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Design and additive manufacturing of nerve guide conduits using triple periodic minimal surface structures

Üçlü periyodik minimal yüzey yapilar kullanarak sinir kilavuz kanallarinin tasarimi ve eklemeli imalatı

Yazar(lar) (Author(s)): *Aybegüm Numanoğlu[1], İsmail Şahin[2], Neslihan Top[3]*

ORCID[1]: 0000-0001-9425-1756

ORCID[2]: 0000-0001-8566-3433

ORCID[3]: 0000-0002-0771-6963

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Design and Additive Manufacturing of Nerve Guide Conduits Using Triple Periodic Minimal Surface Structures

Highlights

- *Porous Nerve Guide Conduits (NGC)*
- *Triple Periodic Minimal Surfaces based NGC design*
- *3D Printing of Nerve Guide Conduits*
- *Characterization of Nerve Guide Conduits*

Graphical Abstract

Three different nerve guide conduit structures were designed, produced and analysed.

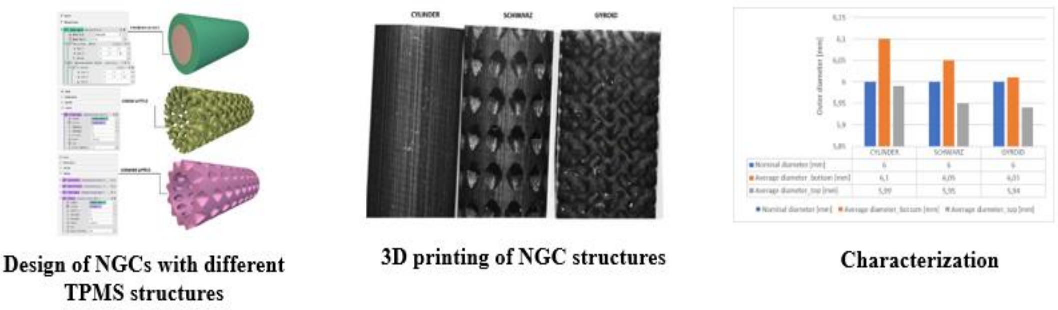

Figure. Scope of the study

Aim

The aim of this study is to investigate the applicability of 3D printing, in the production of TPMS based porous nerve guide conduits, and to assess how accurately 3D printing can produce the geometric properties of porous nerve guide conduits.

Design & Methodology

Three different unit cell designs were developed and the models were fabricated using photopolymerization technology. The dimensional accuracy, pore size, and porosity parameters of the samples were measured.

Originality

In this study, triple periodic minimal surfaces were used in porous nerve guide conduit design to resolve the conflict between structural parameters.

Findings

The parameter with the lowest average deviation was the diameter. For the Schwarz model, the deviation measured from the lower surface was 0.83%, and for the Gyroid model, it was 0.17%.

Conclusion

The study demonstrates that the geometric properties such as the designed height and outer diameter of the produced nerve guide conduits can be achieved with deviation by <2% from the nominal value in the STL model.

Declaration of Ethical Standards

The author(s) of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Design and Additive Manufacturing of Nerve Guide Conduits Using Triple Periodic Minimal Surface Structures

Araştırma Makalesi / Research Article

Aybegüm NUMANOĞLU[1,2]*, İsmail ŞAHİN[3], Neslihan TOP[3]

[1]Graduate School of Natural and Applied Sciences, Gazi University, Türkiye
[2]Arçelik A.Ş., Yukarısoku Mh. Arçelik Sk No:1, Bolu, Türkiye
[3]Department of Industrial Design Engineering, Gazi University, Türkiye

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ABSTRACT

One important area of research in tissue engineering is scaffold design. Before isolated cells are transplanted, a scaffold is a three-dimensional scaffolding that offers temporary support for the development of new tissue. The goal of scaffolds used in tissue engineering is for cells to colonize them. Scaffolds must deliver the required chemical and physical signals to guarantee adequate tissue growth. The most straightforward of all nerve guide conduits (NGC) designs is the hollow and non-porous design, which consists of a hollow tube composed of either natural or artificial polymers. Negative aspects due to its non-porous nature, the capacity for nerve regeneration will be impacted by the permeability of growth agents and nutrients into and out of the channel. Mass cannot be transferred across the membrane since it is non-porous. This permeability issue has led to the development of porous NGCs. The porous NGC designs based on Triply Periodic Minimal Surfaces (TPMS), which are produced additively utilizing photopolymer resin material and photopolymerization technique, are examined in this work. Three distinct NGCs in all, two of which have pores with pore sizes between 150 and 350 μm and porosity greater than 60%, were taken into consideration. This work represents one of the first to report on the 3D printing of NGCs based on TPMS utilizing photopolymer resin. Our results suggest that TPMS-based structures can potentially be adopted to fabricate porous structures suitable for mass transfer required for nerve regeneration.

Keywords: Triple Periodic Minimal Surfaces, Nerve Guide Conduit, Scaffolding.

Üçlü Periyodik Minimal Yüzey Yapılar Kullanarak Sinir Kılavuz Kanallarının Tasarımı ve Eklemeli İmalatı

ÖZ

Doku mühendisliğindeki önemli araştırma alanlarından biri iskele tasarımıdır. İskele yeni dokunun gelişimi için geçici destek sunan üç boyutlu bir yapıdır. Doku mühendisliğinde kullanılan iskelelerin amacı hücreleri kolonileştirmektir. İskelelerin yeterli doku büyümesini garanti etmek için gerekli kimyasal ve fiziksel sinyalleri iletmesi gerekir. Tüm sinir kılavuzu kanalları (NGC) arasında en yaygın olanı, doğal veya yapay polimerlerden oluşan içi boş tüpten oluşan gözeneksiz yapılardır. Gözeneksiz kanallarda büyüme ajanlarını ve besinlerin kanala giriş çıkışı sağlanamadığından sinir yenilenme kapasitesi olumsuz etkilenmektedir. Gözeneksiz kılavuzlarda kütle membrandan aktarılamaz. Bu geçirgenlik sorunu gözenekli NGC'lerin gelişmesine yol açmıştır. Bu çalışmada, fotopolimer reçine malzemesi ve fotopolimerizasyon tekniği kullanılarak üretilen üçlü periyodik minimal yüzeylere (TPMS) dayalı gözenekli NGC tasarımları incelenmiştir. Çalışmada gözenek boyutları 150 ile 350 μm arasında ve gözeneklilik oranı %60'ın üzerinde olan üç farklı NGC dikkate alınmıştır. Bu çalışma, fotopolimer reçine kullanılarak üretilen TPMS'ye dayalı NGC'lerin 3 boyutlu baskısını rapor eden ilk çalışmalardan biridir. Sonuçlar, TPMS bazlı yapıların, sinir rejenerasyonu için gereken kütle aktarımına uygun gözenekli yapıların üretilmesi için potansiyel olarak benimsenebileceğini göstermektedir.

Anahtar Kelimeler: Üçlü periyodik minimal yüzeyler, Sinir Kılavuz kanalları, İskele yapıları.

1. INTRODUCTION

Nerve tissue injuries are prevalent and widespread conditions that necessitate long-term care, resulting in substantial treatment expenses. Traditional procedures for treating peripheral nerve injuries (Figure 1) involve (allografts) [1]. Nerve allografts can reduce sensory loss in bridging nerve gaps with nerve guide conduits made from the patient's own body (autografts) or donor nerves the donor location produced by autografts and have a wider range of clinical applications. However, immune suppression is a prerequisite for allografting treatment, and health issues associated with immune-suppressive therapies are the main disadvantage of allografting [2]. Nerve autografting is the primary treatment used fornerve tissue injuries. This treatment leads to functional losses in the area where the nerve is harvested, causes injury to the patient for opening the donor area, and requires long surgery times. Nerve guidance conduits (NGCs) have proven to be the most promising way to overcome the drawbacks of autografting [3].

