Enhancing bone regeneration: A mechanobiology-centric approach to TPMS-based bone replacements

by

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Abstract

Cellular porous structures are increasingly used for biomedical applications. Triply Periodic Minimal Surfaces (TPMS) are mathematically defined cellular structures whose geometry can be quickly adjusted to achieve the desired mechanical response (structural and fluid). This has made them desirable as bioinspired materials for bone replacement. Scaffolds for bone replacements should be designed to respond to the mechanical environment: they should provide enough structural support during bone regeneration, while also enabling nutrient diffusion through its pores to allow cell proliferation and differentiation. Mechanobiology plays an important role in bone regeneration and understanding the interaction between the scaffold's geometry, its material, and the mechanobiological environment is required to improve tissue regeneration. The main purpose of this dissertation is to improve the understanding of the mechanical behavior and mechanobiological properties of TPMS structures to design bone replacements that can accurately mimic bone properties.

The design of TPMS scaffolds was parametrized and automated to target bone porosity and pore size while maintaining a good manufacturability. Then, the structural and fluid flow properties of the scaffolds were assessed using Finite Element (FE) and Computational Fluid Dynamics (CFD) models respectively. The results were introduced into an uncoupled tissue differentiation model to predict the TPMS architectures that could be most promising to induce bone differentiation. Finally, a previously validated mechanobiological computational model (FE) was enhanced to evaluate the bone regeneration potential of complex porous structures and integrate the influence of patient-specific properties and clinical strategies to maximize bone regeneration.

The obtained results showed that the permeability of the studied TPMS architectures was affected by pore distribution and architecture. In addition, a novel analytical
model that enables the prediction of the permeability values of TPMS structures based on geometrical parameters was developed. The results also indicated that the TPMS Gyroid architecture was the most suitable for promoting tissue differentiation when considering both the structural and fluid flow properties.

Furthermore, the computational mechanobiological model successfully assessed the ability of various scaffolds to promote bone regeneration, emphasizing the importance of scaffold's geometry and material. The bone ingrowth within the scaffold pores demonstrated that the scaffold's geometrical properties influence cellular diffusion and strain distribution, resulting in differences in regenerated bone volume and distribution. Furthermore, bone ingrowth was found to be material-dependent, implying that the material can be used to fine-tune strain distribution and improve bone growth. Similarly, the use of clinical strategies and consideration of the host's physiological characteristics resulted in variations in bone regeneration, emphasizing the importance of incorporating such parameters into the design process of bone substitutes.

In conclusion, this dissertation provides a framework for designing optimal patient-specific strategies to promote bone regeneration, thereby improving the conceptualization and design of bone replacements.
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# Nomenclature

<table>
<thead>
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<th>Symbol</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>BV</td>
<td>Bone volume</td>
</tr>
<tr>
<td>$C_f$</td>
<td>Level-set constant</td>
</tr>
<tr>
<td>$C_k$</td>
<td>Fitted permeability coefficients</td>
</tr>
<tr>
<td>$c_i$</td>
<td>Fitted constants of the parametrical equations</td>
</tr>
<tr>
<td>$\bar{c}$</td>
<td>Cell concentration</td>
</tr>
<tr>
<td>$D$</td>
<td>Diffusion constant</td>
</tr>
<tr>
<td>$D_p$</td>
<td>Pore size</td>
</tr>
<tr>
<td>$E$</td>
<td>Elastic modulus</td>
</tr>
<tr>
<td>$E_0$</td>
<td>Material’s elastic modulus</td>
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<tr>
<td>$H$</td>
<td>Mean curvature</td>
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<tr>
<td>$k_p$</td>
<td>Permeability</td>
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<tr>
<td>$L$</td>
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<td>Wall thickness of sheet-based TPMS structures</td>
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<tr>
<td>$t_{sk}$</td>
<td>Wall thickness of skeletal-based TPMS structures</td>
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<tr>
<td>$TV$</td>
<td>Total defect volume</td>
</tr>
<tr>
<td>$U$</td>
<td>Strain energy density</td>
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<tr>
<td>$\dot{V}$</td>
<td>Bone deposition rate</td>
</tr>
<tr>
<td>$\dot{V}_{max}$</td>
<td>Maximum bone deposition rate</td>
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</tbody>
</table>
\( \nu \)  
Inlet velocity

\( \alpha \)  
Location-specific reduction factor

\( \bar{\varepsilon} \)  
Effective tissue microstrain

\( \varepsilon_t \)  
Principal strain

\( \mu \)  
Dynamic viscosity

\( \rho \)  
Density

\( \dot{\rho} \)  
Density rate

\( \phi \)  
Porosity

\( \Psi \)  
Mechanical strain stimulus

\( \Psi_{local}^* \)  
Local stimulus at the implant region

\( \gamma \)  
Octahedral shear strain

\( \tau \)  
Fluid shear stress

\( \Delta P \)  
Pressure drop
Chapter 1.

Introduction and objectives

There has been a significant increase in the number of bone fractures worldwide in recent decades, especially in aging populations with a higher frequency of skeletal disorders. This trend is aggravated by the increasing rates in obesity and decreasing levels of physical activity, which lead to changes in bone structure, ultimately affecting its mechanical integrity. Consequently, individuals with bone affections often experience a decrease in mobility, independence, and overall quality of life.

The American Joint replacement Registry (AJRR) reported in 2023 3,149,042 primary and revision hip and knee arthroplasty procedures performed between 2012 and 2022. This represents a 23% increase of cases from the previous report (2,550,232 from 2012 to 2021). Primary knee procedures accounted for 51.0% of the cases, while primary hip arthroplasties were 33.4%. This means that 15.6% of the total procedures were revision surgeries due to complications related to implant failure or its interaction with surrounding tissue. Although technological and material science advancements have extended the functional lifespan of implants up to 20-30 years, it is still projected that 10% to 20% of these implants will require a revision surgery within the next 15 years after implantation. Therefore, younger patients who need replacement surgeries are at a significant risk of requiring at least one additional revision surgery to address implant failure.
Orthopedic implants and bone scaffolds are one of the primary treatments used to address musculoskeletal conditions and provide patients with significant pain relief and restoring functional mobility. These load-bearing devices are designed to withstand the mechanical demands of daily activities while remaining functional for as long as possible, thereby improving the patients’ quality of life.

Complications such as implant wear, corrosion, and stress shielding can lead to osteolysis, aseptic loosening, and ultimately, implant failure. These concerns highlight the need for more research to optimize implant design and material composition to overcome the challenges. The lifespan of each orthopedic implant is determined by the material’s durability, the implant’s design and its interaction with biological factors such as the patient’s age, activity level, bone health and the mechanobiological environment of the implant site.

The development of orthopedic scaffolds and load-bearing implants has recently focused on cellular porous structures such as Triply Periodic Minimal Surfaces (TPMS) and lattice designs. These structures have the potential to closely mimic the mechanical properties of the trabecular bone, thereby enhancing osseointegration and minimizing complications such as stress shielding. In addition, their tunable nature allows the optimization of porosity and pore size to provide an adequate mechanical stability and promote bone ingrowth, which is essential for successful osseointegration, bone healing and tissue regeneration.

In silico modeling has become a key part in the design process of scaffolds, providing insight into the mechanical behavior of the scaffolds under physiological loads and help predict the biological response. In addition, the development of these devices can be significantly accelerated by simulating and refining the designs in a virtual environment. The integration of cellular porous structures into the design of orthopedic implants represents a promising approach to achieve patient-specific solutions.

Recent developments in orthopedic research have made significant progress in integrating mechanobiological principles into the scaffold’s design. However, a deeper
understanding of the interplay between the scaffold’s geometry, its material and the mechanobiological environment is still necessary to improve tissue regeneration. Elucidating this relationship is key to optimize scaffold performance. In addition, clinical strategies can potentially enhance the regenerative capabilities of scaffolds. Approaches such as pre-seeding scaffolds and refining post-operative care can support cellular activities, induce tissue growth and improve healing.

Accommodating the patient-specific requirements within the scaffold design is equally essential. Younger patients may apply higher mechanical loads on the implants because of their more active lifestyle, while older patients may have a weaker bone structure, requiring different design considerations. Considering biological variables such as age, bone quality, and individual mechanosensitivity, help to obtain ideal designs that meet the requirements of each patient and bone site. Integrating personalized aspects into the design is essential to maximize the performance of bone scaffolds.

This dissertation investigates the use of cellular porous structures in the design of optimal orthopedic scaffolds and implants, emphasizing the role of mechanobiology in the bone regeneration process. The overall aim is to broaden the knowledge of the mechanical and mechanobiological behavior of TPMS structures and advance their computational modeling. This will improve the conceptualization and design of orthopedic implants and provide a framework to design optimal patient-specific strategies to promote bone regeneration. Hence, five main objectives have been established.

**Parametrization of the design methodology of TPMS structures.**

A parametric methodology is investigated to design optimal TPMS structures to target specific mechanical properties and bone mimicking features (porosity and pore size). Furthermore, this approach has been refined to consider restrictive parameters for additive manufacturing (wall thickness and cell size) to ensure feasible fabricability.
Assessment of the suitability of TPMS structures as bone replacements from a mechanical perspective.

Structural and fluid flow analyses were performed as part of an uncoupled fluid-structure model for tissue differentiation. The model aimed to predict which TPMS structures could be most promising to induce bone differentiation based on the mechanical stimulus at their surface.

Characterization of the fluid flow properties of TPMS structures.

A limitation in the literature on the fluid flow properties of TPMS structures is the inconsistency of the studied parameters. Variations in cell sizes, porosity levels, and the number of cells examined in the flow direction present significant challenges when comparing and evaluating the results of different studies. The lack of standardized parameters prevents a consistent understanding and discussion of the results presented in literature.

This research develops a comprehensive study of the influence of different TPMS design parameters and CFD modelling approaches on their fluid flow properties. This will enable more consistent and comparable analyses across different studies.

Development of a generalized analytical model to estimate the permeability of TPMS structures.

Incorporating permeability models into optimization algorithms allow to tailor and optimize the scaffold’s design for each individual patient requirements. Designing scaffolds that match the mechanical needs of patients is a burdensome process. Numerical simulations do not offer the speed needed to efficiently design the structures. In contrast, analytical models provide a more efficient alternative and can be seamlessly incorporated into optimization algorithms.

While existing literature presents several analytical models to characterize the permeability of TPMS structures, these models are limited to a single cell size. This dissertation proposes a generalized analytical model to predict the permeability of TPMS across any given pore size and porosity.
Study of mechanobiologically-induced bone regeneration integrating clinical approaches.

A computational mechanobiological model is used to assess the potential to promote bone regeneration of various porous scaffolds, emphasizing the role of the scaffold’s geometry and material. The study examines how the diffusion patterns and load distribution properties, in combination with the local biological environment and the host’s response, affect the regenerative process. In addition, the design of porous scaffolds for bone regeneration presents several limitations that require further investigation. The induction of bone ingrowth at the core of the scaffolds remains an elusive goal, and the integration of patient-specific parameters into the scaffold design has not been adequately addressed.

The objective is to establish a fundamental framework for future patient-specific bone replacement development that effectively enhance bone repair and regeneration. To this end, this dissertation examines bone formation within the pores of the scaffold, as well as the potential for enhancing this process through the integration of clinical strategies within the predictive model.

1.1. Thesis structure

This dissertation is structured into seven chapters:

Chapter 1 provides an introduction and outlines the objectives of the thesis.

Chapter 2 reviews the current state of the art of cellular porous structures and Triply Periodic Minimal Surfaces. It then examines bone structure and the techniques used to engineer bone substitutes, concluding with a review of mechanobiology and its role in bone tissue engineering applications.

Chapter 3 analyzes the key parameters involved in designing TPMS structures and features the methodology used to model the structures studied in this thesis.

Chapter 4 presents a mechanobiological study to determine which TPMS architectures are best suited for bone replacements using an uncoupled fluid-structure analysis. To
Chapter 1

this end, the TPMS architectures that most closely resemble bone morphology were selected (Gyroid, Fisher-Koch S and Diamond).

**Chapter 5** presents a study of the fluid flow properties of TPMS structures and a novel analytical model to predict the permeability of TPMS structures. To ensure that this model can be applied to any TPMS, architectures with very different geometries have been studied (Gyroid, Fisher-Koch S and Schwartz Primitive).

**Chapter 6** presents a study on the mechanobiological interaction of scaffolds with the host's tissue after implantation. Gyroid scaffolds are studied as they were found to be the most promising TPMS to induce bone differentiation in Chapter 4. A framework for evaluating their performance is presented, including clinical strategies to enhance their osseointegration.

**Chapter 7** outlines the main contributions of this dissertation, and the ongoing and future work.

Portions of the work presented in this dissertation are adaptations, with permissions of the rights-holder, of the published research articles included in Appendix B.
Chapter 2.

Background

This chapter aims to review the nature of cellular porous structures and their relevance in various fields, with a special focus on Triply Periodic Minimal Surfaces (TPMS) and their mechanical properties, fluid flow dynamics, and mechanobiological behavior for their applications to act as bone substitutes.

First, the unique geometric features of cellular porous structures are discussed. Their influence on the mechanical stability and fluid flow properties is then evaluated, considering how they can be tailored to meet the requirements of specific applications.

Bone, its properties and bone substitutes are then reviewed, detailing the basics of bone tissue engineering and the design challenges that arise in the creation of orthopedic implants. This analysis aims to highlight the potential advantages that TPMS structures can offer when replacing bone.

Finally, the fundamentals of mechanobiology and relevant models of bone mechanobiology are summarized. The mechanobiological response induced by cellular porous structures on tissue regeneration is reviewed, emphasizing their potential to mimic the cellular environment and promote desirable biological responses.
2.1. Cellular porous structures

Cellular porous structures are strong yet lightweight materials with applications in diverse fields such as healthcare, engineering or military [1]. Their characteristics are attributed to the existence an interconnected structure of both solid and void networks within their composition [2]. These structures can be found in natural systems such as bone or honeycombs and human beings have incorporated them into engineering designs for their functionality [3] (Figure 2.1). In addition to their light weigh, which is ideal for material saving, they have other outstanding properties such as sound attenuation, vibration damping and energy absorption [2].

![Figure 2.1](image-url) A. Naturally occurring cellular structures: human bone, honeycomb structure and Voronoi structure in bubbles. Image adapted with permission of the rights holder [4]. B. Example of human-designed cellular structures. From left to right: TPMS Gyroid, honeycomb (closed-cell) and lattice diamond [5].

Cellular porous structures have different classifications in the literature. According to the cell type they can be divided into open-cell and closed-cell. Open-cell structures have cells connected only by the edges, whereas in closed-cell structures, they are isolated by solid faces (Figure 2.1-B) [6]. Depending on their porous structure they can be made out of plates (intersecting plates in different orientations), struts (organized
and interconnected beams) or surfaces [2] (Figure 2.2). Based on the periodicity cellular structures can be stochastic or periodic. Periodic structures are characterized for being defined by a unit cell repeated in space to assemble the structure. The modeling of cellular porous structures can be parametric or non-parametric depending on whether or not they can be generated by equations or algorithms [1]. Lattice structures are an example of periodic structures, while Triply Periodic Minimal Surfaces (TPMS) are an example of periodic and parametric structures.

![Figure 2.2. Example of cellular structures made out of plates, struts and surfaces.](image)

### 2.1.1. Triply periodic minimal surfaces

Triply periodic minimal surfaces (Figure 2.3) have become increasingly popular for their multifunctionality. TPMS are self-standing structures, free of self-intersections, with highly ordered and interconnected pores, which makes them lightweight and suitable for Additive Manufacturing (AM) [7], [8]. By definition, they possess zero-mean curvature while exhibiting a substantially enhanced surface area-to-volume ratio.

![Figure 2.3. Common TPMS structures used in the literature. Image adapted with permission of the rights holder [9].](image)

TPMS geometries can be defined by the Enneper-Weierstrass representation, also known as their parametric form. However, this form can only represent few types of TPMS geometries [10]. A more general approximation is the periodic surface form (Eq.
[2.1]). This mathematical model represents TPMS as a derivation of a sum of Fourier series where \( r \) represents the location in the Euclidean space, \( h \) is a reciprocal vector, \( A_h \) is a magnitude factor that determines the amplitude, \( \alpha(h) \) is the phase shift and \( C_f \) is a constant [10], [11].

\[
\Psi(r) = \sum_h A_h \cdot \cos[2\pi h \cdot r - \alpha(h)] = C_f
\]  

TPMS are implicit surfaces that can be reduced to the form \( f(x, y, z) = C_f \), where \( C_f \) is a level-set constant. Their full geometric structure can be represented by algebraic equations, which also qualifies them as isosurfaces. They exhibit periodic behavior in three orthogonal directions, with both the magnitude and the periodicity adjustable via the function parameters. They can be divided into two types of structures, skeletal-based (or solid), where the volume enclosed by the surface’s geometry forms the structure; and sheet-based (or laminar), where the main surface acts as a layer that is then extruded to obtain the final structure (Figure 2.4). Each configuration requires a different design approach, as it will be further explained in Chapter 3.

