10.30699/jovm.002.e0103

Journal of Visualized Medicine

Design and Fabrication of Structured Tissue Engineering Scaffolds with Different Porosity Architecture

Hekmat Farajpour[1], Babak Sabet[2], Arash Khojasteh[*3]

1. *Department of Artificial Intelligence, Smart University of Medical Sciences, Tehran, Iran*
2. *Department of Surgery, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
3. *Department of Oral and Maxillofacial Surgery, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

ABSTRACT

Scaffold design is a critical aspect of tissue engineering, as it directly influences the success of regenerative therapies. This article focuses on the scaffold geometry and porosity architecture design, and its impact on tissue regeneration, highlighting the significant advancements achieved through the integration of Computer-Aided Design/Computer-Aided Manufacturing (CAD/CAM), and 3D printing. CAD/CAM software facilitate precise scaffold design, allowing control over geometry and pore size distribution. By closely mimicking the natural extracellular matrix (ECM), these scaffolds create an ideal microenvironment for cellular adhesion, proliferation, and tissue formation. The integration of 3D printing technology enables the fabrication of complex structures layer-by-layer, ensuring high accuracy and reproducibility. The resulting scaffolds exhibit well-defined microarchitecture and porosity. In conclusion, scaffold design plays a pivotal role in tissue engineering success. CAD/CAM, and 3D printing techniques hold great promise in advancing regenerative medicine, offering personalized therapeutic solutions and transformative advancements in tissue engineering and regenerative medicine applications.

Keywords: 3D printing, Tissue Engineering, CAD/CAM, Porosity, Scaffold

Received: 11 November 2023; **Accepted**: 07 January 2024; **Published Online:** 22 February 2024;

Corresponding Information: **Arash Khojasteh**, *Department of Oral and Maxillofacial Surgery, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran* & Email: arashkhojasteh@sbmu.ac.ir

1. Introduction

Tissue engineering has emerged as a promising field in modern medicine, using engineered constructs called scaffolds to regenerate damaged tissues or organs. These scaffolds provide a 3D framework resembling the natural extracellular matrix, enabling cell growth and tissue formation **(1)**. Customized scaffold designs with diverse porosity architecture are essential to cater to specific tissue regeneration needs. Each tissue type has unique requirements, necessitating tailoring of scaffold porosity for successful outcomes. Porosity greatly influences biocompatibility and functionality, affecting cell infiltration, nutrient diffusion, waste removal, and mechanical properties of the scaffolds **(2, 3)**.

CAD/CAM (Computer-Aided Design/Computer-Aided Manufacturing) and 3D printing have revolutionized tissue engineering, particularly in scaffold design. CAD software allows researchers to create intricate 3D models with precise control over geometry, enabling customized scaffolds for different tissues. The iterative design process ensures constant refinement based on research findings **(4)**.

3D printing is a game-changing technology that brings CAD-designed scaffold geometries to life. It allows for the layer-by-layer fabrication of scaffolds with high precision and accuracy **(5)**. One of the key advantages of 3D printing is the ability to customize scaffold porosity according to the regenerative requirements of different tissues. By adjusting printing parameters such as infill density and layer thickness, researchers can control the size and distribution of pores within the scaffold **(6)**. This level of control is critical in promoting specific cellular responses, such as cell adhesion **(7)**, migration **(8)**, and proliferation **(8, 9)**. Additionally, 3D printing permits the creation of scaffolds with gradient porosity, enabling a smooth transition from one region to another, which is beneficial for tissue interfaces or complex tissue regeneration **(10, 11)**.

The aim of this article is to explain the process of designing and manufacturing scaffolds with distinct pore architectures using CAD/CAM techniques, coupled with 3D printing technology. By investigating the interplay between design parameters, fabrication methods, and resultant porosity, this study aims to provide valuable insights into optimizing scaffold construction for enhanced tissue engineering outcomes.

2. Protocol

For designing and fabricating structured tissue engineering scaffolds with varied porosity architecture we use CAD/CAM software, and 3D Printing.