**Corresponding Author*
e-posta : aybegum.numanoglu@gazi.edu.tr

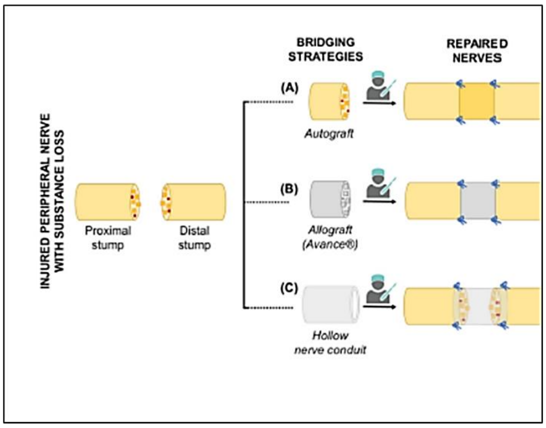


Figure 1. Bridging applications applied in peripheral nerve injuries [1]

Tissue engineering scaffolds called nerve guide conduits are used to restore damaged nerve tissue. They are used to treat damage to peripheral nerves and perform the role of physically guiding nerve regeneration along lesions. Numerous experiments with porous, hollow, multi-conduited, lumen-filled structures [4], as well as different materials like chitosan [5], silk fibroin [6], and gelatin methacrylate (GelMA) [7], and production methods like electrospinning [9], Freeze-drying [8], and 3D printing [10], have been carried out in an effort to improve the properties of nerve guide conduits. Nonetheless, further investigation and advancement are necessary to integrate these design specifications into a single optimal nerve guide conduit. Tissue engineering routinely employs additive manufacturing techniques because of theincreased design freedom they offer [11–14]. Numerous benefits, like high precision,repeatability, controlled porosity, and a large selection of materials, come with additive manufacturing. It also offers benefits in scaffold structure management and facilitates the creation of intricate microstructures [15]. Since additive manufacturing techniques are predictable, controllable, and effective, nerve guide conduits have also been made with them in recent years, coinciding with the growth of additive manufacturing applications in tissue engineering [4–16]. Although the method of additive manufacturing offers benefits in terms of increased design flexibility, scaffold design is subject to stringent limitations. The degree of production technique adequacy limits the intricacy of the scaffold structure. Nerve guide conduits produced by additive manufacturing methods offer lower costs, higher efficiency, and ease of preparation compared to traditional manufacturing methods, and can also be used as carriers for growth factors or bioactive substances. Nerve guide conduits produced by additive manufacturing methods both solve the problems of autografting and serve as carriers for bioactive substances [17]. The capacity to "customise" any desired shape and the flexibility to include appropriate active cells are the two primary benefits of neural guide conduits made using additive manufacturing techniques. Scaffold design therefore becomes less important when adequately made scaffolds that have been computationally developed and optimised cannot be achieved. The advancement of additive manufacturing techniques over traditional scaffold production processes is seen in the ability to build scaffolds rapidly, accurately, and with high flexibility. This is made possible by the advent of 3D printing. Many porous structures are produced using traditional methods such as electrospinning and gas foaming. It is not easy to control the pore structures with traditional production methods. As a result, cage constructions made of nodes and struts have been created. According to [18], these are regarded as the second class of permeable structures. Strut lengths, diameters, and the topology of strut connections can all be readily changed to regulate geometry and performance in porous systems. The majority of cage structures can be manufactured via additive manufacturing because of the nested struts [19–20]. On the other hand, stress concentration may happen at the strut connecting sites. Consequently, three-dimensional minimum surfaces, the third class of porous structures, are used in the design of cage structures in order to enhance design performance [21]. Three-dimensional minimum surfaces in the construction of porous structures offer two significant benefits: (1) Mathematical functions provide for an exact expression of the complete structure. By modifying function parameters, basic characteristics like porosity or specific surface area can be directly changed. Two-dimensional minimum surfaces are extremely smooth and devoid of any roughness, such as junction points or sharp edges found in cage constructions. These benefits make three-dimensional minimal surface structures exceptional for research in a variety of domains, including production, acoustic and optical fields, and other fields [22–24]. Although triply periodic minimal surfaces (TPMS) have been an interesting research topic in various fields, their advantages are not fully utilized. Interdisciplinary studies are needed to promote the applications TPMS. In addition, improvements in manufacturing methods are required for the design and production of TPMS. Despite current manufacturing technologies, there are significant challenges in producing complex TPMS. For example, one of them is the necessity of considering more manufacturing constraints in the design process to further enhance production quality. More research is needed to identify critical issues in the process from design to production and applications of triply periodic minimal surface (TPMS) structures.This paper deals with nerve guide conduit design and additive manufacturing. The dimensional accuracy, porosity, and pore sizes between the design and production of nerve guide conduits produced by additive manufacturing were observed to assess the performance of additive manufacturing in the production process of NGCs. A significant innovation of the study is the use of TPMS structures in the design of nerve guide conduits. In the literature review, it was found that there are no applications of nerve guide conduits designed and produced with TPMS in nerve guide conduit applications. The study contributes to the field by providing information on the manufacturability of TPMS-based nerve guide conduits. The study's analysis of porous structures in the creation and manufacture of NGCs is another contribution to the body of literature.

2. NERVE GUIDE CONDUITS

Nerve guide conduits are tubular tissue engineering scaffolds that mimic the endoneurium structure surrounding axons [2]. When nerves are damaged, nerve guide conduits are used as functional tubes for nerve regeneration. Nerve guide conduits are primarily used to concentrate neurotrophic substances generated by injured nerve ends and to guide emerging axons between nerve ends [2,3]. The length of reparable nerve damage and its healing impact both rise when nerve guide conduits are paired with cells and growth factors. In addition to having biomimetic features that offer structural support for axon growth, the "ideal" nerve guide conduit should also include conductivity, biocompatibility, and biodegradability. It ought to offer nutritional support throughout the duration of nerve regeneration [4,5].

NGCs must be created and manufactured with the necessary qualities, as indicated in Figure 2, in order for them to carry out all of these tasks during the regeneration process and perhaps result in optimal nerve regeneration [16].

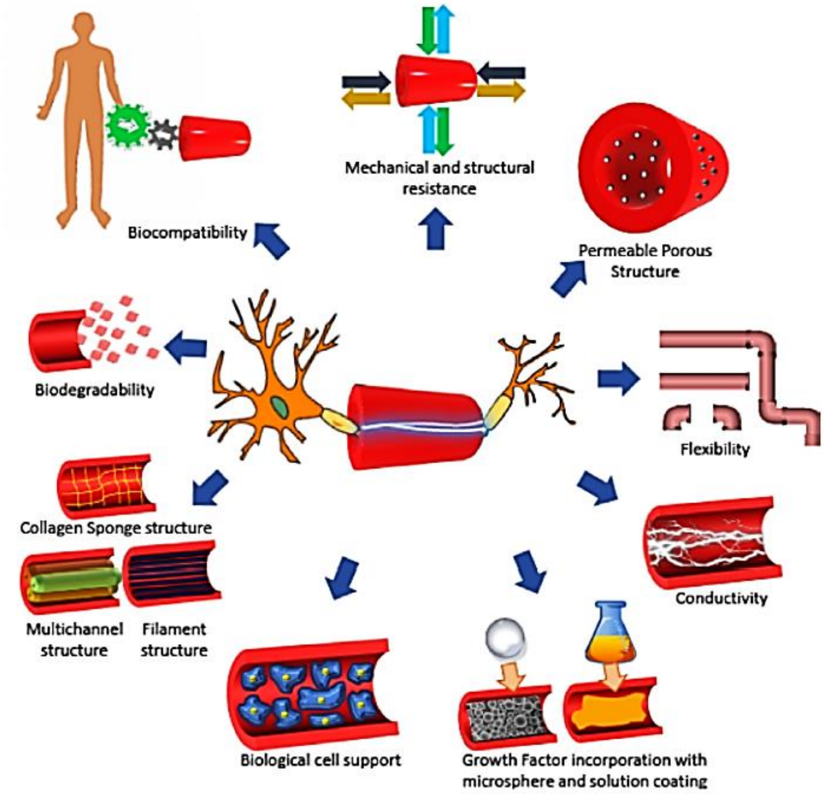


Figure 2. Characteristic features of ideal nerve guide conduits [16]

To promote nerve regeneration, NGCs should provide the following, as listed below [25]:**Biocompatibility:** NGCs should minimize adverse tissue reactions and be biocompatible to support cell adhesion and proliferation [2, 25]. **Biodegradability:** From a biological perspective, nerve guide conduits should be made of biologically degradable materials [10]. **Mechanical Properties:** Nerve guide conduits should have appropriate mechanical properties to withstand physiological loads and provide structural support during the healing process [10, 25]. **Porosity:** To support cell proliferation, nutrition transport, and metabolite release, NGCs should have the right amount of porosity. They should consist of interconnected porous networks of three-dimensional porous structures [8]. **Flexibility:** NGCs should be flexible to prevent mechanical injuries to surrounding tissues and axons during the regeneration process. **Surface Microtopography and Chemistry:** Protein molecules can be absorbed, and how cells adhere and position themselves are determined by the proper surface microtopography and chemistry that promote cell adhesion, proliferation, and differentiation [9]. **Electrical Conductivity:** Biological reactions are improved by surface charges. Cell settling is influenced by the conductivity or electrical charge of a substance [16, 25].The non-porous design, which consists of a hollow tube, is the most basic design. Non-porosu NGCs provide the benefits of reproducibility and ease of production. Its drawback is that, because it is non porous, the rate at which nerve regeneration occurs is negatively impacted because it prevents nutrients and growth factors from entering and leaving the canal. By offering permeability, the porous architecture can address the mass transfer issue needed for NGC growth [3].

