The Effect of Pore Size in 3D-Printed Porous Titanium Implant on Osseo-Integration: (An in Vivo Study)

Abstract

Background: Porous titanium structures have recently gained considerable popularity among researchers in studies examining bone ingrowth and osseointegration. Porous implants fabricated using triply periodic minimal surface design (TPMS) and designed through 3D printing techniques exhibited remarkable mechanical strength and cell viability compared to conventional implants. This study aimed to evaluate the effect of pore size of titanium implants with gyroid structure.

Methods: This study was conducted on Adult male Wistar rats weighing 350 and 450 g for the animal study by the calvarial defect model to investigate bone regeneration. Three disk-shaped implants were designed using a gyroid structure with pore sizes of 400, 500, and 600 micrometers. All implants were made by additive manufacturing (Selective Laser Melting) using Ti6Al4V medical-grade powder. Animals were sacrificed after 12 weeks, the skin was removed from the calvaria, and the implants were removed for histological examination.

Results: Gyroid structures had a high surface-to-volume ratio and pore connectivity, facilitating cell adhesion and ossification. A significant amount of bone ingrowth was observed in the 400 mm group, so that bone penetrated into pores significantly more than in the other groups. However, the vascularization was more pronounced in the 600 µm group than in the other groups.

Conclusion: According to the results, there was a positive effect of porosity in titanium implants in encouraging bone ingrowth. The porosity size of 400 µm was more suitable for the differentiation and proliferation of bone cells and thus the osseointegration in porous titanium implants with gyroid structure.

Keywords: Osseointegration, Bone Regeneration, Titanium, Prostheses and Implants, Bone-Implant Interface

Received: 3 months before printing; Accepted: 1 month before printing

Ghazaleh Moradkhani, MSc[1]; Morad Karimpour, PhD[2]; Mahmoud Taheri, PhD[3]

[2]Assistant professor,

[3]Professor, School of Engineering Science, College of Engineering, University of Tehran, Tehran, Iran.

[1, 2]School of Mechanical Engineering, College of Engineering, University of Tehran, Tehran, Iran.

Corresponding author:
M. Karimpour, PhD
Email Address:
m.karimpour@ut.ac.ir

Introduction

The skeletal structure of the human body is supported by bone, which acts as a frame. Bones protect the body's vital organs, store minerals and fat, assist with movement, and produce blood cells. As a dynamic organ, bone tissue undergoes constant remodelling and self-healing. Trauma, cancer, and tumours may affect bone tissue's ability to perform its usual functions, and the bone may be unable to repair the lost tissue. Therefore, the need for bone implants has increased widely in recent decades (1). The biocompatibility and biomechanical properties of bone implants are important factors for medical applications. Biological compatibility refers to the relationship between the implant material and the biological host tissue, which interacts the activity of living cells, increases their activity to form new tissue, and expresses the non-toxicity of implants (2). Titanium is widely used in load-bearing implants, in addition to its biocompatibility, due to its excellent mechanical characteristics, including high strength, suitable elastic modulus, fracture toughness, and fatigue strength (3). Titanium has a high elastic modulus, which removes stresses from the bone that cause self-resorption of the bone and stress shielding as per Wolff's law (4). Implant loosening and revision surgeries are mainly caused by this phenomenon (5). Porosity design in metal implants has recently been proposed as a solution to this problem.

Solid materials have a higher elastic modulus than porous materials [6, 7]. Interconnecting porous structures promotes osseointegration and biological fixation by allowing large amounts of bodily fluid to flow through the implant pores, which increases the contact area between the implant and the host tissue [8]. The microstructure and architecture of porous implants, including the size and shape of the pores, their interconnectivity, and the void volume fraction, are important factors in mechanical strength, cell differentiation and proliferation, cell migration, and bone regeneration [9]. The structure porosity is a significant parameter in enhancing cellular interaction. The porosity should support cell mobility and ossification and provide mechanical stability [10]. Triply periodic minimal surface structures (TPMS) are proper for bone implant applications due to their unique properties. The mean curvature of these structures is zero at any point, and the concave and convex curvatures are symmetrical [11, 12]. Triply periodic minimal surface structures have a high surface-to-volume ratio [13] and pore connectivity, facilitating the ossification and bone ingrowth [14]. Structures with smooth convergence and no sharp corners minimize stress concentration and shielding [15]. Different ranges of pore sizes have different effects on the bone regeneration process. Cell differentiation occurs when the pore size is smaller than 188 micrometers, whereas bone enlargement occurs when the pore size is greater than 390 micrometers [16]. The results of previous studies have not been able to determine the optimal pore size. There are several reasons for inconclusive results, including the neglect of other structural parameters, such as porosity, pore geometry, and specific surface area, which also play an important role in osteogenesis [17-20]. Orthopaedic implants that use porous structures to increase osseointegration have recently gained much attention. For example, porosity in Acetabular cups was used to help bone penetration and increase fixation [21]. Another study used porous structures in orthopaedic implants, including spinal cages [22], tibial components in total knee replacement surgery [23], and metal cones and augments in revision total knee surgeries [24]. This study aimed to preclinical investigates the effect of porosity size on bone cohesion in 3D printed porous titanium implants.