2-1- Scaffold Design using CAD software

The first step in the fabrication process is the design of the tissue engineering scaffold using CAD software. CAD allows for precise control over the scaffold's geometry, pore size, and interconnectivity. During this stage, the specific requirements of the target tissue or organ are taken into consideration to customize the scaffold accordingly. Solidworks, Materialise Mimics, Materialise 3-matic, and etc. are the common software for designing custom scaffolds.

2-2- CAM software (Prusa Slicer)

Using CAM Software (Prusa Slicer) for 3D Printing Tissue Engineering Scaffold:

1) Launch Prusa Slicer and Import STL Geometry: Click on "Add" or drag-and-drop the STL file of your scaffold design (designed in CAD softwares like Solidworks, MIMICS, 3-matic, …) into Prusa Slicer's interface. The software will load and display the 3D model.
2) Printer and Material Selection: Choose your 3D printer model from the list of supported printers. Select the appropriate material (e.g., biocompatible filament) from the material profiles available, and choose 0.5 mm for nozzle diameter.
3) Temperature Settings: Navigate to the "Filament" settings. Set the nozzle and bed temperatures according to the recommendations for your chosen material. Proper temperatures ensure optimal adhesion and print quality. For PLA material nozzle and bed temperature set to 190 ℃ and 30 ℃ respectively.
4) Layer Thickness and Print Speed: In the "Print Settings" section, adjust the layer thickness (also known as layer height) based on your desired scaffold porosity and print resolution (For our study layer thickness was 0.2 mm). Modify print speeds to balance speed and print quality.
5) Pore Design and Density: Prusa Slicer provides you with the flexibility to finely tailor the pore design and density of your tissue engineering scaffold through precise adjustment of the infill settings. This essential feature empowers you to create scaffolds with optimized structural characteristics. Within Prusa Slicer, choose from a variety of infill patterns to define the spatial arrangement of pores within your scaffold. Options such as grid, honeycomb, gyroid, cubic, and more are available. Select an infill pattern that aligns with your scaffold's intended function and the specific cellular responses you seek to encourage. After choosing infill pattern, finelytune the infill density. Higher infill density translates to a more solid scaffold with fewer voids, suitable for load-bearing applications. Lower infill density promotes higher porosity, facilitating cellular infiltration and nutrient diffusion for tissue regeneration. Depending on your tissue engineering goals, you can adapt the infill settings to create scaffolds with porosity optimized for different tissues. For example, bone scaffolds may benefit

from a denser infill to mimic natural bone density, while softer tissues like cartilage could thrive in a scaffold with higher porosity.

6) Visualize Before Printing: Use Prusa Slicer's 3D preview to visualize how the scaffold will be printed layer by layer. This helps you identify potential issues and make adjustments before initiating the printing process (Figure 1).
7) Support Structures (Optional): If your scaffold design requires support structures, enable them in the settings. Prusa Slicer will automatically generate supports to ensure overhangs and complex geometries are properly printed.
8) Generate G-code: Once you're satisfied with the settings, click on "Slice Now." Prusa Slicer will process your model and generate G-code instructions that your 3D printer will follow.
9) Export G-code: After slicing is complete, click on "Export G-code" and choose a location to save the G-code file. This file contains the detailed instructions for the printer, including movement paths, temperatures, and extrusion rates.

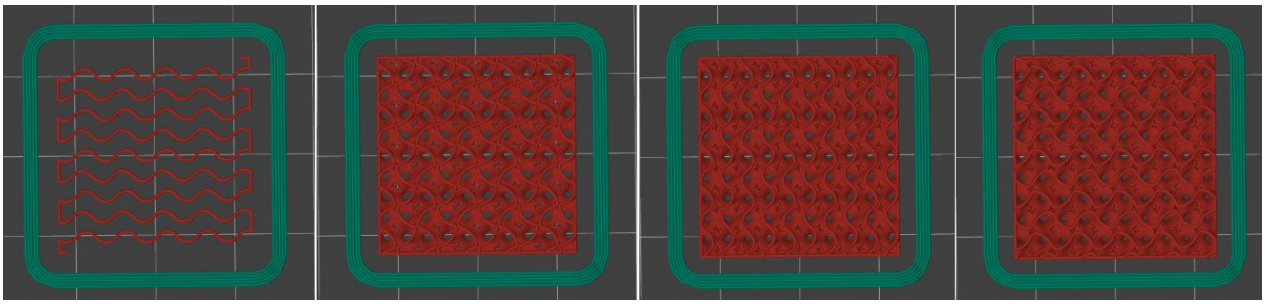

Figure 1. Different layers of scaffold formation in the Prusa Slicer (visualize printing path before real printing) (Design by Authors - 2024)