Wall thickness and permeability have an impact on the conduit's mechanical attributes, including strength and flexibility. It should therefore be prioritised in design processes that serve as guidelines. Axonal development is inhibited in conduits whose walls are thicker than 0.8 mm [26]. Reduced porosity and permeability, which are critical for nerve regeneration, have been linked to this decline. Transparency of nerve guide conduits is a preferred feature by surgeons because it allows surgeons to place nerve ends optimally into the conduits during nerve repair surgery.

A perfect biomaterial for nerve guide conduit manufacturing and tissue engineering should balance a number of characteristics, including biocompatibility, biodegradability, permeability, suitable biomechanics, and surface qualities. Conduit production techniques should be used to modify these attributes. For a design to be implemented in commercial settings, it must be practically manufacturable (Figure 3) [27].

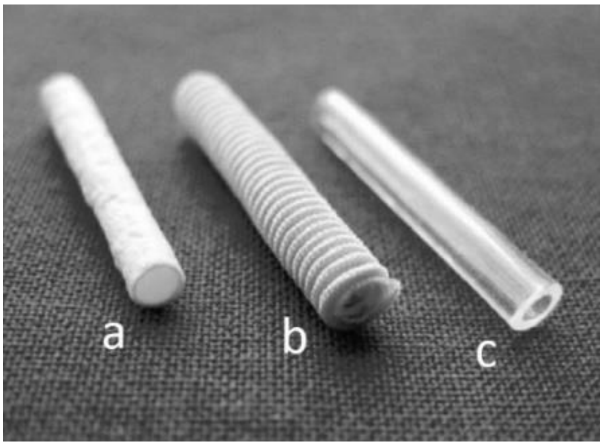


Figure 3. Commercial nerve guide conduit examples (a) Collagen I NeuraGen (b) Poly(glycolic acid)(PGA) NeuroTube (c) poly-L-lactic acid (PLACL) Neurolac [27]

3.MATERIALS AND METHODS

Two different designs of NGCs were made, based on the three-dimensional minimum surfaces of the unit cell structure. The TPMS structures that were employed in this study were Schwarz and Gyroid. The Schwarz Primitive, Gyroid, and Schwarz Diamond surfaces are the most often utilized structures in TPMS-related research [28]. Using photopolymerization, nerve guide conduit models were produced based on these unit cells. We examined the porosity and dimensional accuracy of the nerve guidance conduits that were constructed.

3.1. Materials and Printers

NGCs were 3D printed by the photopolymerization process. For the 3D printing of nerve guide conduits, a Photocentric LC Opus 3D printer was utilized. During printing, a layer thickness of 100 microns and 5 base layers were employed. BASF Ultracur3D ST 45 B photopolymer resin was used as the printing material. ST 45 B photopolymer resin was chosen for its biocompatibility. The detailed mechanical properties of the material are provided in Table 1.

Table 1. The Ultracur3D ST 45 B photopolymer resin's mechanical characteristics

	Standard	Unit
Tensile strength	ASTM D638	60 MPa
Elongation at break	ASTM D638	25%
Elastic modulus	ASTM D638	2300 MPa
Bending strength	ASTM D790	110 MPa
Density	ASTM D4052-18	1.12 gr/cm^3
Biocompatibility	ISO 10993	

The photopolymerization method was preferred for 3D printing because it does not require support material during the printing process. For samples designed with pore sizes between 150 and 350 microns, support-free printing was preferred to achieve the desired porosity. Additionally, another reason for its preference is that the photopolymer resin does not require fine adjustments in its physicochemical properties such as surface tension, viscosity, and volatility [11, 29].

3.2. Design

NGCs are typically designed as empty luminal [30-32], multi-conduited [33, 34], and microgrooved structures [35]. Studies have reported that NGCs do not meet all requirements. Nerve guide conduit design is a process that involves many contradictions. For example, the high porosity of nerve guide conduits can weaken their mechanical properties [36]. To concurrently improve all nerve guide conduit qualities, these conflicts that make the design process difficult and complex must be properly handled throughout the design stage.In this study, the use of TPMS helped to resolve a conflict between structural variables in the nerve guide conduit design. TPMS have advantages in geometric and biological regularity due to their porous qualities. Triple periodic minimum surfaces can be used to get geometric and thermal qualities, mathematical accuracy, multifunctionality, and topology tailored for mechanical applications [37, 38]. Based on the unit cell structure's TPMS, two distinct nerve guide conduit designs were created. In this study, Schwarz and Gyroid were the TPMS structures used. The most often used surfaces in TPMS-related research are the Schwarz Primitive, Gyroid, and Schwarz Diamond surfaces [39]. Alan Schoen made the initial discovery of the Gyroid structure in 1970 [40]. The only known embedded TPMS without symmetry lines and with triple connections is the Gyroid structure. The Gyroid surface can be obtained trigonometrically from its equation [41, 42]. Sinx Cosy+Siny Cosz+Sinz Cosx=t (1)

The Schwarz minimal surface is a TPMS structure developed by Hermann Schwarz [43]. There are different types of Schwarz minimal surfaces. In this study, the Schwarz P surface was utilized. The Schwarz P surface, which can be trigonometrically generated with the equation provided in Equation 2, has cubic symmetry, making it useful for creating prototypes of tissue scaffolds (Figure 4).$Cosx + Cosy + Cosz = t$ (2)

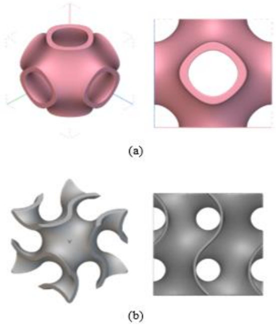


Figure 4. (a) Schwarz (b) Gyroid unit cells

When creating the design volume, different cell types were defined as unit cells. The radius of the unit cells is determined as 2 mm, the height is determined as 2 mm and the thickness is determined as 0.4 mm. Unit cells were applied to cylindrical channels divided into 12 equal parts from the center circulary. The conduits' outer diameter (d) was adjusted to 6 mm, wall thickness (t) to 1 mm, and height (h) to 15 mm when designing NGCs (Figure 5).

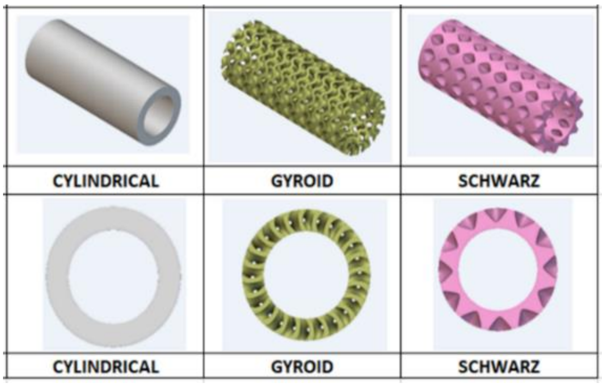


Figure 5. Perspective and top views of nerve guide conduits design models

Three distinct nerve guidance conduits were designed using the nTopology software, and the design procedure described here were used independently for the Gyroid, Schwarz, and cylindrical models (Figure 6).

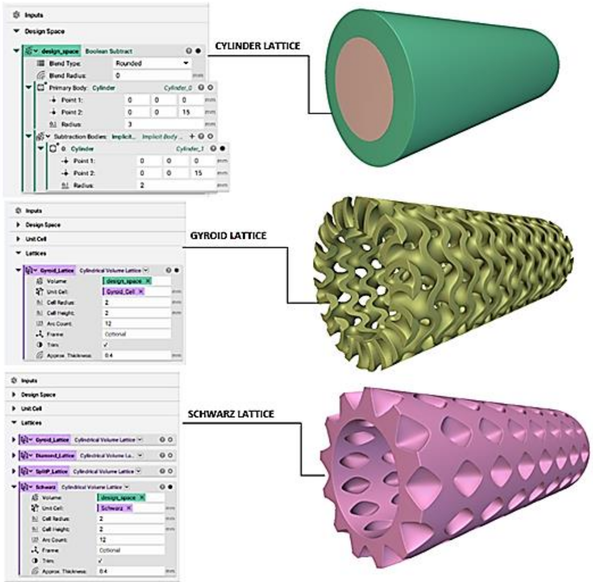


Figure 6. Nerve guide conduits design parameters

3.3. 3D Printing of Nerve Guide Conduits

In the study, nerve guide conduits were produced using BASF Ultracur3D ST 45 photopolymer resin with photopolymerization technology (Figure 7). The nerve guide conduits that are created have an outer diameter of 6 mm, a wall thickness of 1 mm, and a height of 15 mm (Figure 8). Five base layers and a layer thickness of 100 microns were used for the 3D printing process. The curing time was adjusted to 40 minutes. Two exposure periods, 40 and 60 seconds, can be used. The producer of the samples recommends a duration of 40 seconds. The samples underwent a 10-minute washing process in isopropyl alcohol using an ultrasonic cleaner.