Furthermore, TPMS are characterized by their minimal surface property, which means that their mean curvature, at any point of the surface, is zero \((H=0)\). The mean
curvature $H$ is calculated as per Eq. [2.2], where $k_1$ and $k_2$ are the principal curvatures in two orthogonal planes [12].

$$H = \frac{k_1 + k_2}{2} \tag{2.2}$$

TPMS structures are attractive for many engineering applications thanks to their versatile architecture. They possess adaptable pore sizes and porosities, along with their strong mechanical (specific elastic modulus and yield strength) and mass transport (permeability and diffusivity) properties [13], [14], [15], [16], [17]. In biomedical engineering, they are expected to be especially promising as bone replacements, both as permanent implants, where non-biodegradable materials (ceramics or metals) are usually used [18], [19], [20], as well as temporary scaffolds for tissue engineering, in which a biodegradable material is preferred [21], [22], [23], [24]. Their complex architecture can mimic trabecular bone and it can be adjusted to obtain desirable porosity and pore sizes to match those of the host tissue. However, a deeper understanding of their mechanical properties is required to optimize their designs [25].

### 2.1.2. Mechanical properties of TPMS structures

#### Structural behavior

The structural properties of TPMS structures have been widely studied in recent years. The works in the literature have mainly focused on their tensile or compressive behavior [26], [27], [28], but there are some works that have also investigated bending and shear properties [29], [30].

TPMS's structural properties are defined by three main factors: the TPMS geometry, the porosity and the material used. Al-Ketan et al. [26] found that sheet-TPMS scaffolds had superior mechanical properties compared to strut-TPMS scaffolds under uniaxial compression. They also reported that there is no trend exhibiting that one geometry is superior to another (Figure 2.5). Instead, their relative behavior is porosity dependent. Regarding the stress plateau and the energy absorption characteristics, the works of AlMahri et al. [31] and Qiu et al. [9] reported that Diamond and F-RD geometries exhibited the best performance. The work of Feng et al. [28] compared the
performance of TPMS structures under quasi-static and dynamic conditions. They reported that the specific strength of highly porous structures (80%) was higher under quasi-static conditions, while for lower porosities (60%), the strength values were higher when tested under dynamic loading. As for the fatigue behavior, Soro et al. [32] investigated the Gyroid, Diamond and Schwartz skeletal-based architectures reporting that their performance was better than strut-based lattices under cyclic loads. They also observed that fatigue cracks initiated at the surface of nodal points due to surface roughness and defects, coinciding with the findings of Yánez et al. [33] for Gyroid structures. Yang et al. [34] also reported that graded porosity can improve the fatigue behavior of Gyroid TPMS structures in comparison with uniform porosity.

Figure 2.5. Nominal stress-strain curves under quasi-static compression for different TPMS architectures. Image reproduced with permission of the rights holder [9].
One of the advantages of cellular structures is that they follow analytical curves for the calculation of mechanical properties based on the Gibson-Ashby model [35], [36]. The stiffness of TPMS structures can be expressed as a function of the porosity or relative density \( \rho/\rho_0 \) by fitting two variables \((C, n)\) (Eq. [2.3]) where \( E_0 \) is the elastic modulus of the constituent material. Similarly, their strength can be expressed as per Eq. [2.4].

\[
\frac{E}{E_0} = C_E \left(\frac{\rho}{\rho_0}\right)^{n_E} \tag{2.3}
\]

\[
\frac{\sigma}{\sigma_0} = C_\sigma \left(\frac{\rho}{\rho_0}\right)^{n_\sigma} \tag{2.4}
\]

**Fluid flow properties**

TPMS structures are also interesting for their fluid flow properties thanks to their interconnected pore network. They are promising for applications in the energy, aerospace and biomedical engineering fields [37], [38]. Investigating their mass transport properties for biomedical applications, such as bone scaffolds for tissue engineering, has led to a rapid increase in permeability studies in the recent years [27], [39], [40]. Davoodi et al. [41] studied the permeability of lattice and skeletal-based structures and reported a porosity dependent behavior, with the latter being more permeable (Figure 2.6). The works of Karaman et al. [42] and Foroughi et al. [43] reported that skeletal-based scaffolds have higher permeability compared to sheet-based ones.

![Figure 2.6. Permeability values for different lattice and TPMS scaffolds. Image reproduced with permission of the rights holder [41].](image-url)
Among the studies of sheet-based TPMS structures found in the literature, Santos et al. [44] compared various approaches to calculate the experimental permeability of three TPMS architectures (Schwarz D, Gyroid, and Schwarz P) with a 3.25 mm cell size and a range of porosities from 50% to 80%. Subsequently, Pires et al. [45] validated those results with Computational Fluid Dynamics (CFD) simulations. Despite the discrepancies between computational and experimental results, the latter being about four times higher, a linear correlation was found between the two for all cases. Ma et al. [46] explored how different pore sizes (500-1300 μm) affected the permeability of Gyroid TPMS architecture both experimentally and computationally, yielding to experimental values that were half of those measured computationally. Ali et al. [47] carried out CFD simulations of Schwarz Primitive, Gyroid, F-RD and Double Diamond structures, considering fixed cell sizes for each structure of 1, 1.56, 1.84 and 2.54 mm respectively, a fixed thickness value and porosity of 80% for all. Different applications might require different pore sizes and porosities and previous studies have focused on analyzing permeability for a specific cell size, porosity or considering only one type of structure. As a consequence, a direct comparison cannot be done between most of the works that have studied the permeability of TPMS structures; therefore, a more parametric methodology to calculate permeability in TPMS structures is needed to obtain more general solutions.

Darcy’s law is usually used to measure the permeability of a laminar flow through a porous medium but it requires a previous experimental or computational study to obtain all the parameters required. Computational analyses are a great alternative to the costly and time-consuming experimental tests, but they can also be simplified into analytical models. An analytical model can predict the permeability of the structure avoiding the need of tedious modelling and simulating. It must be noted that the main difficulty behind CFD of TPMS structures is not the simulation itself, as the analysis is conducted under simple conditions; the real inconvenient is obtaining high-quality Computer-aided design (CAD) models and meshes for an optimal study, which can be very cumbersome in such geometrically complicated structures.
A similar analytical approach to the Gibson-Ashby model would also be desirable to describe the permeability of these structures. In porous media, permeability is critical as it provides a quantitative characterization of the ability of the fluid to flow through the porous network [48]. Measuring permeability is relevant in the design of scaffolds for bone tissue engineering, as it is of paramount importance to match the permeability of the scaffold to the one of the host tissue, as the diffusion of nutrient and cell proliferation and differentiation are directly related to the permeability of the scaffold [49], [50]. Different factors like the age of the patient [51], the density and load bearing capacity of the bone that is being replaced, the bone defect geometry or underlying conditions (such as arthritis or osteoporosis) cause variability in the targeted structural and permeability needs [52], [53].

An analytical permeability model would avoid the need of tiresome tests [54], [55], [56]. Some analytical models have been reported in the literature for solid-based TPMS structures but these models were fitted for one specific cell size. In those models the permeability was presented to be a power function of the porosity [41], [57], [58], [59]. Hagen-Poiseuille’s law is a simpler way to evaluate permeability in porous media, where the permeability is described by the medium properties and no further parameters are needed. This model has limitations as it simplifies the pore distribution of the medium into a series of unconnected tubes; and hence, it cannot be applied universally [60]. However, it can be hypothesized that a similar simplification to Hagen-Poiseuille’s law may be appropriate for TPMS architectures since their pore network can be reduced to a group of tubes crossing a volume due to the periodicity and interconnection of the pores.
2.2. Bone structure and bone replacements

2.2.1. Bone structure

Bones are complex biological organs with a porous hierarchical structure. Their primary functions are to support the mechanical loads and body movement as well as the protection of internal organs. They also serve as a storage for minerals and help in the endocrine regulation of the metabolism [61].

Bone’s hierarchical structure can be divided into different levels (Figure 2.7). At the macroscopic level, bones can be classified into cortical and trabecular bone. Cortical bones are dense and compact structures (3% to 12% porosity) formed by osteons, whereas trabecular bones have a highly porous structure (50% to 90%) formed by trabeculae [62]. Lamellae are the main components of both osteons and trabeculae and consist of aligned nanostructures named mineralized collagen fibrils. At the molecule level, two main components are found in the bone’s mineral composition: hydroxyapatite (HA) gives bones their strength and type I collagen provide them with flexibility [63].

Figure 2.7. Hierarchical organization of bone tissue. Image reproduced with permission of the rights holder [63].
Bone tissue undergoes a constant adaptation known as bone remodeling. Throughout the bone remodeling, opposite processes of bone formation and resorption take place to adapt its morphology and structure. Healthy individuals have similar rates of bone formation and resorption to maintain an unvaried bone balance. However, age and certain pathologies induce higher resorption levels than formation, resulting in a negative bone balance, and consequently, in bone loss [64].

Cortical and trabecular bones have different mechanical properties due to their structural differences. The stiffness values of cortical bone range between 3 GPa and 30 GPa, the compressive strength varies between 100 MPa and 230 MPa and its strain to failure is 1-3% [62], [65], [66]. On the other hand, trabecular bones exhibit values of stiffness between 0.02 GPa and 6 GPa, a compressive strength in the range 2-12 MPa and a strain to failure of 5-7% [62], [65], [66]. In addition, changes due to bone remodeling also affect bone’s mechanical behavior, reducing its properties with age or disease [67].

Bone regeneration is the process of tissue self-reconstruction after a bone defect. These defects can be caused by a number of reasons including severe fractures, trauma, infections and tumors [68]. Bone defects with a gap greater than 2.5 cm are considered critical size defects (CSD) [69]. They prevent bones from self-healing and require an additional support to regenerate. These defects have remained a surgical challenge, where in fractures such as the tibial diaphysis, the bone non-union rate is up to 21% of the cases [70]. Therefore, new bone replacement solutions need to be engineered to improve surgery success and patient comfort.

2.2.2. Orthopedic implants and bone tissue engineering

Orthopedic implants and bone tissue engineering scaffolds represent the forefront of medical innovation for addressing musculoskeletal disorders. Orthopedic implants are biocompatible medical devices designed to support or replace damaged bone structures by providing mechanical stability. They can range from plates and screws to total joints replacements such as hip, knee or shoulder (Figure 2.8). Bone tissue engineering explores the regeneration of tissue by combining cells, scaffolds and
nutrients (Figure 2.9). These two techniques have the potential to expand the boundaries of orthopedic treatment and to improve the quality of life for patients with orthopedic disorders.

![Fixing plates and screws (top), and hip and knee replacement systems (bottom) (Zimmer Biomet) [71].](image)

Figure 2.8. Fixing plates and screws (top), and hip and knee replacement systems (bottom) (Zimmer Biomet) [71].

![Additively manufactured stochastic scaffolds for bone tissue engineering.](image)

Figure 2.9. Additively manufactured stochastic scaffolds for bone tissue engineering.

The development of novel orthopedic implants is focused on guiding bone healing and inspiring innovative solutions in the orthopedic regenerative medicine field [72], [73]. Improving implant durability is important to decrease the number of revision procedures and limit healthcare costs [74]. Ulrich et al. [75] reported that out of 225 participants, half needed a revision surgery within 5 years following hip arthroplasty.
The most common reason for this prevalence was aseptic loosening, especially in older patients (>50 years-old), covering over 50% of the cases and highlighting the need to improve the long-time osteointegration and stability of the implants. Another significant drawback of orthopedic implants is stress shielding, which is produced by a stiffness mismatch between the implant and the surrounding bone [76]. The most common material used for biomedical implants is titanium (Ti) and its alloys because of their high biocompatibility and corrosion resistance. However, their elastic modulus (100-120 GPa) is much higher than the one of cancellous (0.02-6 GPa) and cortical bone (3-30 GPa) [65], [66].

Additively manufactured (AM) porous metallic structures have been proposed to address the lack of osteointegration in orthopedic implants [77], [78], [79] (Figure 2.10). Amongst their benefits, porous surfaces at the bone-implant interface enhance the biological interaction of the implant with the surrounding bone tissue. They can facilitate new bone tissue growth and support vascularization and nutrient transport, which are essential for bone ingrowth. Their effectiveness in promoting new bone depends on the pore shape, size, and overall porosity. Smaller pores allow for greater initial cell attachment, while larger pores are better for vascularization and nutrient delivery, enhancing bone formation in later stages [80]. Pore sizes larger than 250 μm are typically recommended, with some studies suggesting an optimal upper limit of 800 μm, although there is no definitive agreement on this in the scientific literature [81].

Figure 2.10. Design approach for additively manufactured porous hip implants. Image adapted with permission of the rights holder [57].
2.2.3. Bone tissue engineering

Bone tissue engineering (BTE) investigates state-of-the-art techniques to induce bone regeneration by combining the principles of biology and engineering. For BTE devices to be successful, three key factors need to be combined: cells, a matrix for the cells to settle and a bioreactor environment to provide the nutrients for cells to grow and proliferate [82]. Scaffolds for BTE offer a promising alternative to bone autografts, which are considered the gold standard. They can be custom-designed to match clinical needs, while autografts can cause pain to the patient and have limited availability [83]. The sought characteristics of bone scaffolds are to provide an optimal mechanical stability, to allow an adequate mass transport for cell proliferation and differentiation, and to promote tissue regeneration [84] (Figure 2.11).

![Figure 2.11. Design requirements for BTE scaffolds.](image)

The optimization of 3D printed bone scaffolds relies on controlling their mechanical properties to sustain the loads they are subjected to while satisfying the local biomechanical demand [77]. Many works have focused their study on geometrical parameters of lattice bone scaffolds [81], [85], [86], [87]. Triply periodic minimal surfaces (TPMS) have shown promising behavior for bone engineering applications in both their skeletal and sheet configurations [59], [88], [89], [90], [91]. Besides their outstanding mechanical performance, their architectural features may have a crucial impact on the diffusion of nutrients and the proliferation and differentiation of cells.
[12], [92], [93]. Also, their versatility allows to easily adapt their porosity and pore size to match a desired stiffness and permeability [94], [95].

A recurrent limitation in scaffold guided bone regeneration is the lack of bone formation in the core of the scaffold [96], [97]. The impact of different strategies to overcome this challenge has been addressed in the literature. Several works have investigated the implantation of pre-seeded scaffold to improve the performance of acellular scaffolds [98], [99], [100]. The strategies included stem cell therapy, growth factor delivery, or a combination of the two. Huang et al. [101] compared in an *in vivo* rat study the bone regeneration efficiency of a scaffold with and without pre-seeded bone marrow mesenchymal stromal cells and reported that osteogenesis was promoted with a pre-seeded scaffold. Secondly, the effect of increasing the healing period after the surgery was investigated. Li et al. [102] and Henkel et al. [103] studied the effect of extending the post-operative period to three weeks of rest before allowing major loads on the limb, resulting in an increase of the implant osseointegration. Yet, there is no a clear consensus on the optimal strategies to follow in each case.

**2.2.4. Patient-specific substitutes**

One of the primary limitations to tissue engineering products' clinical translation is intra-subject variability. Inconsistencies in their efficiency and outcomes are primarily due to uncertainties in patient response. Certain parameters such as age or health of the patient can affect both the mechanical properties of bones and the mechanosensitivity of the subject [104], [105], [106].

In preclinical models, testing small groups of young and healthy animals may overestimate a regenerative product's clinical efficacy [107]. Small ruminant models are often used in orthopedics research because of the resemblance of their stifle joint to the human knee and compatibilities in bone size and thickness with humans [108], [109], [110]. Recent studies have reported that aging affects the bone adaptive response of individuals [111]. Nafei et al. [112] studied the relationship between age and the mechanical properties of sheep bones. The work reported that older sheep
had values of elastic modulus up to 1.3 times higher than the studied case; while, younger sheep reported values up to 0.5 times lower. The works of Nasello et al. [113] and Sanz-Herrera et al. [114] investigated the accuracy of mechanosensitivity thresholds in translating clinical results into in silico mechanobiological models. These results highlight the significance of adapting the design variables of bone scaffolds to each patient’s physiological requirements in order to enhance their performance. Therefore, changes in the mechanical properties of the bone and the host’s mechanosensitivity require more in-depth research.
2.3. Mechanobiology and bone regeneration

The structural and material properties of bone undergo constant adaptation and remodeling in response to mechanical loading, resulting in changes in its microarchitecture [115]. Mechanobiology is an emerging field that involves the detailed analysis of how mechanical loads affect bone biology for which engineers and biologists collaborate to unravel this complex interplay [116], [117]. This interdisciplinary approach allows researchers to investigate and quantify how mechanical forces ranging from the macroscopic to the cellular and molecular levels, can influence cellular behavior and tissue development and repair. Experimental approaches such as animal models and cell culture methods produce mechanical signals that are converted into chemical and structural reactions. These responses are quantified using methods including biochemical tests, tissue marking and imaging. Mechanobiological theories use computational models to combine them all and simulate the in vivo and in vitro processes that occur within living organisms across different tissue scales (Figure 2.12) [118].

![Figure 2.12. Multiscale mechanobiology modeling. Image reproduced with permission of the rights holder [118].](image-url)
Scaffold design and material properties must be optimized to enhance the scaffold’s osteointegration and promote bone regeneration [117], [119], [120]. To effectively develop better bone substitute implants and scaffolds for bone tissue engineering, it is essential to thoroughly comprehend and control the various mechanical characteristics of the bone matrix and their biological importance to create biomimetic materials and bone-inspired solutions [121]. Adjusting the apparent stiffness of the scaffold is targeted as it is believed to be one factor that encourages bone ingrowth given the intrinsic relationship between mechanical stimulation and bone adaptation [122]. This requires an exhaustive study of the performance of different scaffold designs.