2-3- 3D Printing Process

1) Material Selection and Preparation: Choose a suitable filament material for your tissue engineering scaffold. We used PLA filament (Gando). Ensure the filament is stored in a dry and controlled environment to prevent moisture absorption.
2) Load Filament: Insert the chosen filament into the 3D printer's filament guide or extruder assembly. If material are in granule type, load the granules into the printer's material feeding system such as metal syringe. The granules will be fed into the extruder for layer-by-layer deposition.
3) Printer Setup: Power on the FDM (Fused Deposition Modeling) 3D printer machine (Sizan ECO, Sizan Co., Kashan, Iran) and ensure it is properly calibrated. Make sure the print bed is clean and level, and the nozzle is at the appropriate starting position.
4) Transfer G-code to 3D Printer: Transfer the exported G-code file form Prusa Slicer to your 3D printer using a USB drive, SD card, or other appropriate method.
5) Start Printing: Initiate the 3D printing process through the machine software interface. The printer will start heating the nozzle and bed to the specified temperatures. After heating completed, the printer will follow the instructions in the G-code to create your scaffold layer by layer.
6) Printing Progress: Monitor the printing progress closely, especially during the initial layers. Ensure that the filament adheres properly to the print bed and that the layers are being deposited accurately.
7) Post-Printing Care: Once printing is complete, allow the print to cool before removing it from the print bed. Carefully detach any support structures, if present, using appropriate tools.
8) Finishing Touches: Inspect the printed scaffold for any imperfections, such as stringing or layer inconsistencies. Trim or sand any rough edges if necessary.
9) Quality Control: Perform quality checks on the printed scaffold, ensuring that dimensions and structural integrity meet your specifications.
10) Storage and Handling: Store the printed scaffold in a controlled environment to prevent exposure to moisture or UV light, which can degrade the material over time.

3. Results and Discussion

Successful 3D printing of diverse scaffold designs has yielded a range of fabricated structures characterized by varying pore shapes and sizes (Figure 2). Notably, the print times for each scaffold exhibit a significant disparity, spanning from mere minutes to several hours. The scaffolds were tailored to cater to the regeneration needs of various tissues, including bone, cartilage, and skin.

Figure 2. Left: Scaffolds on 3d printing bed at the end of the printing process, Right: Different scaffolds with diverse shape and pores. (Design by Authors - 2024)

Our study presents a notable advancement in the field of tissue engineering through the precise customization of scaffold architectures. By meticulously manipulating pore shape and size, we offer a pathway to fabricating tissue-engineered constructs that closely emulate the intricate microenvironments of natural tissues. Fine-tuning of scaffold mechanical properties enhances the potential for generating constructs with tailored strength and structural integrity, thereby catering to diverse tissue applications such as bone and cartilage regeneration. Opting for granule-type materials or hydrogels in 3D printing, as opposed to conventional filaments, could potentially limit the array of biomaterials accessible for the fabrication of the intended scaffold designs. This limitation arises from the possibility that these alternatives might not fully replicate the intricately designed pore structures and shapes as designed.

However, the successful design and fabrication of tissue engineering scaffolds with diverse porosity architecture using CAD/CAM, and 3D printing demonstrate the immense potential of this approach in the field of regenerative medicine **(10-14)**. As these fabrication techniques continue to evolve, we can expect significant advancements in tissue engineering and regenerative medicine, ultimately benefiting countless patients in need of effective and personalized therapeutic solutions.

Acknowledgment

The authors are grateful to the Department of Infrastructure, Smart University of Medical Sciences for technical support.

Ethical Considerations

Not applicable.

Conflict of Interest

The authors declared no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public.