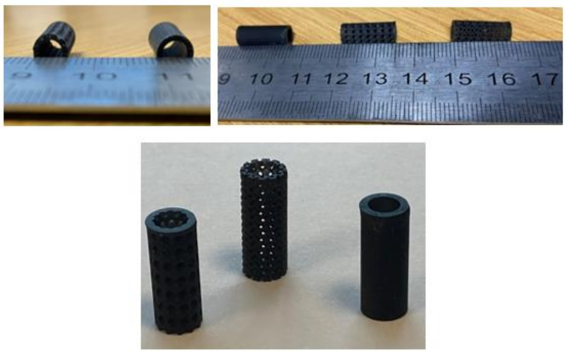


Figure 7. 3D printed nerve guide conduits

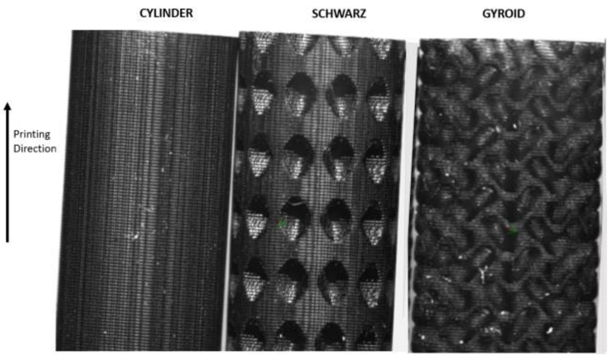


Figure 8. 3D printed TPMS based nerve guide conduits

3.4. Characterization

The Nikon VMA-2520 optical imaging instrument was used to measure the outside diameters of the NGCs at both top and bottom points, the thickness of the conduit walls, and the conduit heights.Three measurements were taken from each sample for each dimension, and the average of the measured values was calculated. The upper, lower, and side surfaces, as well as the pores of the nerve guide conduits, were imaged at 24x magnification using the Nikon VMA-2520. The nominal outer diameter, nominal wall thickness, nominal height dimensions, and porosity ratio of the NGCs were determined as design parameters before the design process; they were then verified based on the deviation between design and 3D printing. Additionally, the surface morphology of the produced nerve guide conduits was examined

3.5. Porosity and Pore Size

The volumes of the samples were calculated using the Archimedes principle (Figure 9). The weights of the samples were measured using the OHAUS PX85 (0.0001g) analytical balance.

Figure 9. Volume measurement of samples

In the context of this study, the bulk volumes of the cellular structures (V_B) (Equation (3)) had to be established before the porosity ratio in the specified scaffold structures could be found. Subsequently, the volumes of the created porous units (V_sk) were calculated using the Archimedes principle. Finally, the porosity ratio was computed using Equation (4) [11, 44].

$$V_B = \pi * (r_d - r_i)^2 * h \qquad (3)$$

$$Porosity\ (\%) = \frac{V_B - V_{sk}}{V_B} \times 100 \qquad (4)$$

4.RESULTS AND DISCUSSION

This section examined the dimensional accuracy, pore size, porosity, and weight properties of flat cylindrical, Gyroid, and Schwarz nerve guidance conduits made using the photopolymerization process.

4.1. Dimensional Accuracy

The diameter of NGCs should be compatible with the size of the injured nerve's proximal and distal ends. Conduits that are not compatible with nerve endings adversely affect nerve regeneration. Small-diameter nerve guide conduits can lead to chronic nerve compression. On the other hand wide-diameter nerve guide conduits may allow unwanted cells to enter the conduit while permitting the escape of growth factors [45-47]. In the Ni et al. (2013) study, a "critical length" of 15 mm was established by testing NGCs in rats with sciatic nerve injury. Given that the target organ should be taken into consideration when determining the conduit's inner diameter in order to maximise healing [48], 4 mm was selected as the inner diameter, falling between the lowest and highest diameters of commercial guide conduits displayed in Figure 10 [49].

Product name	Material	Diameter × length
Neurotube™	PGA	2–8 mm × 4 cm
NeuroMatrix™, Neuroflex™	Type I collagen	2–6 mm × 2.5 cm
Neurolac®	Poly(DL-lactide-caprolactone)	1.5–10 mm × 3 cm
Neuragen®	Type I collagen	2–7 mm × 2 cm
SaluBridge®	Polyvinyl alcohol hydrogel	2–10 mm × 6.35 cm

Abbreviations: FDA, US Food and Drug Administration; PGA, polyglycolic acid.

Figure 10. Commercial nerve guide conduit examples and measurements approved by the American Food and Drug Administration [47]

The nominal height of the NGCs in the study was found to be 15 mm, in accordance with the literature. After printing, the height of the straight cylinder measured 14.85 mm, while it was 14.77 mm for the Schwarz model and 14.79 mm for the Gyroid model (Figure 11). For each model, there were discrepancies in the measured average heights and the intended heights. These height discrepancies are assumed to result from variations in the indentations and slits on the models' upper surfaces.

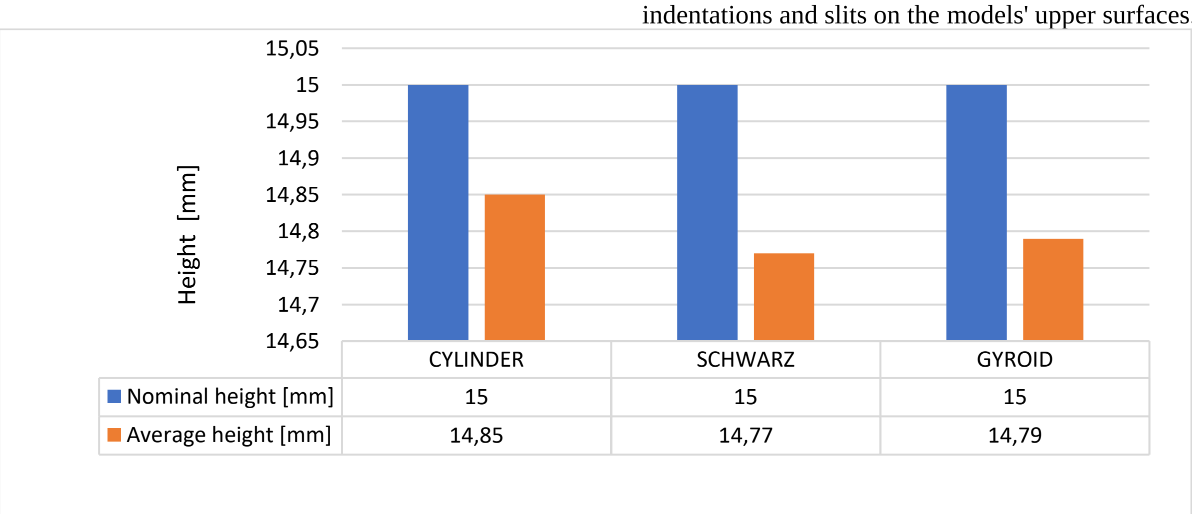


Figure 11. Nominal heights and measured average heights of the nerve guide conduit models determined during the design

The indentation on the upper surface of the Gyroid model is larger compared to the Schwarz model (Figure 12). Consequently, the height of the Gyroid model (14.79 mm) was measured to be lower than the height of the Schwarzmodel(14.77mm).