Methods

Sample preparation

The gyroid sheet-based structure was utilized to design the porous implant. Three porous gyroid disks with pore sizes of 400, 500, and 600 micrometers were designed. The wall thickness in all samples was kept at 0.17 mm, the diameter of the designed implant was 5 mm, and its height was 1.5 mm.
All implants were made by additive manufacturing (Selective Laser Melting) and Ti6Al4V medical-grade powder (Figure 1).

Figure 1: Printed Titanium samples with three pore sizes of 400, 500, and 600 micrometers from left to the right, respectively

Since the porosity of the porous structure affects the osseointegration, the porosity of each sample was calculated after fabrication using Equation 1:

$$P = \left(1 - \frac{W_p}{W_s}\right) \times 100 \quad (1)$$

Where W_p and W_s are the weight of porous and solid sample, respectively, calculated through the Ti6Al4V density. Each printed sample was weighed separately and sterilized by autoclave prior to implantation.

Animal and surgical procedure

In the animal study, seven adult male Wistar rats weighing 350 to 450 grams were used. Anesthesia was induced by intramuscular injection of a combination of Ketamine and Xylazine. The animals were positioned in a stereotaxic frame, and the hair over the skull was shaved and disinfected with Betadine. A circular drill bit was used to create defects in the dorsal part of the skull. The animals were divided into three groups of two with a porous implant with a pore size of 400, 500, and 600 µm. The control group also included one rat, and the defect was left empty. The periosteum was repositioned after implantation and sutured with a 4-0 Vicryl suture, and the skin was sutured with 3-0 silk. The rats were housed in a temperature-controlled room with a 12h/12h light/dark cycle with free access to food and water. All animals survived by the end of the study except for one specimen in the 500 µm group. Animals were sacrificed after 12 weeks, the skin was removed from the calvaria, and the implants were removed for histological examination.

Statistical analysis

The one-way analysis of Variance (ANOVA) with Fisher's multiple comparison test was used to evaluate differences between groups, and $P<0.05$ was considered statistically significant.

Histology at 12 week

The degree of mineralization of osteoblastic cells was assessed using Alizarin Red S staining to identify calcium-containing osteocytes in the differentiated culture of both human and rodent mesenchymal stem cells (MSCs). In other words, the bone tissue penetrated inside the porous structure in contact with alizarin red turns red, and the color spectrum varies depending on the amount of calcium present. Finally, the absorbed color was measured using a spectrophotometer (405 nm).

Results

Macroscopic observation

There were over 60% porosities calculated for all implants. No macroscopic wound infection was observed during the recovery phase, and the wounds were completely healed after four weeks. The implants were firmly attached to the bone. The bone ingrowth was significantly higher in the 400 µm group so that the penetration of bone into the pores was detectable (Figure 2). Nevertheless, the 600 µm group shows the greatest degree of vascularisation.