References

1. Yi S, Ding F, Gong L, Gu X. Extracellular matrix scaffolds for tissue engineering and regenerative medicine. Curr Stem Cell Res Ther. 2017;12(3): 233-46. [PMID] [DOI:10.2174/1574888X11666160905092513]
2. Forrestal, D.P., T.J. Klein, and M.A. Woodruff, Challenges in engineering large customized bone constructs. Biotechnol Bioeng. 2017;114(6): 1129-39. [DOI:10.1002/bit.26222] [PMID]
3. Wang C, Huang W, Zhou Y, He L, He Z, Chen Z, et al. 3D printing of bone tissue engineering scaffolds. Bioact. Mater. 2020;5(1):82-91. [PMID] [DOI:10.1016/j.bioactmat.2020.01.004] [PMCID]
4. Naujokat H, Rohwedder J, Gülses A, Cenk Aktas O, Wiltfang J, Açil Y. CAD/CAM scaffolds for bone tissue engineering: Investigation of biocompatibility of selective laser melted lightweight titanium. IET Nanobiotechnol. 2020; 14(7):584-9. [PMCID] [PMID] [DOI:10.1049/iet-nbt.2019.0320]
5. Su X, Wang T, Guo S. Applications of 3D printed bone tissue engineering scaffolds in the stem cell field. Regen. Ther. 2021;16:63-72. [PMCID] [DOI:10.1016/j.reth.2021.01.007][PMID]
6. Kelly CN, Miller AT, Hollister SJ, Guldberg RE, Gall K. Design and structure-function characterization of 3D printed synthetic porous biomaterials for tissue engineering. Adv Healthc Mater. 2018; 7(7):1701095. [DOI:10.1002/adhm.201701095] [PMID]
7. Cunha-Reis C, TuzlaKoglu K, Baas E, Yang Y, Haj AE, Reis RL. Influence of porosity and fibre diameter on the degradation of chitosan fibre-mesh scaffolds and cell adhesion. J Mater Sci Mater Med. 2007;18:195-200. [DOI:10.1007/s10856-006-0681-x] [PMID]
8. Mandal BB, Kundu SC. Cell proliferation and migration in silk fibroin 3D scaffolds. Biomaterials. 2009;30(15):2956-65. [PMID] [DOI:10.1016/j.biomaterials.2009.02.006]
9. Samourides A, Browning L, Hearnden V, Chen B. The effect of porous structure on the cell proliferation, tissue ingrowth and angiogenic properties of poly (glycerol sebacate urethane) scaffolds. Mater Sci Eng C. 2020;108:110384. [DOI:10.1016/j.msec.2019.110384] [PMID]
10. Tan Y, Richards DJ, Trusk TC, Visconti RP, Yost MJ, Kindy MS, et al. 3D printing facilitated scaffold-free tissue unit fabrication. Biofabrication. 2014;6(2):024111. [PMCID] [DOI:10.1088/1758-5082/6/2/024111] [PMID]
11. Jariwala SH, Lewis GS, Bushman ZJ, Adair JH, Donahue HJ. 3D printing of personalized artificial bone scaffolds. 3D Print Addit Manuf. 2015;2(2): 56-64. [PMID] [PMCID] [DOI:10.1089/3dp.2015.0001]
12. Wang C, Meng C, Zhang Z, Zhu Q. 3D printing of polycaprolactone/bioactive glass composite scaffolds for in situ bone repair. Ceram Int. 2022; 48(6):7491-9. [DOI:10.1016/j.ceramint.2021.11.293]
13. Murphy CM, Haugh MG, O'brien FJ. The effect of mean pore size on cell attachment, proliferation and migration in collagen-glycosaminoglycan scaffolds for bone tissue engineering. Biomaterials. 2010;31(3):461-6. [DOI:10.1016/j.biomaterials.2009.09.063] [PMID]
14. Salama M, Vaz MF, Colaço R, Santos C, Carmezim M. Biodegradable iron and porous iron: mechanical properties, degradation behaviour, manufacturing routes and biomedical applications. J Funct Biomater. 2022;13(2):72. [DOI:10.3390/jfb13020072] [PMID] [PMCID]