2.3.1. Bone mechanobiological models

Mechanobiological models are mathematical frameworks used to predict how bone cells respond to mechanical stimuli, leading to the adaptation of bone tissue. These models are able to characterize bone at organ, tissue and cell levels [115]. Amongst them, most characterize bone remodeling based on the mechanical stimuli at the organ level using macroscopic tissue strains to regulate bone response [123], [124]. Two prominent models are those developed by Huiskes et al. [123] and by Prendergast et al. [124]. The work of Huiskes et al. [123] proposed a model where bone remodeling is driven by the local differences in strain energy density (SED), suggesting that bone tissue adapts its structure to maintain an optimal strain energy density range. The work of Prendergast et al. [124] defined a model that related the biophysical stimuli of regenerating tissues to the cell differentiation. This model hypothesized that musculoskeletal tissue regeneration is a continuous process of tissue differentiation controlled separately by tissue shear strain and interstitial fluid flow at bone-implant interfaces.

Other models consider bone as a poroelastic material where in addition to macroscopic strains, they consider the effects of the interstitial fluid to regulate the stimulus [125]. Lacroix and Prendergast [126] modeled fracture healing by describing the tissue differentiation that takes place within the fracture callus based on the shear strain and the interstitial fluid flow. Additionally, some other models have coupled mechanical stimuli with agent based models accounting for cellular activities where
cell’s biochemical and bioregulation factors, such as oxygen supply and angiogenesis, are as important as mechanical ones [127], [128].

When studying scaffold induced bone regeneration, some models use their mechanical behavior (such as strain rate, SED or wall shear stress) under isolated tests (tensile, compression, fluid flow) to relate to the ideal values for bone regeneration (Figure 2.13). Multiscale models investigate the relationship between the mechanical and physical reactions inherent to the bone’s structure and its microarchitecture when in contact with scaffolds. They study the localized cell response due to deformations at tissue level resulting from the applied mechanical forces at the organ level (Figure 2.14) [113], [117].
A few recent works have studied the mechanobiological behavior induced by TMPS geometries. Kelly et al. [40] studied *in vivo* the effect of pore size of gyroid-sheet scaffolds in large femoral defects and concluded that scaffolds with smaller pores induced higher bone regeneration when no osteoinductive factors were added. In the work of Van hede et al. [129], the authors reported that sheet-based gyroid scaffolds are promising for the optimization of the internal design of intra-oral bone defects. Jaber et al. 2022 [130] studied and compared both *in silico* the bone regeneration potential of a strut-based scaffold and a sheet-based gyroid, reporting that the data did not indicate a better performance for gyroid scaffolds (Figure 2.15). Therefore, more studies are needed to assess the suitability of gyroid scaffolds for bone replacement applications.

Although some experimental and numerical works have studied the gyroid architecture in recent years [26], [130], [131], none of those works compared the potential to induce mechanobiologically-driven bone regeneration between sheet-based and skeletal-based TPMS architectures. These works showcase the need to further investigate complex porous structures for bone regeneration applications, with all the image-processing and computational difficulties that this entails.
Figure 2.15. Scaffold induced bone regeneration model of a large bone defect. Image reproduced with permission of the rights holder [130].

Computational modeling has become a common approach in addressing experimental difficulties and enhancing the comprehension of bone mechanobiology [52], [126], [132], [133]. The integration of computer simulation techniques and mathematical models has created numerous opportunities for better understanding the mechanobiological behavior of bone tissue [114], [134], [135]. The complexity of computational mechanobiology models is increasing as the field is progressing towards the use of patient-specific models in clinical settings. However, despite the progress, these approaches still require resolving challenges regarding the computational costs, and the size and intricacy of the CAD models. Consequently, there is still potential for improvement until these models are ready to be used safely in the clinical context [136].

As technology advances, *in silico* modelling will allow to use initially calibrated mechanobiological models to run infinite tests in a time and cost-efficient way, enabling the evaluation of potential treatment strategies, thereby diminishing the need for pre-clinical animal experiments [137].
Chapter 3.

Parametric design of TPMS structures

This chapter defines the main geometrical parameters of the Triply Period Minimal Surfaces (TPMS) studied in this dissertation and describes the methodology used for their design. Design parameterization is studied with the aim of automating and optimizing the process. Furthermore, the modelling for the structures used for the computational analyses in Chapters 4, 5 and 6 is described.
3.1. Geometrical parameters

TPMS geometries are characterized by certain geometric parameters that make them valuable for different applications. The most representative parameters of TPMS geometries are the type of configuration (sheet- or skeletal-based), $C_f$ parameter, cell size ($L$), pore size ($D_p$), relative density ($\rho^*$), porosity ($\phi$) and wall thickness ($t$) (Figure 3.1). The accurate definition of parameters such as pore size or wall thickness is critical for several applications and for understanding the manufacturability possibilities of these structures, but it is often unclear how to define such parameters [46]. The purpose of this section is to describe how to define these parameters to facilitate subsequent design parameterization and to ensure that the resulting structures meet the intended specifications.

The TPMS geometries investigated in this dissertation are Gyroid (G), Schwarz Primitive (SP), Fisher-Koch S (FKS) and Diamond (Dia), as they were found to have most interest for biomedical applications [91], [138]. Table 3.1 shows the implicit equations that define the surfaces of G, SP, FKS, and Dia, where $C_f$ represents the level-set constant. The isosurfaces divide the domain into two regions based on the offset parameter $C_f$. When $C_f$ equals 0, the main domain is divided by the TPMS surface into two subdomains of equal volume. For any other value of the $C_f$ parameter, the surface
is offset within the domain. The volume of one subdomain increases while the volume of the other decreases, as shown in Figure 3.2.

Table 3.1. Implicit equation and surface representation of each TPMS architecture.

<table>
<thead>
<tr>
<th>TPMS architecture</th>
<th>Equation ( f(x, y, z) = C_f )</th>
<th>Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>G ( G )</td>
<td>( \cos(x)\cdot\sin(y)+\cos(y)\cdot\sin(z)+\cos(z)\cdot\sin(x) )</td>
<td><img src="image" alt="G surface" /></td>
</tr>
<tr>
<td>SP ( SP )</td>
<td>( \cos(x)+\cos(y)+\cos(z) )</td>
<td><img src="image" alt="SP surface" /></td>
</tr>
<tr>
<td>FKS ( FKS )</td>
<td>( \cos(2x)\cdot\sin(y)\cdot\cos(z)+\cos(2y)\cdot\sin(z)\cdot\cos(x) +\cos(2z)\cdot\sin(x)\cdot\cos(y) )</td>
<td><img src="image" alt="FKS surface" /></td>
</tr>
<tr>
<td>Dia ( Dia )</td>
<td>( \sin(x)\cdot\sin(y)\cdot\sin(z)+\sin(x)\cdot\cos(y)\cdot\cos(z) +\cos(x)\cdot\sin(y)\cdot\cos(z)+\cos(x)\cdot\cos(y)\cdot\sin(z) )</td>
<td><img src="image" alt="Dia surface" /></td>
</tr>
</tbody>
</table>
Figure 3.2. Evolution of the volume distribution of each subdomain created by the isosurfaces evaluated at different level-sets ($C_f$).

The unit cell is defined as a single surface bounded by a cubic domain and periodic in the $x$-, $y$- and $z$-directions. The length of any side of the unit cell is represented by the cell size ($L$) (Figure 3.3).

Figure 3.3. Description of the unit cell design.

Wall thickness ($t$) describes the distance between one external surface of a geometry and its opposing parallel surface (Figure 3.1). Porosity ($\phi$) is defined as the volume of void within the unit cell domain (Eq. [3.1]) while the relative density ($\rho^*$) is the ratio between the volume occupied by the structure and the volume of the bounding box (Eq. [3.2]). Porosity and relative density are complementary variables associated as shown in Eq. [3.3].

$$\phi = \frac{V_{bounding \ box} - V_{structure}}{V_{bounding \ box}} \cdot 100 = \frac{V_{void}}{L^3} \cdot 100$$  \hspace{1cm} [3.1]
Cell size and wall thickness can be combined to design structures with different combinations of porosity and pore size. A structure of wider or narrower pores can be obtained by increasing or decreasing the cell size and wall thickness, maintaining the same porosity. This design approach is interesting when targeting a specific porosity (Figure 3.4).

Similarly, when comparing the performance of structures with varying porosities, it is common to maintain a regular cell size. An increase in porosity requires a decrease in wall thickness, and vice versa (Figure 3.5). Therefore, it is essential to find an optimal balance between cell size and wall thickness to design structures that are not only theoretically sound, but also manufacturable.
Figure 3.5. Representation of the change in porosity and pore size with wall thickness (t) for a given cell size (L).

Figure 3.6. Depiction of the pore size ($D_p$) and wall thickness (t) definition for each TPMS architecture in both configurations.
Wall thickness and pore size of scaffolds intended for bone regeneration resemble the trabecular bone thickness and trabecular bone spacing. Consequently, the pore size is considered a valuable reference parameter when designing this type of scaffolds as it ensures that they mimic the natural bone architecture, thereby facilitating an effective tissue integration and regeneration. Some works define the pore size as the sphere with the largest diameter that can fit into the pores of the structure [139], [140]. Since TPMS structures do not possess a uniform pore diameter, in this thesis, the pore size has been defined as the widest pore within the structure [93], [141] (Figure 3.6). This is also important in the fluid flow analysis (Chapters 4 and 5) because the pore size is directly related to the Reynolds number, and, by considering the widest pore, the most restrictive laminar flow condition is examined.

The main geometric parameters have already been described, and as will be discussed in Chapters 4 and 5, the mechanical properties and permeability are directly related to the porosity and pore diameter. TPMS structures can be optimized to target a specific mechanical property such as the elastic modulus. The Gibson-Ashby model [36], which is often used to parameterize the elastic modulus of lattice structures, is also suitable for TPMS structures [35]. Permeability is another mechanical property of interest in the design of porous structures intended for bone regeneration. Therefore, an analytical model for estimating the permeability of TPMS structures is studied in Chapter 5.

In turn, porosity and pore diameter are interrelated with the cell size and wall thickness. From a manufacturability standpoint, these latter two parameters are the ones to consider due to the limitations of additive manufacturing technologies. They limit the size of structures that can be effectively manufactured and are dependent on the manufacturing technology (Figure 3.7). For instance, it was found that to achieve a TPMS structure with a decent quality with polymeric materials, the unit cell size and wall thickness are limited to 5 mm and 0.3 mm respectively for PolyJet technology (Stratasys, Object Connex 3 260), in agreement with the results of Jones et al. [142]. Stereolithography (SLA) (Formlabs, Form3) was found to allow printing cell sizes that can go down up to 3 mm, similar to the results reported by Luo et al. [143], while the
minimum wall thickness achieved for fused deposition modelling (FDM) (Stratasys, F170) was 0.2 mm. However, the geometrical resolution was very poor compared to the previous technologies (Figure 3.7-A). Furthermore, these limits are a little lower for metallic materials (0.2–0.15 mm of wall thickness) using a powder bed fusion technology (PBF) as reported by Hameed et al. [144]. On the other hand, there are similar limitations with excessively large cell sizes, which may require internal supports to accommodate surface curvature. The limits for feasible scaffold dimensions depend on the constraints imposed by the manufacturing technology, the properties of the materials used, and the capabilities of the equipment. Therefore, further research is needed to refine these techniques and achieve smaller feature sizes.

Figure 3.7. (A) Differences in manufacturing resolution between PolyJet and FDM technologies. (B) TPMS structures printed with PBF technology.
3.2. Parametric study of TPMS structures

The design of TPMS structures involves numerous parameters derived from analytical functions. The relation between the different parameters is investigated to optimize the design process targeting a specific characteristic. First, porosity is parametrized as a function of: i) the $C_f$ parameter for skeletal-based structures, and ii) the cell size and wall thickness for sheet-based structures. Then, an analytical model to predict mechanical properties of TPMS structures is introduced. Finally, a design methodology of medical scaffolds satisfying mechanical, bone mimicking and manufacturing requirements is proposed.

In the design of skeletal-based TPMS structures, the $C_f$ parameter determines the porosity ($\phi$) of the structure. Therefore, a correlation between this parameter and the porosity was investigated. A function (Eq. [3.4]) was fitted where $c_1$ and $c_2$ are constants dependent on the TPMS architecture and sensitive to the resolution (30) used to fit the function. The resolution represents the number of mesh elements per unit cell, and thus, the accuracy and mean curvature ($H$) of the mesh. Figure 3.8 shows that at a resolution of 10 points, the mean curvature distribution has peak values and it is not uniform, while at 30 points, the mean curvature is more uniform. By defining a different resolution, the formulas remain the same but the coefficients need to be slightly adjusted. The obtained values were fitted with a minimum $R^2 = 0.98$ (Figure 3.9). The values of the coefficients can be found in Table A.1 of Appendix A.

![Figure 3.8. Mean curvature change when using two different resolutions: 10 (left) and 30 (right).](image)
Chapter 3

\[ C_f = c_1 \phi - c_2 \] [3.4]

\[ \rho^* = 1 - \frac{\phi}{100} = f(t, L) = c_3 \left( \frac{t}{L} \right) - c_4 \left( \frac{t}{L} \right)^2 \] [3.5]

Figure 3.9. Fitting of \( C_f \) parameter and porosity for each TPMS architecture.

In sheet-based TPMS structures, the \( C_f \) parameter is set to 0 and a thickness value (t) is assigned to the surface. To determine the required wall thickness based on the desired porosity value, a function was derived similar to the approaches used in the literature (Eq. [3.5]) \[145, 146, 147\]. This equation represents porosity (\( \phi \)) as a function of the wall thickness (t) and the cell size (L) ratio (Figure 3.10). The constants \( c_3 \) and \( c_4 \) are dependent on the TPMS architecture and using these functions it was possible to automatically obtain designs with porosity values within 1% of the required values. The fitted coefficients can be found in Table A.2 of Appendix A.
As previously discussed, the design of porous structures for bone regeneration can be optimized for specific mechanical properties (stiffness) and bone mimicking design (pore size), two of the most representative properties sought in this application. Simultaneously, cell size and wall thickness determine the manufacturability limits. Therefore, a design methodology has been optimized to satisfy all these requirements (Figure 3.11). This approach has been fitted to the Gyroid architecture but can be extended to any other TPMS. The Gyroid architecture was studied in more detail because it is the TPMS architecture that has shown the best potential for bone regeneration while also being possible to manufacture via additive manufacturing techniques [131], [148].

Figure 3.10. Multivariable plot combining the wall thickness, porosity and cell size fitted for sheet-based FKS, G and SP.
An elastic modulus similar to that of bone prevents mechanical shielding from occurring, as explained in Chapter 2.2.2. The Gibson-Ashby fit allows to calculate the adequate porosity of the scaffold for it to have the desired stiffness. Establishing parametric relations between the known geometrical requirements (porosity and pore size), the cell size can be obtained (Eq. [3.6]). This parametrization can be used for both configurations (skeletal and sheet). The three variables are linearly related and by knowing any two of those parameters, the third one can be obtained (Figure 3.12). The fitted coefficients can be found in Table A.3 of Appendix A.

\[ L = \frac{D_p}{c_5 \cdot \phi + c_6} \]  

[3.6]
Similarly, thickness of the sheet-based Gyroid ($t_{sh}$) can also be fitted as a function of pore size and porosity (Eq. [3.7]), whereas for the skeletal configuration the thickness ($t_{sk}$) depends on the cell and pore size (Eq. [3.8]). This is especially useful when designing structures that will be additively manufactured as explained in the previous Section. The fitted coefficients can be found in Table A.4 of Appendix A.

$$t_{sh} = (c_7 \cdot \phi + c_8) \cdot D_p$$ \hspace{1cm} [3.7]

$$t_{sk} = L - D_p$$ \hspace{1cm} [3.8]
3.3. Design methodology

All structures were designed using an in-house automated script combining Rhinoceros 6 (Robert McNeel & Associates, USA) with the Grasshopper plug-in (Figure 3.13). The script initially defines the TPMS surface within a cubic unit cell based on the desired TPMS implicit function (Figure 3.3), evaluated in the domain \([-\pi, \pi]\), and cell size (L). Subsequently, the structure configuration (sheet or skeletal) is selected and the porosity (\(\phi\)) is set.

![Figure 3.13. Overview of the Grasshopper script to design TPMS structures.](image)

3.3.1. Design of sheet-based TPMS structures

Sheet-based TPMS structures are designed by setting the level-set parameter (\(C_f\)) to 0. The structure is then extended and assembled by repeating the unit cell in the direction of the corresponding axis (x, y, z) (Figure 3.14-A). After merging the resulting surface, an extrusion of distance ‘t’ is then performed in both directions along the normal vector of the generated surface (Figure 3.14-B). A Boolean operation is performed on the mesh to set its shape as either a prism or cylinder. To perform these operations, the structures are over-dimensioned by one extra cell on each side. This results in a final structure with flat edges instead of the ones that would result from the extrusion. (Figure 3.15-A and B). Then a mesh refinement is performed in
Hypermesh 2021 (Altair Engineering Inc., USA) (Figure 3.15-C). This step is explained in more detail in Section 3.4.