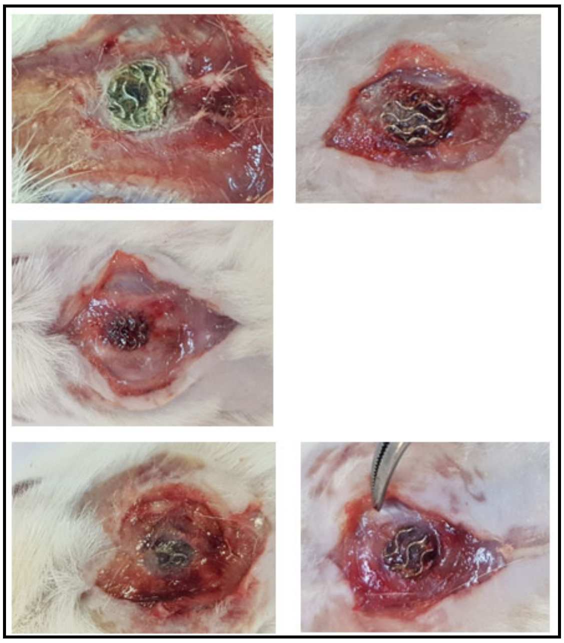

Figure 2: The outcome of bone repair and Osseo integration after 12 weeks in the groups of 400 µm, 500 µm and 600 µm, respectively

Histology

Figure 3 shows the different conditions for inducing ossification in different groups.

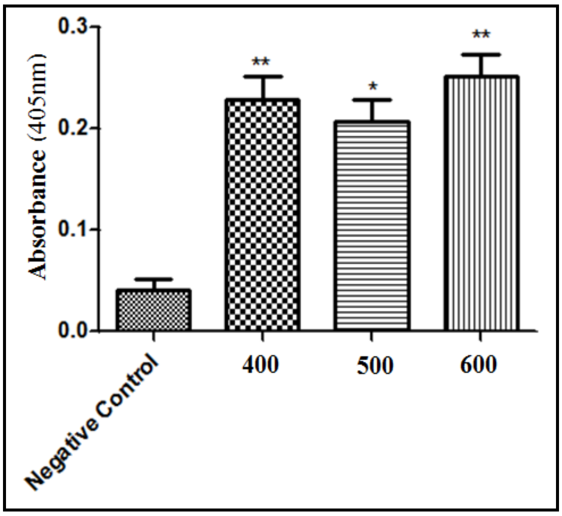

Figure 3: Different osteogenesis induction conditions

According to Table 1, the difference between the 400 and 600 µm groups and the control group is significant.

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
G400 - C	0.1886	0.0272	(0.1021, 0.2750)	6.94	0.006
G600 - C	0.2107	0.0272	(0.1243, 0.2972)	7.76	0.004
G600 - G400	0.0222	0.0272	(-0.0643, 0.1086)	0.82	0.474

Simultaneous confidence level = 90.17%

Table 1: Fisher individual tests for differences of means

Discussion

A minimum porosity of 60% is common in most studies with better bone ingrowth (25). Titanium and titanium alloys are adequate substitutes for lost bone tissue, which are widely used in orthopaedics (26). A minimum pore size of 300 µm is recommended for orthopaedics applications to enhance new bone formation and vascularisation. Porosity size is effective in bone ingrowth, and small pore sizes make it difficult to supply the oxygen needed for bone tissue formation and cause osteochondral formation before bone formation. However, larger pore sizes encourage bone formation before cartilage tissue formation due to angiogenesis (27).

Recent years have seen a great deal of attention given to additive manufacturing due to its ability to control three-dimensional properties such as pores, void volume fractions, and internal structure cohesion (28).

The inconclusive results of previous research were caused by the lack of consideration of other structural parameters such as porosity, pore geometry, and specific surface area, which played a significant role in osteogenesis (17-20). However, the results of this research similarly showed that the pore size of 400 to 600 micrometers is suitable for bone ingrowth (29).

According to observational evaluation results, bone cells could penetrate, differentiate, grow, and proliferate when the gyroid structure has the appropriate porosity and pore size.

Structures should be optimized for their biological characteristics, such as cell growth, and their mechanical properties, such as structural strength.

Experimental data (particularly histological results) indicated that pore size of 400 µm was better suited for bone ingrowth and osseointegration.

There was no significant difference in bone formation between structures with pore sizes of 400 and 600 m according to a statistical analysis.

The porosity size of 400 µm in the Gyroid structure is better suited for use in porous titanium implants due to its higher mechanical strength compared to the pore size of 600 µm (30).

Porous structures, especially the gyroid structure, are widely used in all types of orthopedic implants for osseointegration, implant, and biological fixation due to the mentioned features.

One of the limitations of this research is the impossibility of evaluation using conventional histological methods due to metal implants and impossibility of cutting the thin layer in this type of implant.