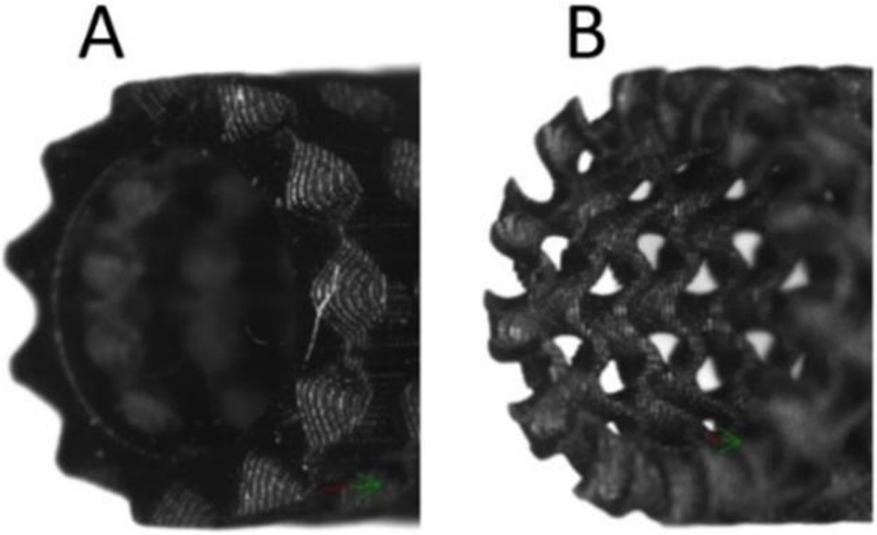


Figure 12. Upper surface of the Schwarz model (A). Upper surface of the Gyroid model (B)

The nerve guide conduits' outer diameter was measured independently of their top and lower surfaces. The nominal outer diameter size is determined as 6 mm in the design. The outer diameter measurements measured from the lower surface were measured to be 0.102 mm higher on average in all models compared to the models measured from the upper surface (Figures 13) which might be attributed to the printing direction. Since the printing starts from the bottom surface, the highest diameter measurements appeared on this surface (Figure 14).

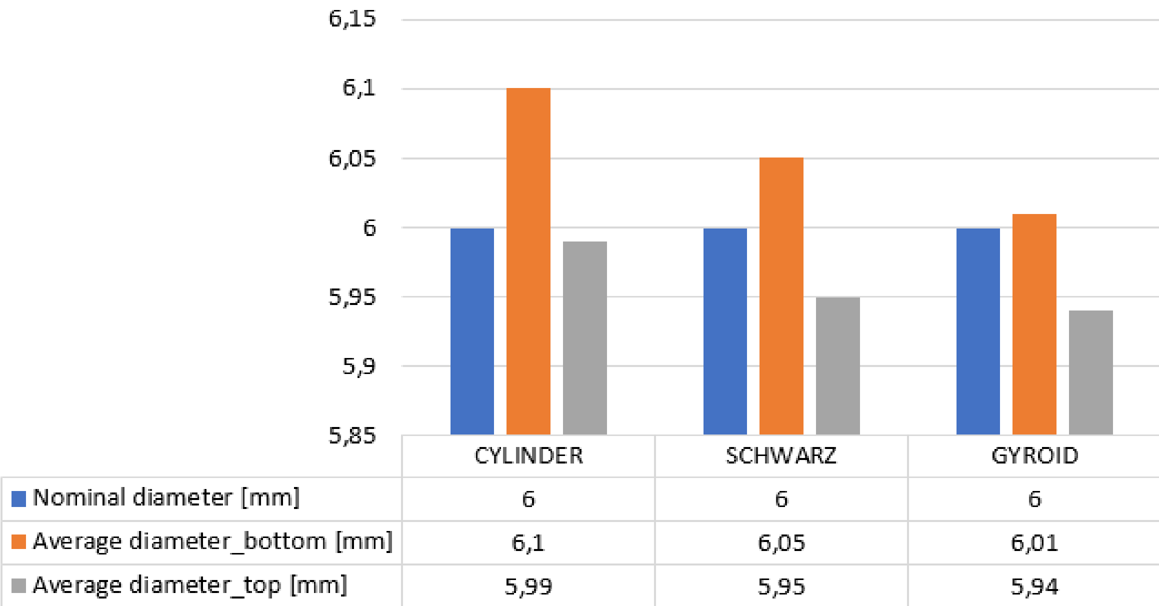


Figure 13. Average outer diameter measured from the lower surface, average outer diameter measured from the upper surface, nominal outer diameter dimensions of all models

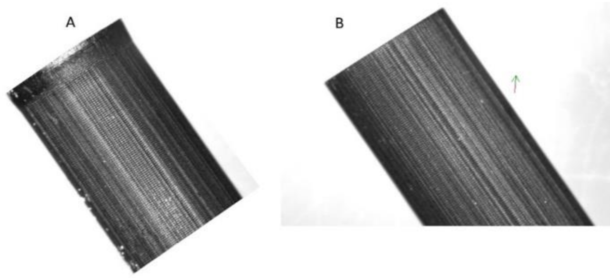


Figure 14. Cylinder Guide conduit lower surface (A) Guide conduit upper surface (B)

One of the factors influencing the stitchability of the guide is the wall thickness of NGCs. A perfect guide should be simple to sew. NGCs should be strong enough to allow the stitch to bind the proximal and distal nerve ends during movement, but also allow the needle to pass through the guide wall, preventing the escape of nerve endings from the guide lumen [50]. Wall thicknesses of less than or equal to 0.6 mm or more than 0.8 mm are unsatisfactory and produce inconsistent results, according to data from in vitro and in vivo tests in a rat sciatic nerve damage model [27, 51]. Nevertheless, inconsistent in-vivo results have been reported in the literature, indicating the need for additional research to establish the optimal wall thickness [52–54]. Nerve guidance conduit wall thicknesses were measured from both the top and bottom surfaces. Visual differences between the bottom and top surfaces were also observed (Figure 15).

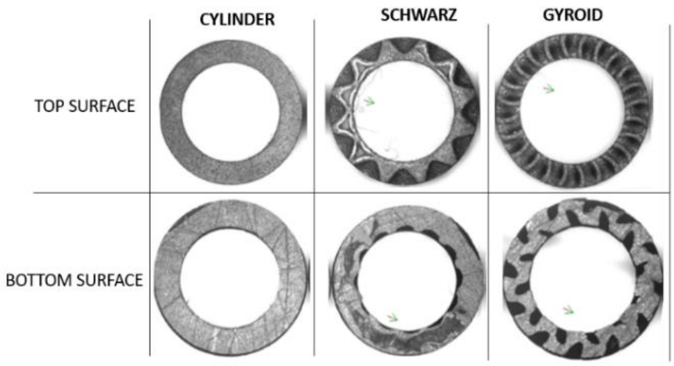
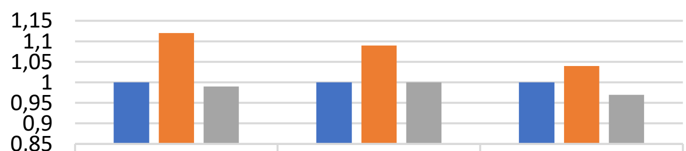


Figure 15. Bottom and top surface images of the produced nerve guide conduits

Since the samples adhere to the printing table from the bottom surface, a flat surface is observed instead of the design pattern on the bottom surface In every model, the measures of wall thickness on the lower surface are greater than those on the upper surface. The difference in exterior diameter measurements and this result are parallel (Figure 16). The reason for this difference is thought to be the direction of printing from bottom to top. Among the TPMS models, the model with the highest wall thickness is the Schwarz model. The Schwarz model has the least surface undulation in terms of surface morphology. Since the Schwarz model's surface undergoes fewer processing operations compared to other models, it is assumed that more material remains in its wall. In the study, the design and production deviation was calculated using the CAD design dimensions of the samples and the post-3D printing measurements (Table 2).



	CYLINDER	SCHWARZ	GYROID
Nominal wall thickness [mm]	1	1	1
Average wall thickness_bottom [mm]	1,12	1,09	1,04
Average wall thickness_top [mm]	0,99	1	0,97

Figure 16. Average wall thickness measured from the bottom surface, average wall thickness measured from the top surface, nominal wall thickness measurements of all models

Table 2. Deviation of produced samples design

	CYLINDER	SCHWARZ	GYROID
Nominal height [mm]	15	15	15
Average height [mm]	14,85	14,77	14,79
Deviation for height	1,00%	1,53%	1,40%
Nominal diameter [mm]	6	6	6
Average diameter_bottom surface [mm]	6,1	6,05	6,01
Average diameter_top surface [mm]	5,99	5,95	5,94
Deviation for diameter_bottom surface	-1,67%	-0,83%	-0,17%
Deviation for diameter_top surface	0,17%	0,83%	1,00%
Nominal wall thickness [mm]	1	1	1
Average wall thickness [mm] Bottom surface	1,12	1,09	1,04
Average wall thickness [mm] Top surface	0,99	1	0,97
Deviation for wall thickness Bottom surface	-12,00%	-9,00%	-4,00%
Deviation for wall thickness Top surface	1,00%	0,00%	3,00%

Equation 5 was used to calculate deviation.

$$\text{Deviation} = \frac{\text{Measured value} - \text{Theoric value}}{\text{Theoric value}} * 100 \quad (5)$$

Theoretical value=Considered as nominal value

The nominal thickness parameter was set to 1 mm for wall thickness of the nerve guide conduits in the study. The wall thickness measurements obtained from the lower surface were 1.12 mm for the cylindrical model, 1.09 mm for the Schwarz model, and 1.04 mm for the Gyroid model. On the other hand, the wall thickness measurements obtained from the upper surface were 0.99 mm for the cylindrical model, 1 mm for the Schwarz model, and 0.97 mm for the Gyroid model.