Figure 3.14. Surface periodization (A) and extrusion along the normal direction (B) of the base surface of sheet-based structures.

Figure 3.15. Mesh refinement details. (A) Edges of the structure after the mesh extrusion. (B) Flatten edges after Boolean operations within the Grasshopper script. (C) Mesh cleanup and refinement.
3.3.2. Design of skeletal-based TPMS structures

Skeletal-based structures use the enclosed volume generated by offsetting the isosurface evaluated at the $C_f$ level-set. This changes the porosity of the generated structure (Figure 3.16-A). Similar to the sheet-based design, the isosurface is then repeated in space (x-, y- and z-directions) to achieve the desired dimensions. The bounding prism or cylinder is constructed to match final structure’s size and an intersection of both volumes is performed (Figure 3.16-B).

![Figure 3.16](image)
(A) Porosity variation of skeletal-based structures. (B) Volume intersection to generate the final structure.

Boolean operations on meshes are a complex procedure that often result in open surfaces or produce non-manifold edges (Figure 3.17). To address these problems, different approaches were taken within the same software or with the use of others. Within the same script, this issue was sometimes resolved by displacing the prism or cylinder an inconsequential distance in the range of $\pm 2 \, \mu m$. If the issue still persisted, the software Autodesk Netfabb (Autodesk Inc., USA) was used to perform the Boolean operation and a better outcome was frequently attained. As Autodesk Netfabb is a
software intended for 3D printing, it also incorporates a tool to check and fix small errors of the mesh such as intersecting triangles.

Figure 3.17. Open mesh after a Boolean operation (A) and the same structure with the resolved issue (B).
3.4. Models for Computer-Aided Engineering

The design methodology generates a low-quality surface mesh that in most of the cases is not suitable for computer-aided engineering applications (CAE) such as a Finite Element Analysis (FEA) or Computational Fluid Dynamics (CFD). Upon the completion of the design process of the structures, the next step is to prepare them for the analysis of their properties using computational tools.

The study of fluid dynamics within porous structures requires a focus on the void volume created by the pores. Therefore, the obtained 3D structure was subtracted from a rectangular prism to obtain the fluid domain (Figure 3.18). Two virtual volumes were created with different proportions depending on the hydrodynamic entry length (Eq. [3.9]), where Re is the Reynolds number and \( D_h \) is the hydraulic diameter. The inlet was extended to allow the flow to fully develop, and the outlet was extended to improve simulation convergence [149]. The final mesh was extracted in STL format for further CFD analysis. It is important to note that this mesh was obtained through an iterative process, as a high-quality mesh is required for CFD analysis, which can be a tedious task for complex geometries.

\[
L_h = 0.0575 \cdot \text{Re} \cdot D_h \quad [3.9]
\]

Figure 3.18. Fluid domain generation for CFD analysis.
The meshes used for FEA were exported as a stereolithography format (STL) and then imported into the software Hypermesh 2021 (Altair Engineering Inc., USA) for further processing to improve their quality (Figure 3.15-C). The meshes were rebuilt and the initial surface mesh (triangles) was turned into a volumetric mesh (tetrahedrons) (Figure 3.19). The outcome was exported as an Abaqus input file (inp) for further simulation in Abaqus 2020 (Dassault Systèmes, France).

Figure 3.19. Mesh rebuild from a surface (A) to a volume (B).

The final designs obtained through this process were then used as inputs for Chapters 4 and 5, where the structural and fluid flow properties were analyzed with FEA and CFD models, and Chapter 6, to explore the bone regeneration induce by porous scaffolds with FEA.
This chapter investigates the mechanical behavior of skeletal-based Triply Period Minimal Surfaces (TPMS) for their use as bone substitutes. Although sheet-based structures exhibit superior mechanical properties compared to skeletal-based ones, the latter are often studied because they are easier to manufacture. The structural behavior and fluid flow-induced wall shear stress are studied as they are both essential for developing bone substitutes that not only facilitate the transport of nutrients but also provide the requisite mechanical support. Both analyses are part of an uncoupled fluid-structure model to predict the tissue differentiation induced by different TPMS architectures. This prediction is based on mechanobiological models that estimate the primary stimulus felt by cells to differentiate into different tissues.

The structural properties are investigated through finite element analysis (FEA). Previously established analytical models (Gibson-Ashby curves) are used to predict elastic properties based on geometrical features. Then, a computational fluid dynamics (CFD) study is performed to obtain the shear stress (τ) distribution over the TPMS structures walls. Lastly, the properties inferred from the fluid flow and structural behavior of TPMS structures are then fed into the mechanobiological analysis. A predictive model is used to determine the primary tissue type — bone, cartilage or
fibrous tissue— that would undergo differentiation. This analysis enables to find the most promising scaffolds geometries that would lead to bone formation.
4.1. Materials and methods

This study analyzed three skeletal-based TPMS architectures: Gyroid (G), Fisher-Koch S (FKS), and Diamond (Dia) (Figure 4.1). These structures were selected to assess the influence of the scaffold morphology over the predicted tissue differentiation. Three porosities were studied ranging from 60% to 80% porosity. The tested scaffolds were prisms according to the ASTM D695 norm with dimensions of 12.7x12.7x25.4 mm compromising 4x4x8 cells. The models were designed and meshed as described in sections 3.3 and 3.4 of Chapter 3.

The mechanobiological assessment consists on an uncoupled fluid-structure analysis. The information from both FE and CFD analyses is extracted and combined to calculate the mechanobiological stimulus at the surface of the scaffold, which regulates tissue differentiation.

Figure 4.1. Representative unit cell models (80% porosity) of each skeletal-based TPMS architecture.

Figure 4.2. Schematic representation of the uncoupled fluid-structure mechanobiological model.
4.1.1. Finite element structural analysis

Structural mechanical properties were obtained via finite element analysis. Quasi-static elasto-plastic compression tests were simulated in ABAQUS 2020 (Dassault Systèmes, France). The material used was polycaprolactone (PCL) and the considered material properties are shown in Table 4.1 [150].

<table>
<thead>
<tr>
<th>Material</th>
<th>E (MPa)</th>
<th>ν</th>
<th>σy (MPa)</th>
<th>εy</th>
<th>σf (MPa)</th>
<th>εf</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL</td>
<td>430</td>
<td>0.3</td>
<td>17.75</td>
<td>0</td>
<td>113.39</td>
<td>1.33</td>
</tr>
</tbody>
</table>

The tests conditions considered that the structure was fixed on its bottom end and a 10% strain displacement control was set from the top to observe the plastic behavior. A mesh sensitivity analysis was performed and an average element size of 0.25 mm was selected for the 60% and 70% porosity models, and 0.15 mm for the 80% models. All the structures were meshed using first order tetrahedral elements (C3D4).

The reaction force (RF), von Misses stresses (σ) and strains (ε) were calculated. Values of the elastic moduli were calculated from the reaction force following Eq. [4.1], where A is the scaffold section and (ΔL/L) is the axial strain. The Gibson-Ashby analytical model (C_E, n_E) was fitted by calculating the normalized elastic moduli (E/E_0) for each

Figure 4.3. Boundary conditions of the FEA models.
relative density ($\rho/\rho_0$) (Eq. [4.2]). To perform a more accurate fitting, scaffolds with 90% porosity were added to the analysis.

$$E = \frac{RF}{\Delta L/L_t}$$  \[4.1\]

$$\frac{E}{E_0} = C_E \left( \frac{\rho}{\rho_0} \right)^{n_E}$$  \[4.2\]

Furthermore, values of the octahedral shear strain ($\gamma$) were calculated as per Eq. [4.3], where $\varepsilon_i$ corresponds to the principal strains.

$$\gamma = \frac{2}{3} \cdot \sqrt{(\varepsilon_1 - \varepsilon_2)^2 + (\varepsilon_2 - \varepsilon_3)^2 + (\varepsilon_3 - \varepsilon_1)^2}$$  \[4.3\]

### 4.1.2. Fluid flow-induced wall shear stress

The CFD study was conducted on ANSYS Fluent 2021 R2 (Ansys Inc., USA) using a model for an incompressible Newtonian fluid with constant density and viscosity and a fully developed flow obeying the Navier-Stokes equation:

$$\rho \frac{\partial u}{\partial t} - \mu \nabla^2 u + \rho (u \cdot \nabla) u + \nabla P = F, \nabla \cdot u = 0$$  \[4.4\]

where $\rho$ is the fluid density, $u$ is the velocity of the fluid, $t$ is the time, $\mu$ is the dynamic viscosity, $\nabla$ is the del operator, $P$ is the pressure and $F$ represents the force, which for this study is 0.

The selected fluid for the analysis was DMEM medium with a 1000 kg/m$^3$ density and 0.00145 kg/m·s viscosity [151], [152]. The inlet velocity was set to 0.1 mm/s as it is reported to be the recommended fluid velocity when investigating bone scaffolds [47], [153].

$$k_p = \frac{v \mu L_t}{\Delta P}$$  \[4.5\]

$$\tau = \mu \cdot \frac{dv}{dx}$$  \[4.6\]
Intrinsic permeability $k_p$ (m$^2$) was calculated using Darcy’s law (Eq. [4.5]) where $v$ is the inlet velocity, $\rho$ the density, $\mu$ the dynamic viscosity of the fluid, $D_p$ the pore size, $L$ the total length of the structure and $\Delta P$ the pressure drop in the model. The fluid shear stress ($\tau$) (Eq. [4.6]) is obtained directly from the CFD analysis of the fluid phase as an output variable, where $\mu$ and $(dv/dx)$ are the dynamic viscosity and the velocity gradient in the axial direction.

It is important to note that meshing these structures (4x4x8 unit cells) can be a time-consuming and resource-demanding process. Periodic boundary conditions (PBCs) are a promising tool to significantly reduce pre-processing and computational time. However, there may be differences in pressure values when analyzing the complete structures or structures with periodic boundary conditions. The sensitivity of using PBCs is later studied in Chapter 5.2.

The 3D models were meshed with poly-hexcore elements in Fluent Meshing and a mesh sensitivity analysis was performed to evaluate the independence of the mesh size. A poly-hexcore mesh was chosen because it provides a lower element count, higher mesh quality and better solver performance [154]. The models were meshed with a maximum element size of 0.3 mm and a minimum of 0.03 mm with a growth rate of 1.2.

**4.1.3. Tissue differentiation from a mechanobiological perspective**

The skeletal-based TPMS scaffolds were investigated from a mechanobiological perspective using the results from the studies detailed in sections 4.1.1 and 4.1.2. An adapted model [153], [155] based on the mechanobiological theory proposed by Prendergast et al. [124] was used to study the estimated tissue differentiation that would occur within the pores of the structures. The model estimates the cell stimulus ($S$) (Eq. [4.7]), where $a$ and $b$ are 0.0375 and 10 mPa respectively [153], [155]. The cell stimulus ($S$) is based on: i) the octahedral shear strain ($\gamma$) at 1% strain displacement and observing only the elastic behavior of the scaffold, and ii) the fluid shear stress ($\tau$) at 0.1 mm/s inlet velocity. The cell stimulus thresholds for tissue differentiation are presented in Table 4.2. The octahedral shear strain was computed from the results of
the structural analysis of the solid phase (Eq. [4.3]), and the fluid shear stress is obtained directly from the CFD analysis of the liquid phase (Eq. [4.6]).

\[ S = \frac{\gamma}{a} + \frac{\tau}{b} \]  

[4.7]

Table 4.2. Cell stimulus threshold for tissue differentiation [153], [155].

<table>
<thead>
<tr>
<th>Threshold</th>
<th>Differentiated tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>S ≤ 0.001 or S &gt; 6</td>
<td>No tissue differentiation</td>
</tr>
<tr>
<td>0.001 &lt; S ≤ 1</td>
<td>Bone</td>
</tr>
<tr>
<td>1 &lt; S ≤ 3</td>
<td>Cartilage</td>
</tr>
<tr>
<td>3 &lt; S ≤ 6</td>
<td>Fibrous tissue</td>
</tr>
</tbody>
</table>
4.2. Results

4.2.1. Mechanical analysis of TPMS structures

Structural properties

The distribution of von Mises stress demonstrates a predictable pattern consistent with a periodic structure, where the stress distribution is uniform across each unit cell and is replicated throughout the entire structure. Each TPMS geometry displays a distinct behavior, with a unique stress distribution and stress concentration points (Figure 4.4-A). For a given TPMS geometry, it was observed that lower porosities resulted in more evenly distributed stresses and less severe stress concentration points (Figure 4.4-B). In the specific case of G structures, the maximum stress ranged from 45 MPa for the 60% porosity model to 113 MPa for the 80% porosity model. The stress values were both above the yield stress of the material (17.75 MPa), indicating the presence of plastic deformation. A qualitative plastic deformation analysis was therefore performed to determine the percentage of the structure that undergoes plastic deformation and its location.

The results of the qualitative plastic behavior analysis demonstrated the distribution of the elements that exhibited plastic deformations within the scaffolds. A pattern consistent with periodic structures was observed across all TPMS architectures (Figure 4.5), with a higher incidence of plastic deformation observed in the lower porosity scaffolds (Figure 4.6).
The Gibson-Ashby fitting was applied to the three studied TPMS (G, FKS, and Dia) with $R^2 \geq 0.99$ (Figure 4.7). The fitted coefficients can be found in Table A.5 of Appendix A. The results indicate an increase in the normalized elastic moduli as relative density increases.
**Fluid flow properties**

The highest wall shear stress was observed in the narrower sections of the scaffold's pore channels. The peak wall shear stress ranged from 17.3 to 75.4 mPa across all designs. The values of average wall shear stress are displayed in Figure 4.8. Dia scaffolds show the highest average wall shear stress values, followed by FKS and G respectively. This behavior is consistent in all studied porosities. Figure 4.9 displays the wall shear stress profile shape on the inner surfaces of the scaffolds with 70% porosity.
The permeability was calculated from the obtained pressure drop values. Each structure showed an increase in permeability when the porosity increased (Figure 4.10). The permeability values ranged from $3.05 \times 10^{-9}$ to $19.5 \times 10^{-9} \text{ m}^2$ for all structures, and the highest permeability was observed for the G architecture, followed by Dia and FKS, respectively.
4.2.2. Scaffold mechanobiological analysis for tissue differentiation

The primary stimulus felt by the cells was predicted based on the fluid shear stress and octahedral shear strain (Figure 4.11). According to the model predictions, G scaffolds are the optimal choice for bone implant applications. It was observed that higher porosity levels can potentially lead to enhanced bone cell differentiation. FKS scaffolds also show good suitability for bone applications for porosities >70%, although lower porosities may induce cartilage tissue differentiation. Dia scaffolds were predicted to primarily promote cartilage growth.

![Graph showing fluid shear stress vs. octahedral shear strain](image)

Figure 4.11. Mechanobiological results for G, FKS and Dia at 60%, 70% and 80% porosity.
4.3. Discussion

4.3.1. Mechanical behavior of TPMS scaffolds

Structural properties

The structural analysis showed that the elastic modulus is highly dependent on porosity, increasing as porosity decreased in all geometries (Figure 4.12). FKS and G scaffolds had similar normalized elastic moduli, with FKS scaffolds being slightly higher for lower porosities, while Dia scaffolds had the lowest values, agreeing with the results reported in the literature [156], [157].

![Gibson-Ashby curve fitting for G, FKS and Dia.](image)

The comparison of all porosities within a single architecture revealed that lower porosity results in a more evenly distributed stress and less critical stress concentration points [32], [158]. This means that higher porosity models distribute the load less effectively and the stresses concentrate at the critical points, resulting in a lower percentage of the scaffold undergoing plastic deformations. Nevertheless, a more realistic analysis of this behavior would involve subjecting the scaffolds to controlled physiological loads rather than displacements. Likewise, the plastic behavior analysis is only a qualitative assessment and does not consider magnitudes. For a more accurate assessment, further investigation is needed performing a quantitative study.

The study found that the elastic moduli obtained for most of the structures were not within the desired range for trabecular bone (0.02-6 GPa) [65], [66]. To improve the mechanical performance of polycaprolactone, it could be combined with hydroxyapatite (HA) or tricalcium phosphate (TCP), which would increase their
stiffness to be closer to bone and also enhance osteointegration. Therefore, the analysis performed in Chapter 6 considers titanium (Ti) and a composite of polycaprolactone and tricalcium phosphate (PCL-TCP) as the scaffold materials.

**Fluid flow properties**

The obtained wall shear stress values for G and FKS architectures (2.7-11.6 mPa) are in the range described in the literature for osteogenic differentiation, as mesenchymal stem cells (MSC) begin osteogenic differentiation when exposed to average WSS values ranging from 0.1 to 10 mPa [47], [159]. The results also agree with the behavior reported in the literature for skeletal-based TPMS structures [42], [153]. Dia scaffolds, on the other hand, reported values to primarily promote cartilage differentiation. Likewise, the obtained permeability values (3.5-19.5x10^{-9} \text{ m}^2) agreed with other studies [29], [42] and are similar to bone’s permeability (0.01-12.1x10^{-9} \text{ m}^2) [160]. The highest permeability was found for G scaffolds, followed by FKS and Dia respectively.