This research could be applied to all titanium orthopedic implants requiring osseointegration. In addition to biological fixation, these implants use porous structures to prevent loosening.

Conclusion

The porous titanium implants were fabricated using Selective Laser Melting (SLM) technology. Gyroid structures were evaluated for biocompatibility, osseointegration, and osteogenesis in three different pore sizes: 400, 500, and 600 µm. The histological analysis revealed that the amount of new bone mass and osseointegration in the 400 µm group was higher than in the other groups. In addition, the porous titanium implants with gyroid has a high potential for orthopaedic clinical applications.

References

1. Yuan L, Songlin D, Cuie W. Additive manufacturing technology for porous metal implant applications and triple minimal surface structures: A review. Bioact Mater .2018; 4:56-70. PMID: 30596158 PMCID: PMC6305839 DOI: 10.1016/j.bioactmat.2018.12.003.
2. Williams DF. There is no such thing as a biocompatible material. Biomaterials. 2014; 35(38):10009-10014. DOI: 10.1016/j.biomaterials.2014.08.035 PMID: 25263686.
3. Bundy K.J. Biomaterials and the chemical environment of the body. Joint Replacement Technology. Woodhead Publishing. 56-80, 2008. DOI:10.1533/9781845694807.1.56
4. Frost H M. Wolff's Law and bone's structural adaptations to mechanical usage: an overview for clinicians. Angle Orthod. 1994; 64(3):175-88. PMID: 8060014 DOI: 10.1043/0003-3219(1994)064<0175: WLABSA>2.0.CO; 2.
5. Sumner D R. Long-term implant fixation and stress-shielding in total hip replacement. J Biomech. 2015; 18; 48(5):797-800. PMID: 25579990 DOI: 10.1016/j.jbiomech.2014.12.021.
6. Lopez-Heredia MA, Sohier J, Gaillard C, Quillard S, Dorget M, et al. Rapid prototyped porous titanium coated with calcium phosphate as a scaffold for bone tissue engineering. Biomaterials. 2008; 29(17):2608-2015. PMID: 18358527 DOI: 10.1016/j.biomaterials.2008.02.021.
7. Ryan GE, Pandit AS, Apatsidis DP. et al. Porous titanium scaffolds fabricated using a rapid prototyping and powder metallurgy technique. Biomaterials. 2008; 29(27):3625-3635. PMID: 18556060 DOI: 10.1016/j.biomaterials.2008.05.032.
8. Dabrowski B, Swieszkowski W, Godlinski D, Kurzydlowski KJ, et al. Highly porous titanium scaffolds for orthopaedic applications. J Biomed Mater Res B Appl Biomater. 2010; 95(1):53-61. PMID: 20690174 DOI: 10.1002/jbm.b.31682.
9. Ghorbani F, Zamanian A. Oriented microstructure in neural tissue engineering: a review. J Tissue Sci Eng. 2016; 7(3):182. DOI:10.4172/2157-7552.1000182.
10. Zhang J, Zhao SH, Zhu M, Zhu Y, Zhang Y, et al. 3D-printed magnetic Fe 3 O 4/MBG/PCL composite scaffolds with multifunctionality of bone regeneration, local anticancer drug delivery and hyperthermia. J Mater Chem B. 2014; 2(43):7583-7595. PMID: 32261896 DOI: 10.1039/c4tb01063a.
11. Lord E, Mackay AL, Ranganathan S. New geometries for new materials. Cambridge University Press, 2006.
12. Sychov M, Lebedev LA, Dyachenko SV, Nefedova LA. Mechanical properties of energy-absorbing structures with triply periodic minimal surface topology. Acta Astronautica. 2018;150:81-84. https://doi.org/10.1016/j.actaastro.2017.12.034.
13. Yoo DJ. Advanced porous scaffold design using multi-void triply periodic minimal surface models with high surface area to volume ratios. Int J Precis Eng Manufactur. 2014; 15:1657–1666.
14. Aremu AO, Brennan-Craddock JPJ, Panesar A, Ashcroft I, et al. A voxel-based method of constructing and skinning conformal and functionally graded lattice structures suitable for additive manufacturing. Addit Manufactur. 2016; 1-13. DOI: 1016/j.addma.2016.10.006.
15. Zhao M, Liu F, Fu G, Zhang DZ, Zhang T, et al. Improved mechanical properties and energy absorption of BCC lattice structures with triply periodic minimal surfaces fabricated by SLM. Materials. 2018; 11(12):2411. PMID: 30501050 PMCID: PMC6317040 DOI: 10.3390/ma11122411.
16. Chang B, Song W, Han T, Yan J, Li F, et al. Influence of pore size of porous titanium fabricated by vacuum diffusion bonding of titanium meshes on cell penetration and bone ingrowth. Acta Biomater. 2016; 33:311-321. PMID: 26802441 DOI: 10.1016/j.actbio.2016.01.022.
17. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials. 2005; 26(27):5474-91. PMID: 15860204 DOI: 10.1016/j.biomaterials.2005.02.002.
18. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials. 2005; 26(27):5474-91. PMID: 15860204 DOI: 10.1016/j.biomaterials.2005.02.002.
19. Zadpoor AA. Bone tissue regeneration: the role of scaffold geometry. Biomater Sci. 2015; 3(2):231-45. PMID: 26218114 DOI: 10.1039/c4bm00291a.
20. Bouët G, Marchat D, Cruel M, Malaval L, Vico L. In vitro three-dimensional bone tissue models: from cells to controlled and dynamic environment. Tissue Eng Part B Rev. 2015; 21(1):133-56. PMID: 25116032 DOI: 10.1089/ten.TEB.2013.0682.
21. Dall'Ava L. Hothi H. Henckel J. Di Laura A. Shearing P. et al. Characterization of dimensional, morphological and morphometric features of retrieved 3D-printed acetabular cups for hip arthroplasty. J Orthop Surg Res. 2020; 15(1):1-2. PMID: 32306995 PMCID: PMC7169042 DOI: 10.1186/s13018-020-01665-y.
22. Wu SH, Li Y, Zhang YQ, Li XK, Yuan CF, Hao YL, Zhang ZY. et al. Porous titanium-6 aluminum-4 vanadium cage has better osseointegration and less micromotion than a poly-ether-ether-ketone cage in sheep vertebral fusion. Artif Organs. 2013;