The deviation for the wall thickness measured from the lower surface were 12% for the cylindrical model, 9% for the Schwarz model, and 4% for the Gyroid model. For the wall thickness measured from the upper surface, the deviation were 1% for the cylindrical model, 0% for the Schwarz model, and 3% for the Gyroid model. The wall thickness measured from the upper surface of the Schwarz model had the lowest deviation, whereas the wall thickness obtained from the bottom surface of the cylindrical model had the most variance.

The parameter with the lowest average deviation was the diameter. The diameter measurements obtained from the lower surface were 6.1 mm for the cylindrical model, 6.05 mm for the Schwarz model, and 6.01 mm for the Gyroid model. The cylindrical model had the highest diameter among the measurements obtained from the lower surface, resulting in the highest deviation of 1.67%. For the Schwarz model, the deviation measured from the lower surface was 0.83%, and for the Gyroid model, it was 0.17%. On the other hand, the diameter measurements obtained from the upper surface were 5.99 mm for the cylindrical model, 5.95 mm for the Schwarz model, and 5.94 mm for the Gyroid model. The deviation measured from the upper surface were 1% for the Gyroid model, 0.83% for the Schwarz model, and 0.17% for the cylindrical model. In contrast to the deviation measured from the lower surface, the cylindrical model had the lowest deviation for the diameter measured from the upper surface.The average deviation of the diameter measurements obtained from the lower surface was higher than the average deviation of the diameter measurements obtained from the upper surface.

The deviation in wall thickness and diameter measurements are directly related to the direction of printing during production because the deviation for measurements obtained from the lower surface are higher than the deviation for measurements obtained from the upper surface.

The heights of the cylindrical, Schwarz, and Gyroid models were 14.85 mm, 14.77 mm, and 14.79 mm, respectively. The height deviation was 1.53% for the Schwarz model and 1.40% for the Gyroid and cylindrical models. The model with the lowest height deviation was the cylindrical model, while the model with the highest height deviation was the Schwarz model. There were differences between the designed heights and the measured average heights for each model. It is presumed that these differences in heights stem from variations in the protrusions and recesses on the upper surfaces of the models.

4.2. Pore Size and Porosity

The structural characteristics of neural guide conduits are mostly determined by their pore sizes. The proximal and distal ends of the damaged nerve's diameters should match those of the NGCs. Nerve regeneration may be adversely affected by incompatible guides. Larger NGCs may allow undesired cells to enter the conduit and promote the release of growth factors, whereas small-diameter conduits may cause chronic nerve compression [45-47]. As pore size increases, neural guide conduit permeability rises [55, 56]. Greater axonal development is supported by NGCs with bigger pores than by those with smaller pores[44, 57]. Generally speaking, the ideal range of pore sizes is between 10 and 20 micrometres. To maintain nutrition flow while blocking extracellular matrix fibroblast entrance and growth factor release, pores should be bigger than 4 micrometres and smaller than 30 micrometres [58]. Current study suggests that porosity and pore size are dependent on the guide conduit's construction process, despite contradictory data surrounding pore size determination [17].

Microscopic examinations of nerve guide conduit models revealed that porosity in the Schwarz model design was very weak (Figure 17). This condition was also reflected in the porosity rates, with Schwarz models measuring a porosity rate of 31%. Thinning of the wall thickness was observed at certain points in the Schwarz models. Even if permeability can be achieved through these points, permeability tests should be conducted to confirm this. The average pore diameter formed in the Schwarz model was determined to be 152 micrometers.

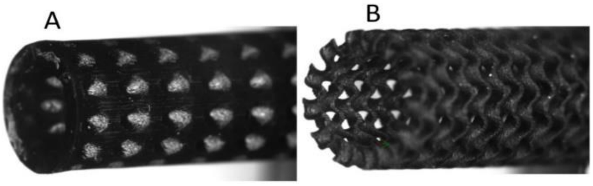


Figure 17. Schwarz model image (A) Gyroid model image (B)

An average of 345 micrometres was found for the pore diameters in the Gyroid model. Table 3 presents the samples' weights, volumes, and densities. Each sample's weight and volume were divided to determine the density value. Porosity rates also showed this condition, with Gyroid models having 65.9% porosity.

Table 3. Weights, volumes and densities of the produced nerve guide conduit samples

Model name	Weight	Volume	Density	Porosity	Pore size
	(gr)	(cm³)	(gr/cm³)	%	(Micron)
CYLINDER	0,2593	0,294	1,2965	-	-
SCHWARZ	0,195	0,2	0,975	31	152
GYROID	0,1109	0,1	1,109	65,9	345

The scaffold needs to be porous and have sufficient pore connectivity in order to guarantee proper nutrition transfer to cells and waste product elimination. Porosity includes the size of the pores, the distribution of pore sizes, and the interconnectedness of the pores. NGCs must have a porosity of more than 50% in order to have mass transfer qualities [17]. Elevated porosity results in elevated permeability, facilitating sufficient oxygen and nutrition delivery to the cells inside the nerve guidance conduits. The model with the highest density is the straight cylinder, while the lowest density is observed in the Schwarz model. To prevent the collapse of nerve guide conduits, which poses a risk to nerve regeneration, a certain level of mechanical resistance is required [18]. Tests need to be conducted to measure the mechanical strength of low-density guide conduits. Surface defects in the samples have been investigated. It was observed that in the porous models of Schwarz and Gyroid, the pores could be produced defect-free. In the straight cylinder model, discontinuities were detected at multiple points (Figure 18). The presence of these defects, particularly at the lower surface where the printing began and in close proximity to each other, suggests that they may be due to some blockage in the printing nozzle. After clearing the blockage in the printing nozzle, no defects were detected at the upper points of the model.

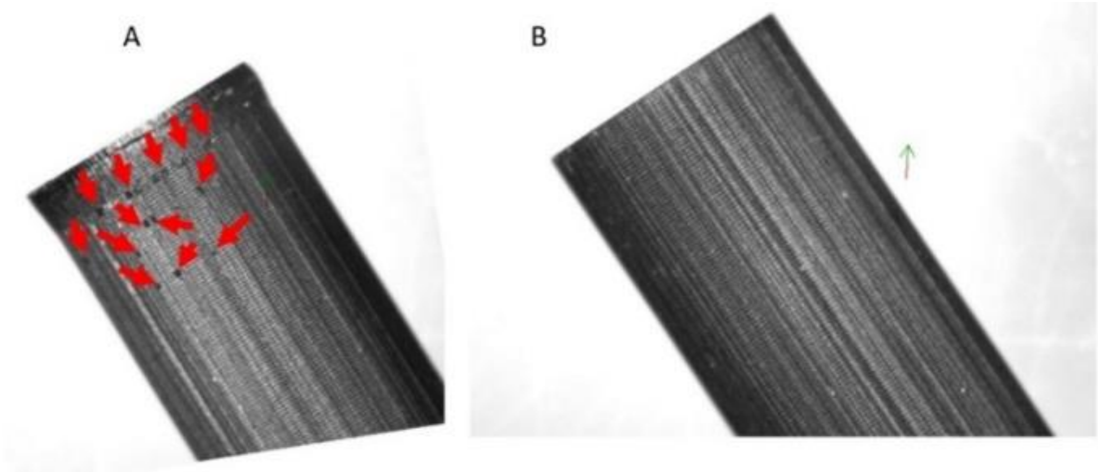


Figure 18. Discontinuities on the flat cylinder model surface (A) Upper region of the model (B)

No macro defects were observed in the connections between the pores and on the surfaces.

5. CONCLUSION

This study examines the TPMS-based design of NGCs using samples made of photopolymer resin. The printed samples showed no macro faults, yet nozzle blockage caused avoidable defects on the surfaces of some structures. The height measurement and NGC diameter in the generated samples deviate by <2% from the nominal value in the STL model. The variance in wall thickness is <12%. The printing orientation is assumed to be the primary source of this large variance. The wall thickness thins out and approaches the STL model size as you proceed up from the direction where the printing begins.

Deviation <2% from the nominal value in height and diameter measurements reflect the stable performance during the production process. However, a detailed examination of the production process is required to optimize deviation. Factors such as material properties, production parameters, and equipment settings affect deviation, and optimizing these factors will lead to more consistent results.Since additive manufacturing offers greater design freedom than traditional production processes, it is a significant factor in tissue engineering. Scaffolds with intricate microstructures can be produced more easily and precisely with 3D printing than with conventional methods, allowing for easy design modifications. Nerve guide conduits made with additive manufacturing have the major benefit of being able to "customise" any shape and include the right active cells.