Similar to the Gibson-Ashby fitting, some analytical models have been proposed in the literature to describe the permeability behavior of skeletal-based TPMS structures [41], [57], [58], [59]. These models estimate the permeability as a function of porosity (\(\phi\)) and two fitting parameters \(C_k\) and \(n_k\) (Eq. [4.8]) (Figure 4.13). The fitted coefficients can be found in Table A.6 of Appendix A. The results showed good agreement with the model as the \(R^2\) values of 0.96, 0.99 and 0.99 were found for G, FKS and Dia respectively. This model has only been investigated for skeletal-based TPMS, therefore a similar approach will be explored for sheet-based TPMS scaffolds in Chapter 5.

\[
k_p = C_k * \phi^{n_k}
\]  

[4.8]
Mechanical behavior of skeletal-based TPMS structures

4.3.2. Mechanobiological model for tissue engineering

Computational mechanobiological model correlates mechanical stimuli with cellular differentiation based on the scaffold structure. Scaffolds with uniform architecture, like TPMS based scaffolds, have morphological features that can tune both the mechanical loading response and the stimuli felt by cells on the scaffold surface. This insight could then be used to guide the design of scaffolds tailored for specific functions, such as promoting the differentiation of bone or cartilage tissue.

The analysis of the mechanobiological model indicates that among the studied geometries, G scaffolds are the most suitable TPMS for bone applications. This finding is consistent with the literature, as the Gyroid architecture is one of the most extensively studied TPMS [144], [161]. This is not only due to its good manufacturability but also from a mechanobiological perspective [130], [162]. Based on the obtained results for these structures, wall shear stress levels exhibit a more relevant role dictating the predominant tissue phenotype differentiation. In contrast, for a sole consideration of the octahedral shear strain, all examined structures would fall within the desired range for osteogenic differentiation. The results also support the data reported in the literature, which suggests that higher porosities are more likely to induce bone differentiation [130]. Chapter 6 comprehensively investigates the mechanobiological properties of G scaffolds based on the insights obtained from these results.
4.4. Conclusions

One of the challenges in bone tissue engineering is the design of structures and biomimetic materials suitable for the regeneration of different bone sites and adapted to patient requirements [63], [163]. TPMS structures have shown to have a promising behavior thanks to their ability to tune their mechanical and fluid flow response [40], [44], [45], [46], [47].

The structural properties of skeletal-based TPMS structures were examined using finite element analyses. Young moduli of PCL structures led to lower values than the ones reported for trabecular bone. The correlation between the apparent moduli and relative density followed a power-law, as reported by Gibson-Ashby.

To determine the average wall shear stress (τ) across the scaffold surfaces, computational fluid dynamics methods were utilized. The obtained wall shear stresses are in the range described for osteogenic differentiation. Analytical models similar to the Gibson-Ashby fitting are reported in the literature for skeletal-based TPMS and agree with the outcome results. Exploring the potential adaptation of these or other models for use with sheet-based scaffolds constitutes interesting prospective research. Therefore, the objective of Chapter 5 is to develop an analytical model for the prediction of the permeability of sheet-based TPMS scaffolds.

The insights gained from the fluid flow and the structural response of TPMS structures were integrated into the mechanobiological study. Only skeletal-based structures were included in this analysis. Future works could also include sheet-based structures and compare the performance of both.

This assessment reported that TPMS Gyroid seems to be the best suited architecture to promote bone growth. Therefore, Chapter 6 will investigate the mechanobiological behavior of TPMS Gyroid scaffolds and the use of materials with a stiffness higher than PCL for bone replacements.
Chapter 5.

Analytical model for the prediction of permeability of sheet-based TPMS structures

A comprehensive computational fluid dynamics (CFD) study is presented in this chapter. Previous studies have analyzed permeability of a few specific TPMS structures, yet differences in the type of TPMS architecture, cell/pore size or porosity hinder direct comparisons between studies hence limiting wider design possibilities. This chapter presents a novel analytical model for predicting permeability based solely on geometric properties. The model is based on a CFD framework and has been developed to be as general as possible, with TPMS structures with very different geometries being used (Gyroid, Fisher-Koch S and Schwartz Primitive). The model could open up new possibilities for design optimization, with special focus on bone replacement applications.
5.1. Materials and methods

5.1.1. Computational fluid flow study setup

The fluid flow behavior of three different sheet-based TPMS geometries were investigated: Fisher-Koch S (FKS), Gyroid (G) and Schwarz Primitive (SP) (Figure 5.1). Those three structures were chosen to represent a range of different geometries. The study initially focused on sheet-based TPMS structures because of their enhanced mechanical properties in comparison to skeletal-based [164], yet it could be expanded to skeletal structures in the future. For each architecture, five porosities (50%, 60%, 70%, 80% and 90%) and five cell sizes (1, 3, 5, 7.5 and 10 mm) were considered for a total of 75 models. Those porosities and cell sizes were chosen to obtain pore sizes in the order of magnitude of trabecular bone (pore sizes from 100 μm up to 1500 μm with porosities from 40% to 90%) [165], [166], [167]. The models were referred to according to their architecture, size and porosity (for example, FKS-80-5 represents a Fisher Koch S architecture with 80% porosity and a 5 mm cell size).

The studied structures (4x4x4 unit cells) were designed as described in Chapter 3 and the pore size ($D_p$) was defined as the widest pore found in the structure (Figure 5.1) similar to the methodology reported by Ali et al. [93]. The pore size is directly related to the Reynolds number, and, by considering the widest pore, the most restrictive laminar flow condition was studied.

![Figure 5.1. Cancellous bone sample (A) and representative unit cell models (80% porosity) of each TPMS architecture: (B) FKS, (C) G and (D) SP; defining the pore size $D_p$ for each architecture.](image-url)
The CFD study was conducted on ANSYS Fluent (Ansys Inc., USA), with the same methodology described in Chapter 4.1.2. The fluid was water (1000 kg/m³ density and 0.001 kg/m·s viscosity). The inlet velocity was adjusted to match a fixed Reynolds number of 1, with the objective of achieving a variable inlet velocity dependent on the model to ensure a laminar flow regime. Reynolds number threshold for laminar flow in porous media is considered to be \( \text{Re} = 1 \) (Eq. [5.1]), where \( v \) is the inlet velocity, \( \rho \) the density, \( \mu \) the dynamic viscosity of the fluid and \( D_p \) is the pore size [168]. The inlet velocity values are presented in Table A.7 of Appendix A.

\[
\text{Re} = \frac{v \rho D_p}{\mu} \quad [5.1]
\]

Intrinsic permeability \( k_p \) (m²) was calculated using Darcy’s law (Eq. [5.2]) where \( v \) is the inlet velocity, \( \rho \) the density, \( \mu \) the dynamic viscosity of the fluid, \( D_p \) the pore size, \( L_t \) the total length of the structure and \( \Delta P \) the pressure drop in the model. A dimensionless permeability was also computed by normalizing permeability with the unit cell cross sectional area (\( k_p/L_t^2 \)) for each structure [16], [169].

\[
k_p = \frac{v \mu L_t}{\Delta P} \quad [5.2]
\]

Meshing 4x4x4 structures can be a time-consuming process. This is due to the need for highly detailed initial geometry, resulting in a very large file with approximately \( 62 \times 10^6 \) elements that can take up to 4 days to import and mesh on a computer with 16 GB of RAM. Periodic boundary conditions (PBCs) are a promising tool to significantly reduce pre-processing and computational time, allowing for models with approximately \( 4.5 \times 10^6 \) elements. However, there may be differences in pressure values when analyzing the complete structures or structures with periodic boundary conditions (PBCs).

Given the repeatability of the structures, a configuration of 1x1x4 unit cells was considered, and PBCs were assigned in the \( x \) and \( y \) directions by projecting the lateral surfaces over the other one in the same axis. This simplification created an infinite structure in both directions. To consider the effects of the chamber walls on the PBCs...
models, the obtained pressure drop was then corrected by multiplying by a factor of 1.2 as reported by Pires et al. [45]. A no slip wall condition was applied and zero-gauge pressure was set on the outlet.

Two sensitivity analyses were performed for the estimation of the pressure drop: one to confirm that the use of periodic boundary conditions was an acceptable simplification; and a second one, to evaluate the number of unit cells required in the flow direction. To evaluate the influence of assigning periodic boundary conditions, the 1.2 factor proposed by Pires et al. was verified by comparing the results from three full 4x4x4 structures (G-50-3, SP-70-1, FKS-70-10) with their respective 1x1x4 structures with PBCs (Figure 5.2). The second analysis was performed on the G-80-5 structure with 2, 3, 4, 6 and 8 cells in the z-direction.

For each design, the obtained 3D structure was subtracted from a rectangular prism in order to obtain the fluid domain as described in Chapter 3.4 (Figure 5.3). A virtual volume was added, based on the hydrodynamic entry length, to extend the inlet and allow the flow to fully develop [149]; and another was added to the outlet to improve simulation convergence. The total length of the 1 mm cell size model was 9 mm, with 4 mm for the structure and two virtual volumes of 2.5 mm each (inlet and outlet). For the rest of the models the lengths varied in proportion.
The 3D models were meshed with poly-hexcore elements in Fluent Meshing (Figure 5.3) and a mesh sensitivity analysis was performed to evaluate the independence of the mesh size. A poly-hexcore mesh was chosen because it provides a lower element count, higher mesh quality and better solver performance [154]. The maximum element size was set to 0.1 mm and the minimum to 0.01 mm with a growth rate of 1.2 for the 1 mm cell size models. For the rest of the cell sizes, the element sizes were scaled proportionally for an equal total element count. Once these parameters were optimized, the 75 models were studied.

Figure 5.3. Definition of cell size (L), total structure length (L_t) and CFD setup with detail view of the mesh with poly-hexcore elements.

5.1.2. Analytical model for the prediction of permeability

Permeability describes the ease with which a fluid passes through a connected porous network, and as such it provides an idea of the suitability of the scaffold to allow the transport of nutrients within the bone. Since computing permeability is a tedious task, a similar simplification to the Hagen-Poiseuille’s law, which relates pore diameter to permeability, would be valuable for TPMS architectures.

According to Hagen-Poiseuille’s equation, permeability $k_p$ (m$^2$) can be expressed as:

$$k_p = C_0 \cdot d^2$$  \[5.3\]
where $C_0$ is a dimensionless constant that describes the configuration of the flow path and $d$ is the pore diameter. This model simplifies the complex pore network of the media into a series of tubes. Since TPMS structures can be ideally described that way thanks to their periodic properties and the interconnection of the pores, the hypothesis for this Chapter is that a similar analytical model will be possible for TPMS structures too.

This is why a characteristic equation was sought for each TPMS architecture in which the permeability $k_p$ ($m^2$) was described as a function of porosity $\phi$ (%) and pore size $D_p$ (mm). As explained in Chapter 3, these two parameters are relevant for the design of scaffolds intended for bone regeneration as they are related to the porosity of the bone and the trabecular bone spacing. In addition, the pore size is also relevant for the CFD analysis to calculate the Reynolds number and to determine the inlet velocity.

The schematic representation of the construction of the analytical model is shown in Figure 5.4. First, an equation was obtained by fitting the CFD permeability results with the measured pore sizes to obtain a coefficient $C_k$ for each porosity level (Eq. [5.4]); then, the $C_k$ parameter was generalized based on the porosity for each TPMS architecture; the final model allowed computing permeability based on porosity and pore size.

\[
k_p = C_k \cdot D_p^2 \quad [5.4]
\]

The difference between the computational ($k_p$) and analytical ($k_p'$) permeability values was calculated as shown in Eq. [5.5].

\[
\text{Difference} \, (\%) = \left| \frac{k_p - k_p'}{k_p} \right| \times 100 \quad [5.5]
\]
Statistical analysis

The statistical analysis was performed in R with RStudio (R v. 4.0.4 R Foundation for Statistical Computing, Austria). The permeability results ($k_p$) were fitted to obtain the parameter $C_k$ with a second order polynomial model (Eq. [5.4]), while the relation describing $C_k$ as a function of the porosity for each architecture was fitted with a linear model. The goodness of the fits was measured with the $R^2$ value (with significant $p < 0.05$). A confidence interval was also calculated for the linear models. The variations in the difference between computational and analytical permeabilities were presented in the form of mean difference ± standard deviation.
5.2. Results

5.2.1. CFD boundary conditions sensitivity

The analysis of the use of periodic boundary conditions resulted in a 11-fold decrease in the number of elements but underestimated the pressure drop. 1x1x4 models with PBCs of G-50-3, SP-70-1 and FKS-70-10 structures yielded differences of 1.16, 1.15 and 1.21 respectively when compared to respective full 4x4x4 models; thus, the 1.2 correction factor adopted by Pires et al. [45] was found to be appropriate also for this study.

Permeability increased with the number of cells in z-direction (Figure 5.5). There was an 8% difference between the values obtained with 2 and 4 unit cell models but the results stabilized from 4 cells onwards, showing differences of less than 2% when increasing the cell number to 8 unit cells. At the same time, the number of elements in the mesh also increased when increasing the cell number. The 8 unit cell model required 1.5 times more elements than the 4 unit cell one. Therefore, the optimal equilibrium between precision and computational efficiency was considered to be 4 unit cells.

Figure 5.5. Sensitivity analysis of the permeability variation \( k_p \) (m\(^2\)) according to the number of cells used in the flow direction (z) with the number of mesh elements in each model
5.2.2. Fluid flow dynamics of TPMS structures

For a given porosity, the pressure drop, velocity contour and streamlines depended on the architecture of the TPMS structure, as shown in Figure 5.6. This is a result that can be extrapolated to all porosities.

Figure 5.6. Pressure contours (Pa), velocity contours and velocity streamlines (m/s) for FKS-80-5 (A), G-80-5 (B) and SP-80-5 (C) simulations.
For a porosity of 80%, the SP exhibited the lowest pressure drop, indicating a less resistance to fluid flow. In contrast, FKS exhibited the greatest pressure drop, resulting in the greatest resistance to fluid flow. To gain further insight into their behavior, the velocity and streamline diagrams were investigated. The fluid exhibited acceleration when flowing through the pores and constricted areas and deceleration in wider zones. This phenomenon can be observed clearly for the SP architecture, where the fluid can pass through the structure in a relatively straight line due to the wide pores. In contrast, the streamlines in the FKS architecture follow a more tortuous path.

Figure 5.7. Permeability values $k_p$ in logarithmic scale calculated from the CFD simulations according to cell size $L$ and porosity $\phi$. 
The permeability results obtained on the CFD simulations are represented in Figure 5.7 and in Table A.8 of Appendix A. All three structures followed the same tendency as for a given cell size, bigger porosity translated into a bigger permeability; and permeability increased with cell size. Permeability values ranged between $1.53 \times 10^{-10}$ m$^2$ for FKS-50-1 and $3.49 \times 10^{-7}$ m$^2$ for SP-90-10. Figure 5.8 shows the normalized permeability by unit cell area for each structure, which led to one single value per architecture and porosity. It can be observed that in all cases permeability increased with porosity, with the SP architecture being the most permeable and the FKS the least on all porosities but the 50% porosity designs, where the G was the most permeable one. Differences in normalized permeability among the different architectures increased with porosity.

![Normalized permeability by unit cell area](image)

**Figure 5.8.** Normalized permeability by unit cell area $k_p/L^2$ (-) vs porosity $\phi$ (%)

### 5.2.3. Analytical model to estimate the permeability of TPMS scaffolds

The correlation between the permeability and pore size followed a second order polynomial curve for each porosity level of each architecture with $R^2 \geq 0.99$ and $p < 0.001$ in all cases (Figure 5.9-A-C). According to Eq. [5.4] this led to the determination of $C_k$ for each porosity level of each TPMS structure. At the same time, for each architecture, $C_k$ was found to be linearly correlated with porosity following Eq. [5.6], as shown in Figure 5.9-D. All three fittings had an $R^2 \geq 0.99$ and $p < 0.001$ (Table 5.1). Therefore, the analytical model was re-written as shown in Eq. [5.7] to calculate permeability as function of porosity, pore size, and two coefficients $C_{k1}$ and $C_{k2}$ whose values depended on the type of TPMS (Table 5.1). A difference between the numerical
and analytical results of 3.31 ± 2.88%, 1.68 ± 1.28% and 0.56 ± 0.43% was found for FKS, SP and G architectures respectively. The resulting model can also be presented as a surface plot fitted with the defined equations relating pore size, porosity and permeability for each architecture (Figure 5.10).

\[
C_k = C_{k1} \cdot \phi - C_{k2} \quad \text{[5.6]}
\]
\[
k_p = (C_{k1} \cdot \phi - C_{k2}) \cdot D_p^2 \quad \text{[5.7]}
\]

![Figure 5.9](image)

Figure 5.9. Correlation between the permeability \( k_p \) (m\(^2\)) and the pore size for FKS (A), G (B) and SP (C). (D) Linear fitting of the coefficients of the permeability curves according to porosity \( \phi \) (%) for each architecture.