37(12):191-201. PMID: 24147953 DOI: 10.1111/aor.12153.

23. Eltlhawy B, Fouda N, Eldesouky I. Numerical Evaluation of a Porous Tibial-Knee Implant using Gyroid Structure. J Biomed Phys Eng. 2022; 1; 12(1):75-82. PMID: 35155295 PMCID: PMC8819261 DOI: 10.31661/jbpe.v0i0.2005-1116.

24. Faizan A, Bhowmik-Stoker M, Alipit V, Kirk AE, Krebs VE, Harwin SF. et al. Development and verification of novel porous titanium metaphyseal cones for revision total knee arthroplasty. J Arthroplasty. 2017; 1; 32(6):1946-53.

25. Ataee A, Li Y. Fraser D, Song G, Wen C. Anisotropic Ti-6Al-4V gyroid scaffolds manufactured by electron beam melting (EBM) for bone implant applications. Materials & Design. 2018; 345-354. DOI: https://doi.org/10.1016/j.matdes.2017.10.040.

26. Alvarez K, Nakajima H. Metallic scaffolds for bone regeneration. Materials. 2009; 23; 2(3):790-832. doi: 10.3390/ma2030790.

27. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials. 2005; 1; 26(27):5474-91. PMID: 15860204. DOI: 10.1016/j.biomaterials.2005.02.002.

28. Patra S, Young V. A review of 3D printing techniques and the future in biofabrication of bioprinted tissue. Cell Biochem Biophys. 2016; 74(2):93-8. PMID: 27193609 DOI: 10.1007/s12013-016-0730-0.

29. Frosch KH, Barvencik F, Viereck V, Lohmann CH, Dresing K, et al. Growth behavior, matrix production, and gene expression of human osteoblasts in defined cylindrical titanium channels. J Biomed Mater Res A. 2004; 1; 68(2):325-34. PMID: 14704974 DOI: 10.1002/jbm.a.20010.

30. Zaharin HA. Abdul Rani AM. Azam FI. Ginta TL. Sallih N. et al. Effect of unit cell type and pore size on porosity and mechanical behavior of additively manufactured Ti6Al4V scaffolds. Materials. 2018; 28; 11(12):2402. PMID: 30487419 PMCID: PMC6317238 DOI: 10.3390/ma11122402.