A limitation of the study is that the experimental data were obtained from a limited number (n=3) of scaffold samples, which limits the generalization of the results. The absence of a larger variety of scaffold designs in the study makes it difficult to make specific evaluations regarding structural properties. The study is limited to in-vitro experiments conducted under laboratory conditions. Therefore, the results obtained in vitro should not be expected to be fully consistent with the performance under real in-vivo conditions.

Another limitation is the lack of evaluation of biological factors such as biocompatibility, degradation properties, and cellular interactions, in addition to the biomechanical performance of the produced nerve guide conduits. This study emphasizes structural and biomechanical aspects while acknowledging this limitation.

Finally, all the samples produced in the study were made from a single type of photopolymer (BASF), which does not provide a comprehensive perspective on the effect of other potential materials on structural design parameters. In the future, combining conventional and technological production techniques like electrospinning, freeze-drying, and 3D printing might make it feasible to precisely create biocompatible NGCs. It is possible to create customised nerve guide conduits that could fully integrate with injured tissues. In the end, the creation of NGCs that facilitate peripheral nerve injury functional recovery will transform clinical applications and enhance patient quality of life for millions of people globally.

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DECLARATION OF ETHICAL STANDARDS

The authors of this article declare that the materials and methods used in their studies do not require ethical committee approval and legal-specific permission.

AUTHORS' CONTRIBUTIONS

Aybegüm NUMANOĞLU: Contributed equally to design, perform the measurements, analyse the results and wrote the manuscript.

İsmail ŞAHİN: Contributed equally to design, perform the measurements, analyse the results and wrote the manuscript.

Neslihan TOP: Contributed equally to design, perform the measurements, analyse the results and wrote the manuscript.

CONFLICT OF INTEREST

There is no conflict of interest in this study.

REFERENCES

[1] Gao, S. vd.,"3D-bioprinted GelMA nerve guide conduits promoted peripheral nerve regeneration by inducing trans-differentiation of MSCs into SCLCs via PIEZO1/YAP axis", ***Materials Today Advances,17***, 100325. (2023).

[2] Dadacı, Mehmet, "Biobozunur "Elektrospun kaprolakton" Sinir Kılavuz Kanallarının Periferik Sinir Sistemi Rejenerasyonlarında Etkinliğinin Değerlendirilmesi", ***Tıpta Uzmanlık Tezi, Hacettepe Üniversitesi,*** (2007)

[3] Vijayavenkataraman, S., Zhang, S., Thaharah, S., Sriram, G., Lu, W. F., & Fuh, J. Y. H., "Electrohydrodynamic jet 3D printed nerve guide conduits (NGCs) for peripheral nerve injury repair", ***Polymers***,*10*(7), 753. (2018).

[4] Wan, T., Wang, Y. L., Zhang, F. S., Zhang, X. M., Zhang, Y. C., Jiang, H. R. & Zhang, P. X., "The porous structure of peripheral nerve guide conduits: features, fabrication, and implications for peripheral nerve regeneration", ***International Journal of Molecular Sciences***, *24*(18), 14132, (2023).

[5] Li, X., Yang, W., Xie, H., Wang, J., Zhang, L., Wang, Z., & Wang, L., "CNT/sericin conductive nerve guide conduit promotes functional recovery of transected peripheral nerve injury in a rat model", ***ACS applied materials & interfaces,*** *12*(33), 36860-36872, (2020).

[6] [6] Ma, Y., Wang, H., Wang, Q., Cao, X., & Gao, H., "Piezoelectric conduit combined with multi-channel conductive scaffold for peripheral nerve regeneration", ***Chemical Engineering Journal,*** *452*, 139424, (2023).

[7] Ye, W., Li, H., Yu, K., Xie, C., Wang, P., Zheng, Y.& Gao, Q., "3D printing of gelatin methacrylate-based nerve guide conduits with multiple channels", ***Materials & Design,*** *192*, 108757, (2020).

[8] Shen, J., Wang, J., Liu, X., Sun, Y., Yin, A., Chai, Y.& Zheng, X., "In situ prevascularization strategy with three-dimensional porous conduits for neural tissue engineering", ***ACS Applied Materials & Interfaces,*** *13*(43), 50785-50801, (2021).

[9] Zheng, T., Wu, L., Xu, J., Sun, S., Guan, W., Han, Q., ... & Li, G., "YR/DFO@ DCNT functionalized anisotropic micro/nano composite topography scaffolds for accelerating long-distance peripheral nerve regeneration", ***Composites Part B: Engineering,*** *246*, 110242, (2022).

[10] Namhongsa, M., Daranarong, D., Sriyai, M., Molloy, R., Ross, S., Ross, G. M.& Punyodom, W., "Surface-modified polypyrrole-coated PLCL and PLGA nerve guide conduits fabricated by 3D printing and electrospinning" ***Biomacromolecules,*** *23*(11), 4532-4546, (2022).

[11] Top, N., Gökçe, H & Şahin, İ., "Additive Manufacturing of Bio-Inspired Microstructures for Bone Tissue Engineering", ***Experimental Techniques,*** *47*(6), 1213-1227, (2023).

[12] Ataollahi, S., "A review on additive manufacturing of lattice structures in tissue engineering", ***Bioprinting,*** e00304, (2023).

[13] Jia, Z., Xu, X., Zhu, D., & Zheng, Y., "Design, printing, and engineering of regenerative biomaterials for personalized bone healthcare", ***Progress in Materials Science,*** *134*, 101072, (2023).

[14] Wei, Q., Zhou, J., An, Y., Li, M., Zhang, J., & Yang, S., "Modification, 3D printing process and application of sodium alginate based hydrogels in soft tissue engineering: A review", ***International Journal of Biological Macromolecules,*** *232*, 123450, (2023).

[15] Mota, C., Puppi, D., Chiellini, F., & Chiellini, E., "Additive manufacturing techniques for the production of tissue engineering constructs", ***Journal of tissue engineering and regenerative medicine,*** *9*(3), 174-190, (2015).

[16] Rahman, M., Mahady Dip, T., Padhye, R., & Houshyar, S., "Review on electrically conductive smart nerve guide conduit for peripheral nerve regeneration", ***Journal of Biomedical Materials Research Part A,*** *111*(12), 1916-1950, (2023).

[17] Stocco, E., Barbon, S., Emmi, A., Tiengo, C., Macchi, V., De Caro, R., & Porzionato, A., "Bridging gaps in peripheral nerves: from current strategies to future perspectives in conduit design", ***International Journal of Molecular Sciences,*** *24*(11), 9170, (2023).

[18] Hanks, B., Berthel, J., Frecker, M., & Simpson, T. W., "Mechanical properties of additively manufactured metal lattice structures: Data review and design interface", ***Additive Manufacturing,*** *35*, 101301, (2020).

[19] Feng, J., Fu, J., Yao, X., & He, Y., "Triply periodic minimal surface (TPMS) porous structures: from multi-scale design, precise additive manufacturing to multidisciplinary applications", ***International Journal of Extreme Manufacturing,*** *4*(2), 022001, (2022).

[20] Top, N., Şahin, İ., & Gökçe, H., "The Mechanical Properties of Functionally Graded Lattice Structures Derived Using Computer-Aided Design for Additive Manufacturing", ***Applied Sciences,*** *13*(21), 11667, (2023).

[21] Rajagopalan, S., & Robb, R. A., "Schwarz meets Schwann: design and fabrication of biomorphic and durataxic tissue engineering scaffolds", ***Medical image analysis,*** *10*(5), 693-712, (2006).

[22] Gan, X., Wang, J., Liu, Z., Zeng, M., Wang, Q., & Cheng, Z., "Numerical Study on Thermal Hydraulic and Flow-Induced Noise in Triply Periodic Minimal Surface (TPMS) Conduits", ***ASME Journal of Heat and Mass Transfer,*** *146*(4), (2024).

[23] Chen, W., Tang, X., Chu, X., Yang, Y., Xu, W., Fu, D., & Zhou, W., "Impact of catalyst carrier with TPMS structures on hydrogen production by methanol reforming", ***International Journal of Hydrogen Energy,*** *58*, 1177-1189, (2024).

[24] Zeng, C., Wang, W., Hai, K., & Ma, S., "Lightweight airborne TPMS-filled reflective mirror design for low thermal deformation", ***Composite Structures,*** *327*, 117665, (2024).