<table>
<thead>
<tr>
<th>Table 5.1. Obtained coefficients ( C_{k1} ) and ( C_{k2} ) for the analytical model, with p-value and 95% confidence interval</th>
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<tbody>
<tr>
<td>Coefficients</td>
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<td>FKS</td>
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<td>( C_{k1} )</td>
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<td>( C_{k2} )</td>
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Analytical model for the prediction of permeability of sheet-based TPMS structures

Figure 5.10. Multivariable plot combining the pore size, porosity and permeability fitted with the reported equation for FKS, G and SP
5.3. Discussion

5.3.1. TPMS fluid flow properties

In this study, permeability of a wide range of TPMS structures was computationally calculated using CFD techniques. 75 different designs were studied, with three different sheet-based TPMS architectures (FKS, G, SP), five cell sizes (1, 3, 5, 7.5 and 10 mm) and five levels of porosity (50%, 60%, 70%, 80% and 90%), which led to pore sizes ranging from 0.32 mm for G-50-1 to 8.50 mm for SP-90-10.

The permeability values obtained computationally agreed with previous numerical studies. Ali et al. [47] reported values of permeability of $5.36 \times 10^{-9}$ m$^2$ for G-1.56-80 and $2.48 \times 10^{-9}$ m$^2$ for SP-80-1 models; while in this work, values of $4.90 \times 10^{-9}$ m$^2$ and $2.52 \times 10^{-9}$ m$^2$ were obtained for the G-80-1.56 and SP-80-1 models respectively. Pires et al. [45] obtained approximate values of permeability of $5 \times 10^{-9}$ m$^2$, $1.2 \times 10^{-8}$ m$^2$, $8.5 \times 10^{-9}$ m$^2$ and $1.25 \times 10^{-8}$ m$^2$ for SP60, SP70, G60 and G70 respectively, considering a 3.25 mm cell size. Ma et al. [46] obtained values of permeability for a G architecture in the range of $0.64 \times 10^{-9}$-$5.33 \times 10^{-9}$ m$^2$ considering a 2x2x2 unit cell structure with pore sizes from 500μm to 1300μm and porosities from 75.1% to 88.8%. A more extensive comparison with those and other works was not possible due to differences in the considered cell sizes, porosities, or even the number of cells considered in the flow direction as it was found in this work to be an influential parameter. Despite these differences, the reported values are all in the same order of magnitude as the ones obtained in this work.

Although only three specific architectures were analyzed, we consider that the methodology can be extended to any sheet-based TPMS, as the studied structures have quite a different architecture (Figure 5.1). The normalized permeability proved that each architecture behaves differently when a fluid flows through their pore network, displaying different permeability properties. The obtained permeability increased with porosity at different rates for each architecture, highlighting the importance of pore distribution and architecture (Figure 5.7). As porosity increased permeability also increased, reaching values at 90% porosity which were 6.4, 4.3 and
6.2 times bigger than those for 50% porosity, for FKS, G and SP respectively. Among the three types of TPMS structures studied here, SP architecture showed to be the most permeable one in most of the analyzed cases. However, for the 50% porosity models, G was the most permeable one. This change might be due to the pore distribution, as in the SP, there is one main pore in the center of the surface and smaller pores on the corners. Because of these geometric properties, the behavior differs when porosity increases from 50% to 90%. With 50% porosity, the central pore in SP is narrower and the wall perpendicular to the flow gets thicker than in FKS and G, causing the flow path to deviate more. This creates an obstruction of the flow and consequently, the pressure drop is higher, making the structure less permeable (Figure 5.11). This phenomenon does not occur with the FKS and G structures as their pore distribution does not cause big changes in the flow path depending on the porosity. This implies that, to fully comprehend the behavior of a TPMS architecture, a wide analysis is needed, including different cell sizes and porosities.

Figure 5.11. Detail of the flow path in FKS, G and SP (from left to right) with 90% (top) and 50% (bottom) porosities in perspective view (A) and frontal view of the streamlines in SP comparing the 90% porosity model (left) with the 50% porosity model (B).
A comparison between the permeability results of the skeletal and sheet-based architectures analyzed in both Chapter 4 and this chapter was also performed. It was observed that both configurations have permeabilities within the same range, and that skeletal-based structures have higher values, which is consistent with the data reported in the literature [42], [43].

5.3.2. Analytical models to estimate the permeability of TPMS scaffolds

One of the main contributions of this thesis is the developed analytical model, which is adaptable to cell size and porosity. Based on the computational permeability values and adapting Hagen-Poiseuille’s law, a novel methodology to build analytical models for the calculation of permeability of TPMS structures was suggested. To the best of the author’s knowledge, this is the first time that such general methodology for TPMS structures is defined.

The analytical model showed to be a good tool to predict the numerical permeability of the studied TPMS structures within a difference < 5% in all cases. Among the benefits of the model, it must be highlighted that it allows to have a first approximation of the required configuration of the structure in terms of size and porosity for a specific application. An immediate use for the methodology could be the design of scaffolds for bone regeneration: for example, a permeability of 2.79 ± 1.91 $\times 10^{-9}$ m$^2$ and 8.05 ± 4.75 $\times 10^{-9}$ m$^2$ [160] is desirable for proximal femur and vertebral body respectively. The reported pore sizes vary from 100 μm up to 1500 μm with porosities from 40% to 90% [165], [166], [167]. This data can be used to design a variety of scaffolds suitable to meet the permeability requirements depending on the mechanical stability sought (Figure 5.12). Likewise, the mechanical stability can be estimated analytically using Gibson-Ashby fittings.
Various analytical models have been reported in the literature to describe the permeability behavior of skeletal-based TPMS structures [41], [57], [58], [59]. These models are limited to one cell size as the unit cell size is fixed, and permeability is described as a function of porosity ($\phi$) and two fitting parameters $C_k$ and $n_k$ (Eq. [5.8]), similar to Eq. [5.4] used in this work, where permeability was described as a function of pore size. However, in the current study the $C_k$ parameter has been generalized to consider all porosities; hence, a model that considers all the design variables has been defined. This model is unique to each architecture and describes all the possible models resulting from considering any porosity and pore size, which are relevant
parameters in the design of bone scaffolds. It must also be noted that, as opposed to the variety found in solid-based TPMS structures, the exponent $n_k$ in Eq. [5.8] has yielded a fixed value of 2 for all three sheet-based architectures, highlighting the different permeability behavior of solid and sheet-based structures.

$$k_p = C_k \cdot \phi^{n_k}$$

[5.8]
5.4. Conclusions

The influence of different CFD modelling parameters on the computed permeability values was explored and a simplified procedure was defined. This will facilitate further CFD studies of these complex structures and will help derive the permeability model constants for other TPMS architectures. It was found that, depending on the porosity level, the relative differences in permeability between TPMS architectures vary. This highlights the importance of studying a wide range of design parameters prior to the choice of the optimal TPMS architecture for the intended application.

Analytical models describing the mechanical behavior of TPMS structures as a function of porosity already exist in the literature [25], [94], [170], [171]. A novel analytical model to determine the permeability of TPMS structures based on the porosity and pore size was developed. This analytical model could be implemented in combination with analytical models for the structural behavior (Gibson-Ashby) which also depends on the porosity of the structure. Together, they could serve as input for topological optimization codes, which could make use of artificial intelligence (AI) and machine learning (ML) techniques [172], for applications where stiffness and permeability must be optimized [173], [174].
Chapter 6.

Cellular porous structures for bone regeneration

In the design of novel bone substitutes different strategies can be followed to mimic the surrounding bone tissue. The first approximation is to match geometrical features, such as pore size or porosity while accounting for manufacturability (Chapter 3). Since bone substitutes need to enable osseointegration and/or bone regeneration while also providing structural stability, the next step includes a thorough analysis of their mechanical and fluid-flow behavior (Chapters 4 and 5). Once the best suited designs have been identified from separate mechanical and fluid-flow analysis (gyroid), mechanobiological models offer the advantage to incorporate the interaction between the scaffold and the newly regenerated surrounding bone tissue.

The overall aim of this chapter is to provide a framework to design and evaluate strategies to promote bone regeneration based on a previously validated mechanobiological model. This mechano-driven model has been applied to investigate the bone regeneration potential of TPMS Gyroid scaffolds, which have been identified in previous chapters as good candidates to act as bone substitutes. Two different biocompatible materials have been studied Titanium and PCL-TCP, which are both manufacturable via additive techniques. Furthermore, the model has been used to evaluate the impact on bone regeneration of different clinical strategies that have
been suggested in the literature, such as pre-seeding the implanted scaffold or increasing the post-operative resting period. Finally, with the aim of simulating patient-specific differences, possible effects of age on the bone regeneration potential have been explored. Therefore, this chapter provides a comprehensive comparison of the bone regeneration potential of complex porous scaffolds considering architecture, material and clinical strategies for patient-specific applications.
6.1. Materials and Methods

6.1.1. Mechanobiological model for bone regeneration

A previously developed *in silico* model for mechanically driven bone regeneration [113] was adapted to assess the bone ingrowth induced by complex porous structures. The work of Nasello et al. [113] developed a subject-specific Finite Element (FE) set-up, where the model was fitted with *in vivo* goat data. On the *in vivo* experiment, a porous simple scaffold was inserted in the epiphyseal area of a goat tibia and the bone regeneration was quantified over a period of 12 weeks.

The computational FE model simulated the experimental conditions. The volume inside the scaffold’s pores was considered as granulation tissue, meaning that bone could be formed within this volume. The model simulated an initial granulation tissue with near-zero density and elastic modulus. To account for new bone ingrowth, both the cell concentration and the mechanical stimulus needed to be within the desired range for bone formation (Figure 6.1).

![Scaffold and Granulation tissue](image)

Figure 6.1. Representation of the granulation tissue and the condition for the update of its mechanical properties.

Bone formation was modeled using a mathematical algorithm that updated the mechanical properties (density and elastic modulus) of the granulation tissue considering both the cellular invasion (diffusion-led cellular concentration) and the mechanical stimulation (tissue strain) (Figure 6.2). The mechano-regulation algorithm...
predicted the tissue differentiation taking place in the pores of the scaffold and updated the values of cell concentration and tissue density iteratively.

Figure 6.2. Overview of the mechanically regulated bone regeneration model. The model considered a simultaneous process of cell diffusion and bone formation to update the mechanical properties of the granulation tissue.

A diffusion process was considered to model the cell migration from the tibia to the granulation tissue, where $t$, $x$ and $D$ are time, space and the diffusion constant respectively (Eq. [6.1]). Cell concentration ($\bar{c}$) was normalized and considered to be 1 at the outer region of the granulation part (tibia-granulation interface) and zero in the scaffold pores.

$$\frac{\partial \bar{c}}{\partial t} = D \cdot \frac{\partial^2 \bar{c}}{\partial x^2}$$  \[6.1\]

To update the mechanical properties of the granulation tissue, the effective tissue microstrains ($\bar{\varepsilon}$) were obtained from the strain energy density ($U$) and the elastic modulus ($E$) (Eq. [6.2]). The daily mechanical stimulus ($\Psi$) (Eq. [6.3]) was then calculated from the strains during the daily load cycles ($n = 10000$), where $m$ is an empirical parameter that quantifies the importance of the number of cycles ($m=4$) [114]; and regulated the bone deposition rate ($\dot{V}$) (Figure 6.2) [113]. The model accounted for two variable parameters representing (1) the mechanosensitivity ($M_s$) that affects the bone deposition rate ($\dot{V}$) and (2) a reduction factor ($\alpha$) for the local
mechanical stimulus in the implant region ($\Psi_{\text{local}}$) that varied based on the implantation site, linearly adjusting $\dot{V}$ from 0 to a maximum rate ($\dot{V}_{\text{max}}$) (Eq.[6.4]) (Figure 6.3). The considered $M_S$ parameter was $1 \cdot 10^{-4} \, [% \cdot \text{μstrains}^{-1} \cdot \text{day}^{-1}]$ [114], [175]; and the reduction factor was set at 50% as fitted to the experimental values [113].

$$\bar{\varepsilon} = \sqrt{\frac{2 \cdot U}{E} \cdot 10^6}$$  \hspace{1cm} [6.2]$$

$$\Psi = \sum_{i=1}^{N} (n_i \cdot \bar{\varepsilon}^m)^{\frac{1}{m}}$$  \hspace{1cm} [6.3]$$

$$\dot{V} = \begin{cases} 0 & \Psi \leq \alpha \cdot \Psi_{\text{local}} \\ M_S \cdot (\Psi - \alpha \cdot \Psi_{\text{local}}) & \Psi > \alpha \cdot \Psi_{\text{local}} \\ \dot{V}_{\text{max}} & M_S \cdot (\Psi - \alpha \cdot \Psi_{\text{local}}) \geq \dot{V}_{\text{max}} \end{cases}$$  \hspace{1cm} [6.4]$$

![Figure 6.3. Correlation between the bone deposition rate ($\dot{V}$) and the mechanical stimulus ($\Psi$) representing the mechanosensitivity ($M_S$) and reduction factor ($\alpha$).](image)

The material properties of the newly formed tissue and the normalized cell concentration ($\bar{c}$) in the granulation tissue were updated after each iteration with a UMAT and UMATHT subroutines in Abaqus respectively. The initial density of the granulation tissue was set to 0.001 g/cm$^3$ and the Poisson’s ratio to 0.3. The elastic modulus was calculated using a correlation between the apparent density and the
Young’s modulus based on existing literature on ovine bone [122]. This correlation was determined by transforming the Hounsfield Units (HU) from the CT images into apparent density in Bonemat (Bonemat v3.2) [176]. The density of the granulation tissue was updated based on Eq. [6.5] and Eq. [6.6] where $\rho_{\text{max}}$ was set to $1.6 \text{ g/cm}^3$ [122].

$$\dot{\rho} = \ddot{c} \cdot \dot{V} \cdot \rho_{\text{max}}$$  \hspace{1cm} [6.5]

$$\rho = \rho + \dot{\rho} \cdot \Delta t$$  \hspace{1cm} [6.6]

### 6.1.2. In silico mechanobiology model set-up

**Tibia modeling and boundary conditions**

The FEM study was performed in ABAQUS 2020 (Dassault Systèmes, France) with a linear stress analysis and using user subroutines (UMAT and UMATHT). The model considered a porous scaffold was inserted in the epiphyseal area of the tibia, to mimic the experimental setup. The tibia was trimmed 10 cm from the proximal region. The trimmed diaphysis had a restricted displacement in the axial direction and 4 nodes along its circumference were tied in all directions. The knee center was tied in the axial and the antero-posterior directions to simulate the ligament insertion [177] (Figure 6.4). The study considered a 3.7 year old goat with a weight of 65 kg and the applied loads were scaled from the animal body weight and distributed over the tibial lateral and medial plateaus [178], [179] (Figure 6.4) (Table 6.1). Figure 6.5 and Figure 6.6 present detail views of the model boundary conditions. As explained earlier, the granulation tissue was defined as the volume inside of the scaffold’s pores (Figure 6.4) and all the interacting surfaces between the tibia, scaffold and granulation part were modeled with a tie constraint.

<table>
<thead>
<tr>
<th>Forces on the medial plateau (N)</th>
<th>Forces on the lateral plateau (N)</th>
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<tbody>
<tr>
<td>Axial A-P M-L</td>
<td>Axial A-P M-L</td>
</tr>
<tr>
<td>-911 -82 -66</td>
<td>-461 -107 13</td>
</tr>
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</table>

Table 6.1. Total applied forces (N) and their directions (axial, antero-posterior and medial-lateral) over the nodes at the medial and lateral plateaus.
Figure 6.4. Model overview with part components and boundary conditions. The magnitude and distribution of the loads is represented in Table 6.1.

Figure 6.5. Diaphysis contact (restricted axial translation) and tied nodes (red).

Figure 6.6. Knee center boundary condition (restricted anteroposterior and axial translations) and load over the medial and lateral plateaus.
The material properties of the tibia were inferred from CT images of the goat tibia used in the experimental validation [180]. These properties were determined with the same correlations previously described for the granulation tissue modeling.

**Scaffold and granulation domain modeling**

Five different cylindrical scaffolds were analyzed in this chapter. The previously experimentally tested scaffold (dodecahedron unit cell) [113] was compared to a Gyroid TPMS geometry. The Gyroid architecture was selected based on findings from Chapters 4 and 5, which demonstrated its potential as an effective bone substitute. It not only mimics the complex geometry of trabecular bone but also encourages bone differentiation and exhibits permeability comparable to natural bone. Additionally, it can be fabricated using metal or polymer additive manufacturing techniques, making it a practical choice for manufacturing. In addition, previous chapters have explored TPMS structures in their skeletal-based (Chapter 4) and sheet-based (Chapter 5) configurations, yet no clear consensus is reached in the literature regarding the superiority of one over the other [26], [29]. Therefore, in this chapter, both sheet and skeletal-based configurations are explored and compared. The geometries were generated in Rhinoceros 6 (Robert McNeel & Associates, USA) with the use of an in-house code in Grasshopper plug-in, following the design methodology shown in Chapter 3. Two porosities (70% and 90%) were considered, 70% to match the initial porosity of the scaffold used in the experimental setup, and 90% to represent the higher limit of trabecular bone porosity [65]. Each scaffold was referred to according to its architecture and porosity. The unit cell size (L) was 1.9 mm and the structures were 8 mm in diameter and 12 mm in height. All the abbreviated names can be found in Figure 6.7. The granulation domain was built by filling the void of the pores of each scaffold (Figure 6.4). Both parts were then meshed in Hypermesh 2021 (Altair, USA) as described in Chapter 3.
As explained previously, TPMS geometries have a complex architecture and possess curved surfaces that need a fine mesh to be accurately characterized, which considerably increases the computational cost. A mesh sensitivity analysis was carried out to find the optimal balance between results accuracy and computational time. Four different mesh sizes were studied using a computer with 64 GB RAM and an 8 core CPU Intel i7-10700X 2.90GHz.

