass changes of three bone scaffold structures in PBS simulated body fluids, the mass changes before and after scaffold degradation were analyzed, as shown in Figure 8. At day 3 and day 6, three samples for each type of scaffolds were taken out and put on a filter paper for seconds to remove the PBS from the scaffolds and then weighed. The results showed that the mass reduction of the 3D printed scaffold with topology structure was the best, followed by gyroid before degradation.

Based on the above analysis, the mechanical properties and degradation properties of the scaffolds with gyroid microstructure are better, and they are more suitable to be biological scaffolds for human bone tissue repair.

**Discussion of scaffold degradation**

Scaffold degradation is the key factor of bone tissue regeneration in BTE. As a kind of temporary scaffolds, bone biological scaffolds will degrade continuously over time. The degradation rate of ideal biological scaffolds should match the tissue regeneration rate and provide early mechanical support. As for the degradation of biological scaffolds in vivo, due to the fluid flow of human tissue and bone mineralization, the degradation rate of scaffolds is predicted to be faster than that of in vitro, and the regeneration rate of bone tissue is also faster. Further studies are needed to determine the mechanical properties of biological scaffolds in the

**Figure 6.** Mechanical testing of 3D printed PLA/β-TCP/HA scaffolds with spherical pore, gyroid, and topology structure. (a) Young’s modulus of 3D printed scaffolds with spherical pore, gyroid, and topology structure. (b) Stress–strain curves before degradation. (c) Stress–strain curves after degradation for 6 days. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $n=3$.**
process of degradation in vivo, as well as the actual efficiency of scaffold degradation and tissue regeneration and biocompatibility.

**Conclusion**

The scaffolds must not only have mechanical strength but also achieve time-controlled bio-absorption. The PLA/β-TCP/HA scaffolds with spherical pore, gyroid, and topology structures were fabricated using 3D printing. Degradation experiments in vitro statistically showed that the mass reduction of the scaffold with topology structure was the best, followed by gyroid and spherical pore after degradation. However, the scaffolds with gyroid microstructure exhibited more enhanced Young’s modulus and many microporous than the others after degradation. Scaffolds with gyroid or topology microstructure might be a promising candidate for BTE application. This work indicated the importance of scaffold micro-structural design for BTE. The next step is to explore the ideal degradation time of the ideal scaffolds and performance in vivo testing.

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**Figure 7.** SEM image analysis of 3D printed PLA/β-TCP/HA scaffolds with spherical pore (a, d, g, and j), gyroid (b, e, h, and k) and topology (c, f, i, and l) structures. (a) SEM image of spherical pore at 100-μm scale before degradation. (b) SEM image of gyroid at 100-μm scale before degradation. (c) SEM image of topology at 100-μm scale before degradation. (d) SEM image of spherical pore at 10-μm scale before degradation. (e) SEM image of gyroid at 10-μm scale before degradation. (f) SEM image of topology at 10-μm scale before degradation. (g) SEM image of spherical pore at 100-μm scale after degradation for 6 days. (h) SEM image of gyroid at 100-μm scale after degradation for 6 days. (i) SEM image of topology at 100-μm scale after degradation for 6 days. (j) SEM image of spherical pore at 10-μm scale after degradation for 6 days. (k) SEM image of gyroid at 10-μm scale after degradation for 6 days. (l) SEM image of topology at 10-μm scale after degradation for 6 days.
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ORCID iD
Hongyu Jin  https://orcid.org/0000-0001-6950-773X

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