[25] [25] Zhang, S., "Computational Design and Optimization of Scaffolds for Tissue Engineering Application",

Doctoral dissertation, National University of Singapore (Singapore), (2018).

[26] [26] Rutkowski, G. E., and Heath, C. A., "Development of a bioartificial nerve graft. II. nerve regeneration in vitro", ***Biotechnol. Prog.,*** 18, 373–379, (2002).

[27] [27] Tian, L., Prabhakaran, M. P., & Ramakrishna, S., "Strategies for regeneration of components of nervous system: scaffolds, cells and biomolecules", ***Regenerative biomaterials,*** *2*(1), 31-45, (2015).

[28] Yeranee, K., & Rao, Y., "Heat transfer and pressure loss of turbulent flow in a wedge-shaped cooling channel with different types of triply periodic minimal surfaces", ***ASME Journal of Heat and Mass Transfer,*** *145*(9), 093901, (2023).

[29] Layani, M., Wang, X., & Magdassi, S., "Novel materials for 3D printing by photopolymerization", ***Advanced Materials,*** *30*(41), 1706344, (2018).

[30] Yu, W., Zhao, W., Zhu, C., Zhang, X., Ye, D., Zhang, W.,& Zhang, Z., "Sciatic nerve regeneration in rats by a promising electrospun collagen/poly (ε-caprolactone) nerve conduit with tailored degradation rate", ***BMC neuroscience,*** *12*, 1-14, (2011).

[31] Gan, L., Zhao, L., Zhao, Y., Li, K., Tong, Z., Yi, L., ... & Chen, Y., "Cellulose/soy protein composite-based nerve guide conduits with designed microstructure for peripheral nerve regeneration", ***Journal of neural engineering,*** *13*(5), 056019, (2016).

[32] Zhu, Y., Wang, A., Patel, S., Kurpinski, K., Diao, E., Bao, X., & Li, S., "Engineering bi-layer nanofibrous conduits for peripheral nerve regeneration", ***Tissue Engineering Part C: Methods,*** *17*(7), 705-715, (2011).

[33] Zeng, C. G., Xiong, Y., Xie, G., Dong, P., & Quan, D., "Fabrication and evaluation of PLLA multiconduit conduits with nanofibrous microstructure for the differentiation of NSCs in vitro", ***Tissue Engineering Part A***, *20*(5-6), 1038-1048, (2014).

[34] Lee, D. J., Fontaine, A., Meng, X., & Park, D., "Biomimetic nerve guide conduit containing intraluminal microconduits with aligned nanofibers markedly facilitates in nerve regeneration", *ACS* ***Biomaterials Science & Engineering,*** *2*(8), 1403-1410, (2016).

[35] Ni, H. C., Tseng, T. C., Chen, J. R., Hsu, S. H., & Chiu, M., "Fabrication of bioactive conduits containing the fibroblast growth factor 1 and neural stem cells for peripheral nerve regeneration across a 15 mm critical gap", ***Biofabrication,*** *5*(3), 035010, (2013).

[36] Chiono, V., & Tonda-Turo, C., "Trends in the design of nerve guide conduits in peripheral nerve tissue engineering", ***Progress in neurobiology,*** *131*, 87-104, (2015).

[37] Kapfer, S. C., Hyde, S. T., Mecke, K., Arns, C. H., & Schröder-Turk, G. E., "Minimal surface scaffold designs for tissue engineering", ***Biomaterials,*** *32*(29), 6875-6882, (2011).

[38] Torquato, S., Hyun, S., & Donev, A., "Multifunctional composites: optimizing microstructures for simultaneous transport of heat and electricity", ***Physical review letters***, *89*(26), 266601, (2002).

[39] Kladovasilakis, N., Charalampous, P., Tsongas, K., Kostavelis, I., Tzetzis, D., & Tzovaras, D., "Experimental and computational investigation of lattice sandwich structures constructed by additive manufacturing technologies", ***Journal of Manufacturing and Materials Processing,*** *5*(3), 95, (2021).

[40] Schoen, A. H., "*Infinite periodic minimal surfaces without self-intersections",* **National Aeronautics and Space Administration**, (Vol. 5541), (1970).

[41] Hoffman, D. A., "*Global Theory of Minimal Surfaces",* ***Proceedings of the Clay Mathematics Institute*** *2001 Summer School, Mathematical Sciences Research Institute, Berkeley, California, June 25-July 27, 2001* (Vol. 2). OECD Publishing, (2005).

[42] Karcher, H., "The triply periodic minimal surfaces of Alan Schoen and their constant mean curvature companions", ***Manuscripta mathematica,*** *64*(3), 291-357, (1989).

[43] Schwarz, H. A., ***"Gesammelte mathematische abhandlungen",*** American Mathematical Soc., (Vol. 260), (1972).

[44] Clements, B. A., Bushman, J., Murthy, N. S., Ezra, M., Pastore, C. M., and Kohn, J., "Design of barrier coatings on kink-resistant peripheral nerve conduits", ***J. Tissue Eng.*** 7, 2041731416629471, (2016).

[45] [45] Daly, W. T., Yao, L., Abu-rub, M. T., O'Connell, C., Zeugolis, D. I., Windebank, A. J., & Pandit, A. S., "The effect of intraluminal contact mediated guide signals on axonal mismatch during peripheral nerve repair", ***Biomaterials,*** *33*(28), 6660-6671, (2012).

[46] Isaacs, J., Mallu, S., Yan, W., & Little, B., "Consequences of oversizing: nerve-to-nerve tube diameter mismatch", ***JBJS,*** *96*(17), 1461-1467, (2014).

[47] Yi, S., Xu, L., & Gu, X., "Scaffolds for peripheral nerve repair and reconstruction", ***Experimental neurology***, *319*, 112761, (2019).

[48] Frost, H. K., Andersson, T., Johansson, S., Englund-Johansson, U., Ekström, P., Dahlin, L. B., & Johansson, F., "Electrospun nerve guide conduits have the potential to bridge peripheral nerve injuries in vivo", ***Scientific reports,*** *8*(1), 16716, (2018).

[49] Ao, Q., "Progress of nerve bridges in the treatment of peripheral nerve disruptions", ***J Neurorestoratology,*** *4*, 107-113, (2016).

[50] Nectow, A. R., Marra, K. G., and Kaplan, D. L., "Biomaterials for the development of peripheral nerve guide conduits", ***Tissue Eng.*** *Part B Rev.* 18, 40–50, (2012).

[51] Stang, F., Keilhoff, G., and Fansa, H., "Biocompatibility of different nerve tubes", ***Materials*** 26, 3083-3091, (2009).

[52] Teuschl, A. H., Schuh, C., Halbweis, R., Pajer, K., Márton, G., Hopf, R., ... & Hausner, T., "A new preparation method for anisotropic silk fibroin nerve guide conduits and its evaluation in vitro and in a rat sciatic nerve defect model", ***Tissue Engineering Part C: Methods***, *21*(9), 945-957, (2015).

[53] Wang, G. W., Yang, H., Wu, W. F., Zhang, P., & Wang, J. Y., "Design and optimization of a biodegradable porous zein conduit using microtubes as a guide for rat sciatic nerve defect repair", ***Biomaterials***, *131*, 145-159, (2017).

[54] Jahromi, H. K., Farzin, A., Hasanzadeh, E., Barough, S. E., Mahmoodi, N., Najafabadi, M. R. H., et al., "Enhanced sciatic nerve regeneration by poly-L-lactic

acid/multi-wall carbon nanotube neural guide conduit containing Schwann cells and curcumin encapsulated chitosan nanoparticles in rat", ***Mater. Sci. Eng.*** *C* 109:110564. doi: 10.1016/j.msec.2019.110564, (2020).

[55] O'Brien, F. J., Harley, B. A., Yannas, I. V., and Gibson, L. J., "The effect of pore size on cell adhesion in collagen-GAG scaffolds", ***Biomaterials*** 26, 433–441, (2005).

[56] Liu, D., Mi, D., Zhang, T., Zhang, Y., Yan, J., Wang, Y., et al., "Tubulation repair mitigates misdirection of regenerating motor axons across a sciatic nerve gap in rats", ***Sci. Rep.*** 8:3443, (2018).

[57] Goulart, C. O., Pereira Lopes, F. R., Monte, Z. O., Dantas, S. V., Souto, A., Oliveira,J. T., et al., "Evaluation of biodegradable polymer conduits - poly(llactic acid) - for guiding sciatic nerve regeneration in mice" ***Methods*** 99, 28–36, (2016).

[58] Du, J., and Jia, X., "Engineering nerve guide conduits with threedimenisonal bioprinting technology for long gap peripheral nerve regeneration", ***Neural Regen***. Res. 14:2073, (2019).