The maximum edge length of the studied meshes of the granulation part were 0.3, 0.25, 0.2 and 0.15 mm; resulting in meshes with an element count ranging from 300,000 to 705,000 elements respectively. The computational time varied from 5h (0.3 mm) to 10.5h (0.15 mm). The difference in the results between the 0.15 mm and the 0.2 mm mesh was under 5% (Figure 6.8), and increasing the number of elements further makes the handling of the mesh very complicated as the resulting file size becomes too big to be postprocessed with ease. Therefore, considering the computational time and accuracy of the results, it was decided that the 0.15 mm mesh was appropriate.
The element type used for the simulation were first order tetrahedral elements (C3D4) for the tibia and scaffold parts and hybrid displacement-temperature first order tetrahedral elements (C3D4T) for the granulation part.

**Scaffold and granulation tissue material properties**

The performance of the designed scaffolds was studied for two different biocompatible materials with different stiffnesses (Table 6.2). The stiffer material was titanium (Ti) as it is one of the most used materials for metallic medical implants. However, the mismatch in the mechanical properties of Ti compared to bone can give rise to stress shielding and a loosening of the implant. This is why medical-grade polycaprolactone and tricalcium phosphate (PCL-TCP) was also considered in the study, due to its wide use for biomedical applications [181], [182].

Table 6.2. Scaffold material properties used in the FE model.

<table>
<thead>
<tr>
<th>Material</th>
<th>Elastic modulus (MPa)</th>
<th>Poisson ratio</th>
<th>Yield strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti</td>
<td>114000</td>
<td>0.3</td>
<td>999&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCL-TCP</td>
<td>400</td>
<td>0.3</td>
<td>12.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a. [183]  
b. [184]

The stress distribution of the scaffolds was studied to ensure that both materials provided enough structural integrity and no structure surpassed the material’s elastic limit [183], [184]. Also, a histology like figure predicting the types of differentiated
tissue was obtained based on their elastic modulus. The different thresholds were adjusted based on the values found in the work of Kelly and Prendergast [185] (Table 6.3).

Table 6.3. Elastic modulus thresholds for histological tissue differentiation [185].

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Granulation tissue</th>
<th>Fibrous tissue</th>
<th>Cartilage</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$ (MPa)</td>
<td>0.2</td>
<td>2</td>
<td>10</td>
<td>1000</td>
</tr>
</tbody>
</table>

6.1.3. Bone ingrowth quantification

A 0.25 g/cm$^3$ was considered to be the density threshold to consider the newly formed tissue as trabecular bone [122]. The condition for the tissue density changes at each element of the granulation tissue was dependent on the cell concentration and the strain stimulus at said element. The normalized cell concentration was analyzed in the range of 0 to 1 to observe the effect of the scaffold geometry on the cellular diffusion process. The evolution of the strain stimulus was observed as the bone density and elastic modulus of the newly formed bone were updated throughout the simulation period. The bone ingrowth volume ($BV$) was obtained by adding up the element volume of all the mesh elements with a density above the threshold to be considered bone (0.25 g/cm$^3$). To calculate the relative bone ingrowth ($BI_{rel}$), the volume of newly formed bone into the scaffolds ($BV$) was measured and divided by the total defect volume ($TV$) minus the scaffold volume ($SV$) in the *in silico* model (Eq. [6.7]). The total bone ingrowth volume percentage ($BI_{tot}$) was also obtained as $BV/TV$ analogous to the measure used clinically (Eq. [6.8]). Three different subregions of the scaffolds were discretized to perform a more refined bone quantification. The medullary, middle and periosteal subregions were 3.7 mm each with the latter being the closest to the external surface of the bone (R1-3 in Figure 6.9).

$$BI_{rel} = \frac{BV}{(TV - SV)} \cdot 100 \quad [6.7]$$

$$BI_{tot} = \frac{BV}{TV} \cdot 100 \quad [6.8]$$
6.1.4. Clinical strategies for enhancing bone ingrowth

As stated in Chapter 2.2.3, the lack of bone formation in the core of the scaffold is a recurrent limitation in the design of scaffold for bone regeneration [96], [97]. This chapter analyses the impact of two strategies to overcome this limitation. Firstly, the effect of increasing the healing period after the surgery was investigated. The effect of extending the post-operative period to three weeks of rest before allowing major loads on the limb has been reported to increase the implant osseointegration [102], [103]. Secondly, the implantation of a pre-seeded scaffold was studied [98], [99]. To improve the performance of acellular scaffolds, strategies such as stem cell therapy, growth factor delivery, or a combination of the two are frequently investigated [98], [100]. Therefore, the effect of a longer post-operative resting period and the implantation of a pre-seeded scaffold were studied to observe if the bone formation at the core of the scaffold could be enhanced. For the post-operative resting period, three weeks before allowing major loads on the limb were examined based on other experimental findings [102], [103]. For the pre-seeded scaffold, in addition to the already considered cell invasion, a diffusion process from the scaffold’s surface to the granulation tissue was modeled with a normalized cell concentration of 1 at the surface. The effect of the pre-seeded scaffold and the three-week resting period were analyzed only in one of the models (D70) but both materials (Ti and PCL-TCP) were considered.
6.1.5. Effect of patient-specific changes in bone ingrowth

To assess the influence of patient-specific parameters, four hypothetical cases were compared by scaling the bone mechanical properties by factors of 1.25 and 0.75, following the findings of Nafei et al. [112] and by modifying the subject’s mechanoregulation factor of bone deposition rate ($M_{S1}, M_{S2}$), according to the values investigated in the literature [113], [114] (Figure 6.10). The case studies considered (1) an elderly subject with stiffer bones by scaling the bone material properties by 1.25, (2) the same elderly subject with a lower mechanoregulation factor ($M_{S2} = 5 \times 10^{-5} \text{[\% \cdot \mu \text{strains}^{-1} \cdot \text{day}^{-1}]}$), (3) a younger subject with more flexible bones by scaling the bone mechanical properties by 0.75 and (4) the same younger subject with a higher mechanoregulation factor ($M_{S1} = 2 \times 10^{-4} \text{[\% \cdot \mu \text{strains}^{-1} \cdot \text{day}^{-1}]}$). These changes were only studied in one of the models (D70) and the study with the fitted parameters was considered as the baseline case to perform the comparison. All the differences were expressed in relative percentages.

![Figure 6.10](image-url)

Figure 6.10. Correlation between the bone deposition rate ($\dot{V}$) and the mechanical stimulus ($\Psi$) representing the mechanosensitivity ($M_{S}, M_{S1}, M_{S2}$) and reduction factor ($\alpha$). Three mechanosensitivity values were considered where $M_{S}$ (orange) is the model fitted value, $M_{S1}$ (blue) is a hypothetical value to represent a higher mechanosensitivity and $M_{S2}$ (green) is a hypothetical value to represent a lower mechanosensitivity.
6.2. Results

6.2.1. In silico mechanically-driven bone regeneration

The Von Mises stress distribution of all of the scaffolds showed that both materials provided enough structural integrity as the yield strength ($\sigma_y$) (Table 6.2) was not reached in any of the studied scaffolds (Figure 6.11). G70 resulted to have the lowest stress concentration for both of the materials, while SK-G70 had the highest values for titanium and G90 for PCL-TCP.

![Stress distribution](image)

Figure 6.11. Stress distribution (A) and maximum stress (B) found for all the analyzed scaffold designs for both materials. From left to right: lattice dodecahedron, sheet-based Gyroid 70% and 90% porosities and skeletal-based Gyroid 70% and 90% porosities.
The bone ingrowth induced by the studied scaffolds is shown in Table 6.4. For the titanium scaffold, the highest bone ingrowth was seen for SK-G90 at 13.7% followed by D70, SK-G70, G90 and G70 at 7.0%, 6.3%, 3.6% and 2.2% respectively. When considering PCL-TCP as the scaffold material, the highest bone ingrowth was seen for the SK-G90 with 77%. SK-G70, D70, G90 and G70, arranged in a decreasing order, reported values of bone ingrowth between 70.9% and 45.1%.

Table 6.4. Bone ingrowth results for the titanium and PCL-TCP scaffolds.

<table>
<thead>
<tr>
<th>Scaffold</th>
<th>Titanium BV (mm$^3$)</th>
<th>BV/TV (%)</th>
<th>BV/(TV-SV) (%)</th>
<th>PCL-TCP BV (mm$^3$)</th>
<th>BV/TV (%)</th>
<th>BV/(TV-SV) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D70</td>
<td>25.1</td>
<td>5.4</td>
<td>7.0</td>
<td>178.5</td>
<td>38.1</td>
<td>49.9</td>
</tr>
<tr>
<td>G70</td>
<td>9.7</td>
<td>2.2</td>
<td>3.2</td>
<td>134.9</td>
<td>30.2</td>
<td>45.1</td>
</tr>
<tr>
<td>G90</td>
<td>14.6</td>
<td>3.2</td>
<td>3.6</td>
<td>193.2</td>
<td>42.7</td>
<td>47.7</td>
</tr>
<tr>
<td>SK-G70</td>
<td>19.7</td>
<td>4.3</td>
<td>6.3</td>
<td>222.5</td>
<td>49.0</td>
<td>70.9</td>
</tr>
<tr>
<td>SK-G90</td>
<td>56.4</td>
<td>12.6</td>
<td>13.7</td>
<td>316.3</td>
<td>70.6</td>
<td>77.0</td>
</tr>
</tbody>
</table>

A different result was observed in each structure based on the studied subregion. Figure 6.12-A shows the percentage of the total bone ingrowth that took place in the medullary, middle and periosteal regions. For the titanium scaffolds, D70 seemed to have the highest relative bone ingrowth in the medullary and middle regions with regard to the total volume of bone predicted for each scaffold. Likewise, G90 had the best relative performance in the periosteal region. G70, SK-G70 and SK-G90 exhibited a similar behavior in all three subregions. The results for the softer material showed a more even distribution of the proportion of regenerated bone over the medullary, middle and periosteal regions (30-40% in each). Figure 6.12-B and Figure 6.12-C present slices from the medullary, middle and periosteal regions at a height of 1 mm, 5.5 mm and 9 mm respectively showing how bone regenerated in each model.
Figure 6.12. (A) Predicted bone ingrowth distribution within the scaffold. The values are a percentage of the total bone ingrowth. Representative slices of the bone ingrowth (brown) in the different regions for each one of the titanium scaffolds (B) and PCL-TCP scaffolds (C).
On the histology like representation of the predicted tissue differentiation (Figure 6.13), it can be observed that the results for the titanium scaffolds primarily showed the presence of granulation tissue due to the scaffold’s high stiffness, whereas the soft material scaffolds predicted a higher volume of bone and fibrous tissue to be differentiated.

![Histology predictions](image)

Figure 6.13. Middle slice with the histology predictions after 12 weeks for the titanium and PCL-TCP scaffolds.

This analysis also reported that D70 had a larger bone ingrowth than SK-G70 for the titanium scaffolds but the outcome was inversed when studying the PCL-TCP material (Table 6.4). It was observed that the geometrical features of the SK-G70 allowed for a better cell diffusion but the titanium material restricted the bone regeneration due to a more localized stimulus. However, when the softer material was used, the mechanical stimulus reached more central areas and the cell diffusion advantage was translated in a higher bone ingrowth (Figure 6.14).
Figure 6.14. Middle slices representing the cell concentration, the areas with a strain stimulus higher than the reference stimulus ($\alpha \cdot \Psi_{local}$) and the bone ingrowth after 12 weeks of the D70 and SK-G70 scaffolds for the titanium models and the PCL-TCP models.

6.2.2. Clinical strategies for enhancing bone ingrowth

The influence of the three-week post-operative resting period studied for the titanium models showed that bone ingrowth took place mainly in the external pores of the scaffold, with a limited ingrowth the core region. The percentage of bone ingrowth increased with regard to considering only one day resting (8.7% vs 7.0%) (Table 6.5). Moreover, when considering the less stiff material, the bone reached a more central part of the scaffold but did not reach the core (Figure 6.15). However, the bone volume increased considerably.
Table 6.5. Observed bone ingrowth for the non-seeded, seeded and 3-week post-operative rest cases for both titanium and PCL-TCP scaffold materials.

<table>
<thead>
<tr>
<th>Scaffold</th>
<th>Titanium</th>
<th></th>
<th></th>
<th>PCL-TCP</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BV (mm³)</td>
<td>BV/TV (%)</td>
<td>BV/(TV-SV)(%)</td>
<td>BV (mm³)</td>
<td>BV/TV (%)</td>
<td>BV/(TV-SV)(%)</td>
</tr>
<tr>
<td>Baseline (D70)</td>
<td>25.1</td>
<td>5.4</td>
<td>7.0</td>
<td>178.5</td>
<td>38.1</td>
<td>49.9</td>
</tr>
<tr>
<td>3-week rest</td>
<td>31.1</td>
<td>6.6</td>
<td>8.7</td>
<td>229.8</td>
<td>49.0</td>
<td>64.2</td>
</tr>
<tr>
<td>Seeded</td>
<td>27.6</td>
<td>5.9</td>
<td>7.7</td>
<td>271.4</td>
<td>57.9</td>
<td>75.8</td>
</tr>
</tbody>
</table>

The results of inserting a titanium pre-seeded scaffold showed that although the total bone ingrowth increased in a 10% with respect to the non-seeded case (Table 6.5), all of the new bone was located in the outer region of the scaffold. For the PCL-TCP scaffolds, the bone ingrowth reached the inner pores of the scaffold and the bone ingrowth had a remarkable increase from 49.9% to 75.8% (Figure 6.15).

Figure 6.15. Middle slices representing the cell concentration, the areas with a strain stimulus higher than the reference stimulus ($\alpha \cdot W_{local}$) and the bone ingrowth after 12 weeks for the baseline, the pre-seeded scaffold and the 3-week post-operative rest studies for both Ti (left) and PCL-TCP materials (right).
6.2.3. Significance of the patient-specific parameters

The study to assess the influence of patient-specific parameters (Figure 6.16) showed that when the bone’s initial elastic modulus is higher (case 1), the resultant bone ingrowth was 6.8% lower than the fitted model (baseline case). The same phenomenon could be observed when, aside from raising the elastic modulus, the mechanosensitivity parameter was lowered, as the results for case 2 showed an 8.8% decrease of the predicted bone ingrowth. However, this tendency was inverted when a lower bone’s elastic modulus was studied as case 3 resulted in a 7.2% increase compared to the baseline case. Likewise, when aside of lowering the elastic modulus, the mechanosensitivity parameter was raised, the predicted bone ingrowth volume reported an 8.0% increase.

![Figure 6.16. Influence of patient-specific parameters in the bone ingrowth predictions with respect to the fitted model (baseline case).](image)
6.3. Discussion

6.3.1. Scaffold’s geometry and material role in bone regeneration

This chapter successfully quantified the mechanobiological potential of TPMS scaffolds and compared the influence of their geometrical properties when considering different stiffness materials.

Five different scaffolds were designed to characterize their bone regeneration capability. The skeletal gyroid scaffold with 90% porosity (SK-G90) consistently showed the highest bone formation in both material analyses. When comparing the performance of each scaffold, it was observed that the cell diffusion was influenced by the geometrical features, but the presence of cells was only translated into bone ingrowth when mechanical stimulus was in the desired range (Figure 6.14). This is the main reason why a considerable difference in the resulting bone ingrowth for both studied materials were observed. The increase in the relative bone ingrowth ranged from fourteen times (G90) to five times higher (SK-G90) when comparing PCL-TCP with Ti. This highlights that both geometry and material need to be tuned to maximize bone regeneration.

To induce bone formation, the osteocyte concentration should be high enough in the scaffold and the mechanical stimulation needs to be in the range for bone formation. Titanium was observed to be too stiff for this application as mechanical shielding limited the strain stimulus which in turn lead to a reduced bone tissue formation. This chapter studied the performance of a softer scaffold to bypass this phenomenon and the results were satisfactory. When considering PCL-TCP the bone ingrowth increased in all the studied cases. In agreement with these results, other works of the literature also reported that softer scaffold material composites have a better osteoregenerative potential as they can match the elastic modulus of cancellous bone [182], [186], [187]. Topological optimization was also investigated by other works to overcome the shielding limitation [78], [188].
Interestingly, results indicate that the skeletal-based Gyroid scaffolds show a higher bone regeneration behavior than the sheet-based ones [29], contrary to what could be inferred from only observing their mechanical performance [26]. One possible explanation is the different diffusion properties of each configuration as the skeletal models reported higher cell concentrations in more central regions of the scaffolds (Figure 6.17). This highlights the need of mechanobiology models that combine mechanics with biological cues to accurately characterize the potential of bone scaffolds.

The geometrical properties of the scaffolds play an important role in bone formation as the distribution of the newly formed bone was different based on the regions of the scaffold. Parameters such as the pore size or shape influence the strain distribution and thus, control the mechanical stimulation to induce bone differentiation. According to the subregion of interest (medullary, middle and periosteal), each scaffold showed a different volume of newly formed bone. All these variables, in addition to the location of the scaffold and the direction of the applied loads suggest that there is no universal...
scaffold design that would be optimal for all applications; instead, the information obtained from these analyses could be used to optimize the implant design to target localized bone formation.

6.3.2. Enhancement of the bone regeneration

Clinical strategies modeling

In some cases, it was observed that no bone formation took place in the core of the scaffold because of a low cell concentration as the cells did not reach the inner pores (Figure 6.14). One investigated approach to improve the cell concentration was extending the recovery time before applying any stress on the affected limb [102], [103]. This allows cells to migrate, proliferate and reach the center of the scaffold, so when the area is mechanically stimulated, either by walking or by physiotherapy, bone ingrowth can be more effective. This approach succeeded in inducing a higher bone ingrowth, but the regenerated bone failed to reach the core of the scaffold.

Another examined approach was the use of a pre-seeded scaffold to allow cells to increase their concentration at the inner pores, as suggested in the literature [101]. This improved the bone ingrowth for the titanium scaffold but mechanical shielding avoided bone formation in the core region (Figure 6.15). However, when considering a pre-seeded scaffold with a softer material, the predicted newly formed bone clearly reached the central part of the scaffold (Figure 6.15).

Patient-specific significance

Age and metabolic diseases such as osteoporosis or osteopetrosis are issues that can affect the bone structure and its mechanical properties. In those cases, the reduction in mechanical properties is accompanied by a change in the normal metabolic balance and affect the bone formation rate [189], [190]. These changes were studied; and although the used parameters only varied slightly as they were hypothetical, the bone regeneration process showed different outcomes. The variation in the patient-specific parameters reported differences of up to 16% in the estimated bone regeneration. This fact highlights the significance of accurately reproducing such parameters in the
analysis in order to use computational models as a presurgical tool to assess what type of scaffold would be more suitable for each application.

### 6.3.3. Model limitations

There are some limitations and assumptions in this study that should be considered. The mechanobiological model only accounts for bone deposition within the granulation area, ignoring the bone remodeling process and its potential consequences. However, due to the scaffolds' size and non-load bearing capacity, it was assumed that this phenomenon would not cause significant variations in the results. In addition, cell diffusion and proliferation were modeled as a simple diffusion process that only considered the tissue density and a diffusion constant. Oxygen concentration, nutrient diffusion and angiogenesis are parameters that can help represent more accurately the cell proliferation and osteogenic differentiation [127], [191]. Nonetheless, these modifications are not expected to greatly alter the comparison between the scaffolds’ bone regeneration potential.

Making use of advanced manufacturing techniques, future lines of work could investigate novel scaffold designs to optimize cell diffusion while also enhancing the stress distributions. The soft material studied in this work seems promising because the scaffolds are not weight bearing. For weight bearing applications, these scaffolds might need an additional support or the study and design of new metamaterials that could provide enough structural integrity whilst maintaining the mechanical stimulus in the desired range of bone differentiation.
6.4. Conclusions

This chapter successfully studied Gyroid based complex scaffold designs using a computational mechanobiology model as a tool to investigate their bone regeneration potential. The scaffold’s mechanical interaction with the subject is determined by factors such as the local environment and the response of the host; affecting greatly the volume and distribution of bone that is formed within the scaffold. The impact of the scaffold’s geometry and material were investigated and proved to affect bone regeneration. The scaffold’s geometrical properties showed different diffusion patterns that affected the regenerated bone volume. The ability to induce mechanobiologically-driven bone regeneration of the scaffolds was also seen to be dependent on the material, suggesting that the material can be used to tune the strain distribution and enhance the bone ingrowth. Likewise, physiological characteristics of the host need to be assessed as variations in the bone mineral density or the mechanosensitivity of the subject can influence the suitability of one scaffold design or another.
This dissertation aimed to investigate the use of cellular porous structures based on Triply Periodic Minimal Surfaces in the design of bone replacements. The research emphasized the role of mechanobiology in the bone regeneration process and broadened the knowledge of the mechanical and mechanobiological behavior of TPMS structures via computational modeling. The main contributions of this work are:

**Parametrization of the design methodology of TPMS structures.**

A parametric design methodology has been developed to meet particular mechanical properties (stiffness/permeability) and bone mimicking features (pore size). These features are among the most critical attributes considered in the design of porous structures for bone regeneration.

The meshes produced are adequate for additive manufacturing applications but typically fall short of the quality standards necessary for Computer-Aided Engineering (CAE) applications. Achieving a higher-quality mesh can be a challenging process, frequently requiring a trial-and-error approach. To complement the design methodology, a meshing procedure has been introduced to create models that are appropriate for Finite Element (FE) and Computational Fluid Dynamics (CFD) analyses.
Assessment of the suitability of TPMS structures as bone replacements from a mechanical perspective.

An uncoupled fluid-structure model was used to determine the mechanical stimulus on the surface for different TPMS structures. Data from both FE and CFD studies was gathered and integrated to correlate mechanical stimuli with cellular differentiation, revealing that the TPMS Gyroid architecture might be the geometry with the greatest potential to induce bone differentiation.

Characterization of the fluid flow properties of TPMS structures.

A comprehensive study of the influence of different TPMS design parameters and CFD modelling approaches on their fluid flow properties (flows velocity and permeability) was performed. The boundary conditions considered in CFD simulations were simplified and optimized to minimize computational costs. The results showed that the fluid flow behavior depended not only on porosity but also on the geometrical features of the different TPMS structures, highlighting the need to accurately investigate and select the most appropriate TPMS configuration for a given application. Furthermore, this work enabled more consistent discussions of the results reported in different studies of the literature, as a wide range of structures varying in architecture, cell size and porosity were studied. The obtained permeability values were in range with the values reported in the literature.

Development of a generalized analytical model to estimate the permeability of TPMS structures.

An analytical model for predicting the permeability of TPMS structures has been obtained and tested for the Gyroid, Fisher-Koch S and Schwarz Primitive architectures. This analytical approach allows for an estimation of permeability based solely on design parameters such as porosity and pore size, allowing for a more flexible design of the structures to match the permeability properties of bone. Incorporating permeability models into optimization algorithms can allow scaffold design to be tailored and optimized for each individual patient's needs.
Conclusions and future work

Study of mechanobiologically-induced bone regeneration integrating clinical approaches.

A previously validated computational mechanobiology model was used to evaluate the potential of various porous scaffolds to promote bone regeneration and integrated the use of different clinical approaches to enhance bone ingrowth. The results indicated that scaffold morphology and material played a significant role in determining their performance. In particular, skeletal-based Gyroid structures were found to exhibit a higher bone ingrowth compared to sheet-based scaffolds. Furthermore, this work has successfully demonstrated that different clinical strategies can be incorporated into predictive patient-specific in-silico models to enhance the bone regeneration induced by porous scaffolds.

These contributions outline a methodology for the design of the most suitable porous structures for bone replacements, integrating both the morphological and mechanical features of the structures and the efficiency of patient-specific strategies to promote bone regeneration. Investigating bone formation within the pores of the scaffold contributes to tailoring the desired biological response to maximize scaffold performance. Concurrently, clinical strategies contribute to the refinement of patient-specific bone replacement solutions.
7.1. Ongoing and future work

As shown in this dissertation, porous structures offer promising properties for orthopedic implants. Nevertheless, their intricate characteristics and incomplete knowledge about certain properties remain obstacles to their widespread acceptance in medical practice. Although this research aims to address the mechanical and mechanobiological features of these structures, there are essential considerations that are not entirely comprehended. These require further investigation to gain a more thorough understanding of the behavior of cellular porous structures for bone replacement applications.

Effect of fatigue on scaffold behavior

The mechanobiological model in Chapter 6 accurately predicts the ability of scaffolds to promote bone regeneration. However, in a clinical setting, their success also relies on their capacity to withstand cyclic loads. Loading conditions, including amplitude, frequency, and type, significantly influence the fatigue behavior and mechanical properties of porous scaffolds. Elevated amplitude and loading ratios increase stress levels, diminishing fatigue life due to increased deformation, plasticity, and the formation of microcracks that may lead to failure [33], [192]. Consequently, the effects of fatigue on scaffolds must not be overlooked. Incorporating fatigue behavior into predictive models would allow for the examination of the dynamic environment and the prediction of not only bone regeneration but also the potential for scaffold failure.

Similarly, the findings from the qualitative plasticity analysis presented in Chapter 4 indicate that skeletal-based structures with high porosity exhibit poorer stress distribution compared to those with lower porosity. This results in more pronounced stress concentration points, potentially leading to scaffold failure under cyclic loading. Future research could concentrate on determining whether sheet-based scaffolds exhibit similar behavior or if local porosity gradients could alleviate the occurrence of high stress concentration points. Exploring local porosity gradients may be interesting when parametric design is integrated into a Design of Experiments (DOE) approach to optimize the structure according to stress distributions.
Physiological loading environment

The studied uncoupled fluid-structure model considers only compression loads from a structural perspective. However, scaffolds subjected to physiological loads may experience additional forces such as bending or torsion, resulting in varying levels of octahedral shear strain. Investigating the effects of these forces separately or in combination could yield insightful information on bone tissue differentiation. Furthermore, combining mechanical testing with digital image correlation techniques (3D DIC) could enhance the accuracy of predictions and validations.

Mechanobiological algorithm

The mechanobiological model presented in Chapter 6 incorporates certain simplifications with respect to the bone formation process, and it does not consider the influence of bioregulatory factors. While these simplifications did not affect the study's primary objectives, they are important from a clinical perspective and must be addressed before these models can be safely applied in clinical settings. Factors such as oxygen concentration and angiogenesis can be as crucial as mechanical stimuli in determining the direction of chondrogenic or osteogenic differentiation. Developing algorithms that can accurately reflect the complexity of biological processes is essential for the advancement of porous structures in orthopedic implants.

Moreover, multiscale models that can accurately transfer the realistic forces applied on bones would allow for a more accurate translation of these loads to the cellular level. To enhance the current model, the diffusion process could be refined by incorporating various cell types, integrating growth factors, and examining the biochemical interactions between cells.

By improving the model to include these factors, researchers can work towards more accurate predictions of tissue formation and response to implants, thereby enhancing the design and performance of bone replacements and improving patient outcomes.
**Experimental permeability setup**

A linear relationship between experimental and computational permeability has been observed in the literature [45], [56]. This suggests that the analytical model developed in this dissertation could also be adapted for experimental settings by using experimental permeability values to calibrate the model, rather than relying solely on computational data.

In an ongoing study, an experimental setup using the pump method has been developed to assess the permeability of TPMS structures (Figure 7.1). The setup consists of a syringe pump (Aladdin Single-Syringe Pump, AL-1000HP, World Precision Instruments) linked to a custom-designed permeability chamber. The pressure differential was determined by measuring the fluid's pressure at the chamber's inlet and outlet. Pressure sensors (BLPR2 Blood Pressure Transducer, World Precision Instruments) were interfaced with a data acquisition board (NI 9201, National Instruments Corp.). The aim of this research is to evaluate the practical application of the analytical model that has been developed. A new parameter might be introduced to establish a correlation between the experimental and computational findings.

![Experimental permeability setup](image)

*Figure 7.1. Experimental permeability setup.*
In vitro and in vivo experimental data

Further in vivo and in vitro experiments are necessary to validate the computational findings and enhance the reliability of the predictions for clinical use. Although analytical and computational models are effective for simulating biological processes, they are still approximations of reality. Combining these models with image analysis techniques, which provide more precise data, represents a promising approach [193]. Moreover, features such as curvature and surface roughness have been shown to influence cellular behavior [194], [195].

An in vitro cell culture study has been proposed to investigate the effects of the TPMS Gyroid curvature and the surface roughness resulting from its manufacturing process. To facilitate this study, four titanium scaffolds have been designed using the methodology described in Chapter 3.2 (Table 7.1). These scaffolds aim to match the elastic modulus of cortical bone (E = 20 GPa) [66], possess pore sizes favourable to cell adhesion and bone formation (500 and 900 μm) [81], and ensure good manufacturability, including achievable wall thickness (Figure 7.2).

<table>
<thead>
<tr>
<th>Desired E (GPa)</th>
<th>Material E (GPa)</th>
<th>Calculated porosity (%)</th>
<th>Desired pore size (mm)</th>
<th>Calculated cell size (mm)</th>
<th>Wall thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>114</td>
<td>65</td>
<td>0.5</td>
<td>1.28</td>
<td>0.16</td>
</tr>
<tr>
<td>20</td>
<td>114</td>
<td>65</td>
<td>0.9</td>
<td>2.31</td>
<td>0.29</td>
</tr>
<tr>
<td>20</td>
<td>114</td>
<td>57</td>
<td>0.5</td>
<td>0.92</td>
<td>0.42</td>
</tr>
<tr>
<td>20</td>
<td>114</td>
<td>57</td>
<td>0.9</td>
<td>1.65</td>
<td>0.75</td>
</tr>
</tbody>
</table>
Figure 7.2. (A) Designed scaffolds for in vitro cell culturing (500 μm pore size): sheet-based Gyroid (left) and skeletal-based Gyroid (right). (B) Additively manufactured scaffolds using PBF technology.

**Perfusion-compression in vitro study**

Another prospective research line could involve integrating both mechanobiological approaches to investigate extracellular matrix (ECM) growth and mineralization over time. The findings of this dissertation, alongside other studies in the literature, have documented an increase in wall shear stress as the porosity of the scaffold decreases. With ECM growth, scaffold porosity decreases, which, under consistent levels of mechanical stimulation (i.e., perfusion and compression), results in elevated stresses and strains. Consequently, mechanical stimuli could surpass the upper threshold for tissue growth, potentially leading to a plateau. Understanding this relationship could help adjust the stimuli to maintain them within the optimal range for tissue growth.

A CFD setup could yield more accurate predictions of cell adhesion zones. By modeling cells as particles within the fluid flow, it is possible to identify regions that not only have appropriate wall shear stress levels for tissue differentiation but also where cells come in contact with the scaffold. This data could then be integrated into a FE analysis, which would apply compression cycles to simulate tissue growth. These simulations could subsequently be validated experimentally within a perfusion-compression bioreactor, allowing for the correlation of ECM growth areas with the computational predictions.
Conclusions and future work
References


References


References


[97] K. S. Rappe et al., «On-Growth and In-Growth Osseointegration Enhancement in PM Porous Ti-Scaffolds by Two Different Bioactivation Strategies: Alkali


[110] A. Chevrier, A. S. M. Kouao, G. Picard, M. B. Hurtig, y M. D. Buschmann, «Interspecies comparison of subchondral bone properties important for cartilage...
References


[184] A. Bruyas et al., «Systematic characterization of 3D-printed PCL/β-TCP scaffolds for biomedical devices and bone tissue engineering: influence of composition and


Appendix A

This Appendix includes the tables with the fitted coefficients for the adjusted equations in Chapters 3, 4 and 5 and the flow velocities for the CFD analyses in Chapters 5.

Table A.1. Fitted coefficients for the correlation between $C_f$ parameter and porosity of skeletal-based TPMS Eq. [3.4].

<table>
<thead>
<tr>
<th></th>
<th>G</th>
<th>SP</th>
<th>FKS</th>
<th>Dia</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_1$</td>
<td>-0.35</td>
<td>-0.45</td>
<td>-1.32</td>
<td>-0.31</td>
</tr>
<tr>
<td>$c_2$</td>
<td>3.22</td>
<td>3.89</td>
<td>5.58</td>
<td>2.44</td>
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</tbody>
</table>

Table A.2. Fitted coefficients for the correlation between wall thickness, cell size and porosity of sheet-based TPMS Eq. [3.5].

<table>
<thead>
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<th>SP</th>
<th>FKS</th>
<th>Dia</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_3$</td>
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<td>3.89</td>
<td>5.58</td>
<td>2.44</td>
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<tr>
<td>$c_4$</td>
<td>1.53</td>
<td>1.67</td>
<td>4.25</td>
<td>0.92</td>
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</table>

Table A.3. Fitted coefficients for the correlation between pore size, cell size and porosity of sheet-based TPMS Eq. [3.6].

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<tr>
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<td>Gyroid</td>
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<td>Sheet</td>
<td>0.34</td>
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<td>Skeletal</td>
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</table>

Table A.4. Fitted coefficients for the correlation between wall thickness, porosity and pore size of sheet-based TPMS Eq. [3.7].

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<td>Sheet-based G</td>
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### Table A.5. Fitted coefficients for the Gibson-Ashby model for skeletal-based TPMS Eq. [4.2].

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</thead>
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<tr>
<td>Dia</td>
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<td>2.06</td>
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</table>

### Table A.6. Fitted coefficients for the analytical permeability model for skeletal-based TPMS Eq. [4.9].

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### Table A.7. Model pore size $D_p$ (mm) and calculated inlet velocity $u$ ($x10^4$ m/s) for Re=1.

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<th>TPMS architecture</th>
<th>Cell size (mm)</th>
<th>Porosity (%)</th>
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</thead>
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<td>60</td>
</tr>
<tr>
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<td>$D_p$</td>
<td>$u$</td>
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Table A.8. Permeability values (x10-9 m²) for all the studied scaffolds.

<table>
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<th>TPMS architecture</th>
<th>Cell size (mm)</th>
<th>Porosity (%)</th>
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</table>
Appendix B

This section outlines the primary contributions of this dissertation, detailing the produced publications:


Furthermore, three conference presentations were also an output of this research:


