Permeability and mechanical properties of triply periodic minimal surface scaffolds for bone regeneration

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Scaffolds for bone regeneration have been investigated as bone substitutes in critical sized bone defects. It is known that the architecture of these scaffolds is important for the bone regeneration process, which depends on cell response and vascular ingrowth into the scaffold. With advanced fabrication techniques, such as selective laser melting, it is possible to manufacture complex geometries. In this study, Ti6Al4V scaffolds based on four different minimal surfaces, primitive, I-WP, gyroid and diamond, with different porosities were evaluated. Of these scaffolds, the morphological and mechanical properties, and the permeability were determined with micro-CT, static compression tests and fatigue tests, and permeability experiments and computational fluid dynamic simulations. Porosities of 71.3-49.2%, 65-44.3%, 65.6-52% and 59.7-44.2% were found for the primitive, I-WP, gyroid and diamond scaffolds, respectively. It was observed that the permeability depends on the shape of the unit cell as well as on the apparent density of the scaffold. Permeability values of $5.48 \times 10^{-11}-6.10 \times 10^{-9} \text{m}^2$ and $1.29-6.96 \times 10^{-9} \text{m}^2$ were obtained from the laminar experimental and computational approaches, respectively. The static compression tests showed that the mechanical properties, such as the plateau stress, quasi-elastic gradient, plateau end stress, and the yield strength depend on the type of unit cell and the porosity. The lowest fatigue lives were found for the primitive unit cells, while the I-WP scaffolds and the diamond scaffolds with the lowest and highest apparent density, respectively, were still intact after $10^6$ cycles. The results in this study suggest that some of the scaffolds evaluated would be suitable as scaffolds for bone regeneration.

1. Introduction
Bone scaffolds act as three-dimensional matrices that guide and promote bone regeneration in order to heal critical sized defects [1-3]. In these defects, caused by trauma, severe fracture, or tumor resection, bone is unable to heal itself. The most common bone substitutes include autografts, allografts, or xenografts, which are pieces of bone taken from another place of the patient's body, another person, or animal, respectively. Because the use of these solutions may result in damage to the body and their supply is limited [4, 5], another solution is needed. Therefore, research has been undertaken to develop new synthetic biomaterials with different architectures to see how these designs influence the bone regeneration process. With advanced fabrication techniques, such as additive manufacturing (AM) or 3D printing, complex structures can be built [2, 6]. With AM, products are created layer by layer based on a Computer-Aided-Design (CAD) model [7]. Selective laser melting (SLM) is an AM technique to build a product layer-wise by selectively melting metal powder layers with a computer controlled, high intensity laser beam [8, 9]. The powder melts due to the energy of the laser and forms a melt pool. The melt pool cools quickly and the consolidated material becomes part of the final product. Every solidified layer represents a cross section of the model. After a layer is completely scanned, a new
powder layer is laid down and scanned according to the CAD model. When this layer gets scanned, the consolidated material below this layer is remelted, making a connection in z-direction between the new layer and the solid part of the product. This process is repeated until the product is finished.

Selective laser melting enables the manufacturing of scaffolds based complex geometries, such as triply periodic minimal surfaces (TPMS). A minimal surface is an infinitely extending surface and can be described by simple trigonometric functions [10]. Minimal surfaces are attractive candidates for scaffolds for bone tissue regeneration, because they have an average mean curvature close to zero [11], which is similar to the average mean curvature of trabecular bone [12]. They also have an open cell structure, a high structural stiffness, and large surface area [13], which are important properties for bone regeneration.

There are two types of TPMS scaffold architectures: 1) minimal surface network solids, where the minimal surface forms the solid/void interface, and 2) minimal surface sheet solids, which are obtained by inflating the minimal surface to a finite, homogeneous thickness, until a solid volume fraction is reached. Both architectures divide space into a single solid domain, and either one or two void domains [13]. These void domains provide the space in which new tissue formation, initial remodeling of this tissue, and angiogenesis occur [14].

The design of a scaffold has influence on the bone regeneration process, which depends on cell response and vascular growth into the scaffold. Parameters that describe the architecture of a scaffold are pore size [15-18], pore shape [18, 19], interconnectivity of the pores [16-18], and the porosity [16, 18, 20]. These parameters affect the permeability and mechanical properties of the scaffold [18]. The permeability describes the rate at which a fluid can flow through a porous structure with interconnected pores or channels, at a given pressure difference. The fluid flow through the scaffold exerts shear stress on the cells within the scaffold, which in turn affects cell response [1].

Mechanical properties, such as compressive stiffness and fatigue strength, are important when the scaffold is placed into a load-bearing area. When the stiffness of the implant is too low, the scaffold will not be able to withstand the forces applied to it. However, when the stiffness is too high, stress shielding might occur, which is the process of bone resorption around the implant, leading to implant loosening [21]. When the scaffold is for instance, implanted into a load bearing area such as the femur, it will be subjected to multiple loading modes, including cyclic loading [22]. The fatigue strength, which is the number of load cycles a scaffold can resist before failure, is one of the important factors for the success of a scaffold in a load bearing area [23].

Not much information is available on Ti6Al4V scaffolds with a minimal surface sheet solid architecture manufactured with SLM. Therefore, the objective of this study was to determine the morphological and mechanical properties, and the permeability of these scaffolds. Four different types of TPMS scaffolds with four different porosities for every type of TPMS were built with selective laser melting. The morphological properties were determined with the dry weighing method and micro-CT scans. The
characterization of the permeability was done using both computational and experimental methods. Static compressive mechanical tests and fatigue tests were performed to determine the mechanical properties of the scaffolds.

The research question of this study is whether these TMPS scaffolds would be suitable tissue engineering scaffolds for bone regeneration based on their architecture, permeability, and mechanical properties.
2. Materials and Methods

2.1. Scaffold design and manufacturing

Four TPMS structures, primitive (P), diamond (D), I-WP and gyroid (G), were designed using K3Dsurf. By keeping the size of the unit cell constant (1.5x1.5x1.5mm) and varying the sheet thickness, four porosities between 43% and 77% per minimal surface type were built. The scaffolds with the highest porosity to the lowest porosity are indicated by the following order of numbers (500, 600, 700 or 800) that follows after P, G, I or D. For example, the P500 has the highest porosity of the primitive scaffolds, and the P800 has the lowest porosity. The created OBJ files were converted to .stl files with IVCon 3D Graphics File Converter, and Magics (Materialise, Belgium) was used to assemble the scaffolds based on the different TPMS unit cells. The open porous titanium (Ti6Al4V Grade 23 ELI) scaffolds were built on support structures by a non-commercial SLM machine at LayerWise N.V. (Belgium). Cylindrical scaffolds with a designed height of 20mm and a diameter of 15mm were produced (Figure 1).

![Figure 1: TPMS scaffolds. Top: STL file assembly of 1.5mm unit cells, bottom: cylindrical scaffolds with a height of 20mm and a diameter of 15mm manufactured with selective laser melting. From left to right: primitive, I-WP, gyroid, diamond.](image)

The quality of an SLM product depends on process parameters, such as laser parameters, scanning parameters, material properties and atmospheric parameters [8, 24, 25]. The laser energy density is the amount of energy applied to a volume of material and is considered as the most important factor for the quality of the SLM product. Areas supported by powder instead of solid material are called overhang zones. These zones have a small heat sink, which causes some powder below the scanned layer to melt. The formed meltpool sinks into the supporting powder due to gravity and capillary
forces, leading to a bad surface quality of the overhang zone [26, 27]. The overhang zones (areas under an angle between the horizontal and about 45-50 degrees from the horizontal) in the scaffolds seemed to be the main cause for the lower porosity of the scaffolds compared to the design. Reducing the energy density in these areas, by increasing the downfacing speed, resulted in a lower strut thickness in these zones (in the yz- and xz-planes). The use of an increased downfacing speed affected the porosity of the diamond scaffolds the most, followed by the I-WP, primitive, and gyroid geometries. This agrees with the amount of downfacing areas within the scaffolds.

The Gaussian intensity distribution of the laser [24] creates a zone around the laser spot in which powder is irradiated, melted and consolidated [28]. Using a beam compensation, which is the offset of the beam inwards from the laser path, the effect of the Gaussian distribution can be diminished. By increasing the beam compensation, the strut thickness in the xy-plane is reduced, which increases the porosity of the scaffold.

One of the limitations of SLM was the minimum melt pool width, which was found to be 100-110µm, making it unable to produce sheets thinner than this size. Due to this limitation, the highest porosities that could be realized were 71%, 65%, 66% and 60% for the primitive, I-WP, gyroid and diamond scaffolds, respectively.

For the final set of parameters for every minimal surface scaffold, a compromise was made between the accuracy of the porosity and the strut dimensions.

2.2. Scaffold morphology

The morphology of the scaffolds was characterized using micro-CT imaging and the dry weighing method. Four samples of every type of scaffold were scanned using a Quantum FX µCT, Caliper system (Perkin Elmer, Waltham, MA, USA). A tube voltage of 90kV, tube current of 180 µA, scan time of 3 minutes, and a resolution of 60µm were used. The 3D images were reconstructed using built-in algorithms of the scanner, and transferred to the Caliper Analyze 11.0 software to obtain 2D slices, representing the cross-sections of the scaffolds. Fiji v.1.49s [29] was used for the segmentation of the images. First, the lower limit for the brightness level was set to 90 to make sure only the Ti6Al4V was visible in the slices. Then the auto local threshold was applied, which computes the threshold for each pixel within a specified radius in an 8-bit image. The Bernsen algorithm was selected with a contrast threshold of 6. This combination was chosen, because it gave the best results for the segmentation of the data when all algorithms available were evaluated. After segmentation, circular regions of interests (ROIs) were selected in which only the cross section of the scaffold was visible. The Fiji plugin BoneJ v.1.4.0 [30] was then used to compute the porosity of the scaffold, the pore size, the strut thickness and the surface area.

The dry weighing method is based on the assumption that Ti6Al4V has a specific density of 4.51g/cc. By weighing the scaffold, and dividing this mass by the mass of a solid cylinder with the same outer dimensions, the material percentage or apparent density (AD) of the scaffold was determined. The porosity \( \varphi \) was defined as \( \varphi = 1 - AD \).
2.3. Permeability

The permeability of the scaffolds was determined using both computational and experimental methods. For both approaches, Darcy's law (Eq. 1) and Eq. 2 were used to determine the permeability and the Reynolds number, respectively. The Reynolds number indicates whether the flow is laminar or turbulent. For porous media, the flow is assumed to be laminar if $1<\text{Re}>10$ [31]. For the determination of the Reynolds number, the micro-CT data for the pore size was used as the pore diameter $d$ (Table 4).

\[
\kappa = \frac{v \cdot \mu \cdot L}{\Delta P} \tag{1}
\]

and

\[
\text{Re} = \frac{v \cdot \rho \cdot d}{\mu} \tag{2}
\]

where

- $\kappa$ Permeability coefficient [m$^2$]
- $\mu$ Dynamic viscosity coefficient of the fluid [Pa·s]
- $L$ Height of the sample [m]
- $v$ Fluid velocity [m/s]
- $\Delta P$ Pressure gradient [Pa]
- $\text{Re}$ Reynolds number [-]
- $\rho$ Density of the fluid [kg/m$^3$]
- $d$ Diameter of the pore [m]

The constant parameters are defined in Table 1.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$</td>
<td>Density of water</td>
<td>$10^3$ [kg/m$^3$]</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Dynamic viscosity coefficient of water</td>
<td>$10^{-3}$ [Pa·s]</td>
</tr>
</tbody>
</table>

Table 1: Constant parameters and their values used in Darcy's law and the equation for the Reynolds number.

2.3.1. Permeability simulations

3D CAD files of the unit cells were used in the flow simulations to determine the permeability of the scaffolds with COMSOL Multiphysics v5.1 [32]. Before importing the .stl files in COMSOL, Rhinoceros 5 [33] was used to scale the geometries to 1.5x1.5x1.5mm (to match the size of the unit cells used to assemble the scaffolds). In COMSOL, a cube with dimensions of 1.55x1.55x1.55mm was created around the unit cell to act as the liquid component of the model. The dimensions of this cube were taken slightly bigger than the dimensions of the unit cell to prevent the intersection of both components, which gave an error in COMSOL. The material was assigned to the cube (water, liquid) and to the unit cell (Ti6-Al-4V). After the materials were assigned, a single phase laminar or turbulent flow were applied for the laminar and turbulent model, respectively. To determine whether the flow was laminar or turbulent, the Reynolds
numbers were computed with Eq. 2 using mass flow rates ($\dot{m}$) from the laminar model after the inlet pressure was specified, with
\[
v = \frac{\dot{m}}{\rho \cdot A}
\]
where
- $\dot{m}$ Mass flow rate [kg/s]
- $A$ Cross sectional area of the box [m\(^2\)]
- $\rho$ Density of the fluid [kg/m\(^3\)]

The top of the cube was specified as the inlet of the flow. In the laminar model, the flow was assumed to be laminar when the Reynolds number was $1<\text{Re}>10$, to match the laminar region of the experiments. With this assumption, inlet pressures of 2Pa, 6Pa, and 10Pa, were applied to the primitive, gyroid, diamond and I-WP geometries, respectively (Figure 2). An inlet pressure of 9000Pa was applied to all unit cells in the turbulent model, which resembled the highest pressure difference in the experiments. The outlet was assigned to the bottom of the cube with a pressure of 0Pa. In the laminar model, the surface of the unit cell was specified as the walls with a no slip condition i.e. the fluid velocity matches (has no slip relative to) the boundary velocity, which is equal to zero. In the turbulent model, the surface of the unit cell was specified as the walls with wall functions i.e. the transitional flow regime from laminar to turbulent is not simulated. Because the scaffolds were assembled by a repetition of unit cells in three directions, and only one unit cell was used for the simulations, periodic flow conditions were assigned to the sides of the cube. These boundary conditions divide the model into a source group and a destination group. Fluid that leaves the domain through one of these planes enters the domain through the corresponding source boundary. A free tetrahedral mesh was generated for both components, with approximately $5 \cdot 10^5$ elements calibrated for fluid dynamics for the liquid component (the cube). Since the element size of the mesh for the unit cell had no effect on the results of the simulations, the default element size for general physics was used for the unit cell mesh. Finally, a stationary study was added to the model, to compute steady flow and pressure fields. The mass flow rate ($\dot{m}$) obtained from the simulations was used to determine the permeability according to Darcy's law (Eq. 1), where $v$ is described by Eq.3. The known values of the used parameters are summarized in Table 2.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L$</td>
<td>Height of the cube</td>
<td>1.55 [m]</td>
</tr>
<tr>
<td>$\Delta P$</td>
<td>Pressure gradient laminar model $1&lt;\text{Re}&gt;10$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Primitive unit cell</td>
<td>2 [Pa]</td>
</tr>
<tr>
<td></td>
<td>Gyroid unit cell</td>
<td>6 [Pa]</td>
</tr>
<tr>
<td></td>
<td>I-WP and diamond unit cells</td>
<td>10 [Pa]</td>
</tr>
<tr>
<td>$\Delta P$</td>
<td>Pressure gradient turbulent model</td>
<td>9000 [Pa]</td>
</tr>
<tr>
<td>$A$</td>
<td>Cross sectional area of the cube</td>
<td>2.4-10^{-6} [m(^2)]</td>
</tr>
</tbody>
</table>

Table 2: Parameters and their values used in Darcy's law and the equation for the Reynolds number for the permeability simulations.
2.3.2. Permeability experiments

The falling head method was used to determine the permeability experimentally. This method involves the flow of water through a relatively short sample connected to a column which provides the water head. A vacuum pump was used to fill the water column above the scaffold. When the column was filled, the air valve was opened and the water flowed back into the tank through the sample (Figure 3).

The water pressure at the bottom of the column was measured just above the scaffold with a pressure gauge and registered every second in LabView (v.11.0). This process
was repeated five times for all four samples of every type of scaffold. Six steps were taken to derive the permeability from the experimental values. The known values for the used parameters are summarized in Table 3.

1. The difference in water level per second within the water column was used to determine the fluid velocity within the column $v_{\text{column}} = \frac{\partial h}{\partial t}$.

2. The volumetric flow rate was computed by multiplying this velocity by the cross sectional area of the column ($A_{\text{column}}$) in the x-y plane.

3. The volumetric flow rate ($Q$) was divided by the cross sectional area of the scaffold in the x-y plane to derive the flow velocity through the sample.

4. In a graph where $\Delta P$ (Eq.5) was plotted as a function of the velocity through the scaffold ($v_{\text{scaffold}}$), a power trendline with $R^2 > 0.998$ was fitted to the data. This trendline was used to extrapolate the data for velocities close to 0.

5. The Reynolds number was computed using Eq. 2, where $v$ is defined in Eq. 4.

6. In the region where the Reynolds number was between Re=1 and Re=10, which is the laminar regime in porous media and Darcy’s law can be applied [31]. For all scaffolds, the slope in this region was computed. As described in Darcy’s law, this slope is equal to the reciprocal of the permeability $\kappa$, multiplied by the dynamic viscosity coefficient ($\mu$) and the length of the scaffold ($L$), i.e. $\frac{\mu \cdot L}{\kappa}$.

The steps described are summarized in Darcy’s law (Eq.1), where $v$ and $\Delta P$ are defined as

$$v = v_{\text{scaffold}} = \frac{v_{\text{column}} \cdot A_{\text{column}}}{A_{\text{scaffold}}} \quad (4)$$

and

$$\Delta P = P_{\text{measured}} - \rho \cdot g \cdot L \quad (5)$$

where

- $v_{\text{scaffold}}$ Fluid velocity through the scaffold [m/s]
- $v_{\text{column}}$ Fluid velocity within the column [m/s]
- $A_{\text{column}}$ Cross sectional area of the column [m$^2$]
- $A_{\text{scaffold}}$ Cross sectional area of the scaffold [m$^2$]
- $h$ Water level within the column [m]

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L$</td>
<td>Length of the scaffold</td>
<td>$20 \cdot 10^{-3}$ [m]</td>
</tr>
<tr>
<td>$A_{\text{column}}$</td>
<td>Cross sectional area of the column</td>
<td>$1.26 \cdot 10^{-3}$ [m$^2$]</td>
</tr>
<tr>
<td>$A_{\text{scaffold}}$</td>
<td>Cross sectional area of the scaffold</td>
<td>$1.77 \cdot 10^{-4}$ [m$^2$]</td>
</tr>
<tr>
<td>$g$</td>
<td>Gravitational acceleration</td>
<td>9.81 [m/s$^2$]</td>
</tr>
</tbody>
</table>

Table 3: Parameters and their values used in Darcy’s law and the equation for the Reynolds number for the permeability experiments.
Because the kinetic term \( \frac{1}{2} \rho \cdot v^2 \) in \( P_{\text{measured}} = \rho \cdot g \cdot h + \frac{1}{2} \rho \cdot v^2 \) was relatively low compared to the pressure of the water within the column, this term was neglected. Hence, \( P_{\text{measured}} = \rho \cdot g \cdot h \).

2.4. Mechanical testing

All scaffolds were mechanically tested to determine their mechanical properties and fatigue strength by static and dynamic tests.

2.4.1. Static mechanical testing

The static mechanical tests were carried out using an Instron 5500R mechanical testing machine with a 100kN load cell and Bluehill v3.61 software to control the machine and record the measurements. According to the standard ISO 13314:2012 [34], a constant deformation rate of \( 10^{-2} \)/s, corresponding to 1.2 mm/min for all samples with a height of 20mm, was applied to three samples of every type of scaffold. The sample was placed between two flat hard metal machine platens and only vertical movement was allowed. When the limit of the 99kN, or a displacement of 16mm was reached, the test was terminated. The strain was measured by the displacement of the crossheads. As described in ISO13314 [34], the plateau stress \( \sigma_{pl} \) was determined as the arithmetical mean of the stresses between 20% and 30% compressive strain. The quasi-elastic gradient was determined by determining the slope between the strains and stresses within the elastic gradient of the stress-strain curve. The plateau end stress \( \sigma_{130} \) is the point in the stress-strain curve at which the stress is 1.3 times the plateau stress (Figure 4). The yield stress \( \sigma_y \) was found by the intersection of the stress-strain curve and the line parallel to the quasi-elastic gradient line at a strain offset of 0.2%. During the static mechanical tests, barreling was observed in some samples. Barreling is a defect caused by friction at the interface of the machine platens and the end surfaces of the scaffold, and causes the sample to become barrel-shaped. To determine if this defect could be reduced by reducing the friction at the interface, tests with and without molybdenum disulfide (MoS\(_2\)) lubricant were performed. For each test, three samples of every type of scaffold were used.
2.4.2. Dynamic mechanical testing

A Materials Test System (MTS) testing machine was used for compression-compression fatigue experiments. Three samples of every type of scaffold were tested at a constant force ratio $R=0.1$ ($R = F_{\text{min}} / F_{\text{max}}$, where $F_{\text{min}}$ and $F_{\text{max}}$ are the applied minimum and maximum forces, respectively [35]), using a sinusoidal waveform at a frequency of 15 Hz. The maximum force ($F_{\text{max}}$) applied during the fatigue tests was defined as 60% of the plateau stress ($\sigma_{\text{pl}}$), which was derived from the static mechanical tests without lubricant. The test was terminated when the sample was fractured, or when $10^6$ cycles were reached without macroscopic failure of the scaffold. To see the cracks within the scaffolds after fatigue testing, one scaffold of every TPMS geometry was embedded. The embedded samples were ground with P320, P800 and P1200 SiC paper, polished with 3µm diamond suspension, and observed using an Olympus BX60M light optical microscope (LOM).
3. Results

3.1. Morphological properties

For every unit cell, the porosity decreases with increasing sheet thickness, and the surface area decreases with decreasing porosity (Table 4). The porosities measured with the dry weighing method lay between 71.3-49.2%, 65-44.3%, 65.6-52% and 59.7-44.2% for the primitive, I-WP, gyroid and diamond scaffolds, respectively. These values are similar to the porosities of the design porosity and the values retrieved from the micro-CT scans (Table 4). A lower surface area was observed for the primitive and diamond scaffolds compared to the I-WP and gyroid scaffolds. During micro-CT, spheres are fitted into the struts (Figure 5 left) and pores (Figure 5 right) to determine their size. In general, the pore size decreases with increasing strut thickness and decreasing porosity. Scaffolds with a similar porosity such as P700, I600, G700, and D600, show different values for the strut thickness, pore size and surface area (Table 4).

<table>
<thead>
<tr>
<th>Porosity $\phi$ [%]</th>
<th>Strut thickness [µm]</th>
<th>Pore size [µm]</th>
<th>Surface area [µm$^2$]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Design</td>
<td>Dry weight</td>
<td>Micro CT</td>
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<tr>
<td>P500</td>
<td>76,64</td>
<td>71,30</td>
<td>71,48</td>
</tr>
<tr>
<td>P600</td>
<td>61,73</td>
<td>59,90</td>
<td>61,03</td>
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<tr>
<td>P700</td>
<td>55,74</td>
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<td>65,01</td>
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<td>51,36</td>
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<td>47,90</td>
</tr>
<tr>
<td>D500</td>
<td>61,29</td>
<td>59,74</td>
<td>46,68</td>
</tr>
<tr>
<td>D600</td>
<td>56,54</td>
<td>52,40</td>
<td>40,18</td>
</tr>
<tr>
<td>D700</td>
<td>48,84</td>
<td>47,77</td>
<td>36,55</td>
</tr>
<tr>
<td>D800</td>
<td>43,05</td>
<td>44,24</td>
<td>35,13</td>
</tr>
</tbody>
</table>

Table 4: Morphological parameters of the different types of scaffolds.
3.2. Permeability

The permeability of the scaffolds was determined using a computational fluid dynamics (CFD) simulations and experiments.

3.2.1. Computational values of permeability

Higher fluid velocities (indicated in red) are found at a larger distance from the pore walls and in areas where the void spaces become narrower (Figure 6). In the primitive unit cell, the fluid was mostly directed in the vertical direction (Figure 6a), while clear flow paths are observed within the other unit cells (Figure 6). The two void spaces in the I-WP unit cell are good to distinguish (Figure 6b). One pathway goes straight down, while the other is a combination of four streams entering the pores from the corners in the top plane. These streams merge in the centre and split again to flow through the pores in the corners at the bottom (Figure 6b). Both the primitive and I-WP unit cell show spaces devoid of fluid in horizontal pathways. The fluid flows within the gyroid unit cell are twisting around each other, with flows merging and splitting in both void spaces (Figure 6c). Similar to the primitive and I-WP unit cells, this unit cell has void spaces that connect the top and bottom of the unit cell in a straight line. However, the direction of the flow is continuously changing due to merging flows from the same void space. In the diamond unit cell, the flows are hard to distinguish. There are two similar flows consisting of a diagonal pathway and a circular hole. In each void space the flows are continuously merging and splitting (Figure 6d).

The lower fluid velocity in the primitive and gyroid unit cells (Figure 6a,c) are caused by the lower inlet pressure applied to these geometries. The computational permeability values found for the different apparent densities varied between $1.29 \cdot 10^{-9}$ m$^2$, and $1.7 \cdot 10^{-10}$-$1.54 \cdot 10^{-9}$ m$^2$ for the laminar model and turbulent model, respectively.
Sixty-four samples were tested (four samples of each of the type of unit cell with four different apparent densities). The measurements were repeated five times for every scaffold, resulting in twenty measurements for every type of scaffold.

A graph with $\Delta P$ as a function of flow velocity $v$ through the sample was used to determine the experimental values for the laminar region (Figure 7). The permeability values (Figure 8) were found to be dependent on the apparent density of the scaffolds, i.e. the permeability decreases with increasing apparent density. Furthermore, the permeability depended on the geometry of the unit cell, regardless of the apparent density of the scaffolds (Figure 8). For example, the I-WP scaffolds have a lower permeability than the diamond scaffolds up to an apparent density of 43%. It was found that the permeability decreases with increasing flow velocities due to growing inertial effects (Figure 8). The highest ($6.10 \cdot 10^{-9} \text{ m}^2$) and lowest ($5.48 \cdot 10^{-11} \text{ m}^2$) permeability values for the laminar regime were found for the P500 and I800 scaffolds, respectively. For the pressure difference of 9000Pa, the permeability varied between $4.92 \cdot 10^{-11} \text{ m}^2$ and $4.77 \cdot 10^{-10} \text{ m}^2$.

3.2.2. Experimental values of permeability

Figure 6: Flow direction and velocity from the laminar COMSOL simulations with a pressure difference of 250 Pa. a. primitive (P500) with $\Delta P=2\text{ Pa}$, b. I-WP (I500) with $\Delta P=10\text{ Pa}$, c. gyroid (G500) with $\Delta P=6\text{ Pa}$ and d. diamond (D500) with $\Delta P=10\text{ Pa}$.
Figure 7: Example of a graph with $\Delta P$ as a function of flow velocity $v$ through the scaffold, based on the experimental data of the P600. For all scaffolds, the slope in the extrapolated region (grey) was determined for Reynolds numbers $1 < Re < 10$ (indicated by the green and red vertical lines). As described in Darcy’s law, this slope is equal to the reciprocal of the permeability ($\kappa$), multiplied by the length of the sample ($L$) and the dynamic viscosity of the fluid ($\mu$), i.e. $(\mu \cdot L)/\kappa$.

Figure 8: Permeability values derived from the experiments for the different apparent densities of the TPMS scaffolds. Green and blue dots represent the permeability values obtained from the laminar region $1 < Re < 10$ and for a pressure gradient of 9000Pa (turbulent flow).

For all geometries, the permeability values of both the experimental and computational approaches are similar in the turbulent regime ($\Delta P=9000Pa$). The computational values
are lower for all geometries, with the exception of the primitive structure (Figure 9). The opposite held for the laminar regime, where the computational values are higher than the experimental values. The difference between the permeability determined for a pressure of 9000Pa and the permeability for the laminar regime with \(1 < \text{Re} < 10\) decreases with increasing apparent density (Figure 9).
Figure 9: Permeability values derived from the CFD simulations and experiments

3.3. Mechanical tests

Nine samples of every type of scaffold were used for the mechanical tests. Six samples were subjected to static compression with or without lubricant (three for each test) and three to compression-compression fatigue tests. The stress-strain curves obtained from the static compression tests were used to derive different mechanical properties. These include the plateau stress \( \sigma_{pl} \), quasi-elastic gradient, plateau end stress \( \sigma_{ples} \), and yield stress \( \sigma_y \). The values shown in the following graphs are the averages of the three samples of every type of scaffold used for the tests.

3.3.1. Static compression tests

Different failure modes were observed for the different types of scaffolds during the static compression tests (Figure 10). These failure modes are dependent on the geometry of the unit cell and could be related to the stress-strain curves (Figure 11, Figure 12). The primitive and gyroid scaffolds, and I-WP scaffolds with a low apparent density showed barreling, where the mid height of the scaffold is bulging out and layers collapsed onto each other (Figure 10b). The I-WP with a higher apparent density and diamond scaffolds failed due to shear band localization (Figure 10a,c). One or multiple shear bands (Figure 10a,c) were observed in these scaffolds. In the primitive scaffolds with the highest density, shear bands were visible, but no shear fracture occurred (Figure 10d).

Figure 10: Different failure modes of samples during the static compression tests. a) two shear lines in different directions, b) barreling, c) one shear line direction, d) diagonal collapsing of layers
The stress-strain curves were typical of porous biomaterials [36, 37] with the same stages of deformation and same features including the linear increase in stress with strain, a relatively long plateau region with and fluctuating stresses, and finally a region of rapid increase in stress (Figure 11, Figure 12).

It was observed that the peaks and valleys in the stress-strain curves (Figure 11, Figure 12) were caused by the formation of shear lines and the build-up of stresses after the load is transferred to neighboring struts or unit cells. The stress-strain curves of the diamond scaffolds (Figure 11, Figure 12) have a short yield plateau, after which the curves demonstrated large levels of irregularity due to shear fracture of the scaffolds. The stress-strain curves remained largely similar when the samples were tested with or without lubricant (Figure 11, Figure 12). Some samples of the D700 and D800 failed under pure shear before 20% strain was reached. Because the plateau stress, \( \sigma_{pl} \), is defined as the mean stress between 20% and 30% strain, this value could not be determined for these samples. An overview of the scaffolds after the compression tests is presented in Appendix A.
Figure 11: Stress-strain curves for the primitive, I-WP, gyroid and diamond scaffolds with four different porosities of the static compression tests performed with a strain rate of 1.2mm/s
Figure 12: Stress-strain curves for the primitive, I-WP, gyroid an diamond scaffolds with four different porosities of the static compression tests performed with a strain rate of 1.2mm/s and MoS2 lubricant.
For all types of scaffolds, the plateau stress, $\sigma_{pl}$, increased with increasing apparent density (Figure 13). A slightly higher plateau stress was observed for the samples tested with lubricant. Because the plateau end stress is defined as 1.3 times the plateau stress, the same relations were found between the stress and apparent density (Figure 14).

Figure 13: Plateau stress ($\sigma_{pl}$) for the different porosities of the primitive, I-WP, gyroid and diamond scaffolds. The ‘+’ sign indicates the values of the tests with MoS$_2$ lubrication. The square red (D700+) and blue (D800+) markers in the diamond figure indicate that these average values are not based on three, but on two and one measurements respectively, due to failure of one or two sample before 20-30% strain.

Figure 14: Plateau end stress for the different porosities of the primitive, IWP, gyroid and diamond scaffolds. The ‘+’ sign indicates the values of the tests with MoS$_2$ lubricant. The square red (D700+) and blue (D800+) markers in the diamond figure indicate that these average values are not based on three, but on two and one measurements respectively, due to failure of one or two sample before 20-30% strain.
The values of the quasi-elastic gradient of the primitive, I-WP, and gyroid samples tested without lubricant increased almost linearly with increasing apparent density (Figure 15). For all scaffolds, the values for the quasi-elastic gradient was reduced with the application of lubricant at the interface of the platens and the end surfaces of the scaffold.

![Figure 15: Quasi-elastic gradient for the different porosities of the primitive, I-WP, gyroid and diamond scaffolds. The '+' sign indicates the values of the tests with MoS2 lubrication.](image)

It can be observed that the yield stress shows slightly higher values for all scaffolds tested with lubricant, with the exception of the gyroid scaffolds (Figure 16).

![Figure 16: Yield stress for the different porosities of the primitive, IWP, gyroid and diamond scaffolds. The '+' sign indicates the values of the tests with MoS2 lubrication.](image)
3.3.2. **Fatigue behavior of the scaffolds**

The primitive scaffolds showed the shortest fatigue life with a maximum amount of approximately $3 \cdot 10^4$ cycles. The fatigue life of the I-WP, diamond and gyroid scaffolds varied between $10^5$ and $7 \cdot 10^5$ cycles. It was observed that the primitive and I-WP scaffolds show a slight increase in cycles to failure as the apparent density increased, while the opposite held for the gyroid and diamond scaffolds (Figure 17). Generally, the fatigue samples of all types of unit cells failed under a $45^\circ$ angle (Figure 18, Appendix C). Two types of scaffolds from the diamond and I-WP structures, namely the D500 and I800, respectively, were still intact after $10^6$ cycles (Figure 17). The graphs of the fatigue tests with stress as a function of cycles to failure can be found in Appendix C (Figure 20).

![Graphs of fatigue behavior](image)

**Figure 17:** Number of cycles to failure for the different apparent densities of the primitive, I-WP, gyroid and diamond scaffolds. The blue and green square markers for the I800 and D500 in the I-WP and diamond figures indicate that these samples were still intact after $10^6$ cycles.

![Failure images](image)

**Figure 18:** Shear failure of scaffolds after fatigue tests. a. primitive, b. I-WP, c. gyroid, d. diamond
In the primitive and gyroid scaffolds, crack initiation was observed at the inside of the unit cells (Figure 19a,c). The optical images of the I-WP show crack development at manufacturing imperfections and small pores that are present in the design (Error! Reference source not found.b). The weak parts of the geometry seem to be the connections between two unit cells, and struts in the vertical direction. Although no macroscopic damage on the diamond (D500) scaffolds was observed after $10^6$ cycles, cracks were present within the scaffold (Figure 19d). These cracks initiated in the struts at the periphery of the scaffold and propagated to the centre via the vertical struts (Figure 19d). Cracks were also visible in the corners that connect the horizontal and vertical struts (Figure 19d).
4. Discussion
In this study, sixteen scaffolds were tested based on four different types of unit cells with four varying porosities. Their morphological properties were determined with micro-CT scans and the dry weighing method. Permeability values were retrieved from experimental and computational approaches, and their mechanical properties were determined with static mechanical tests and fatigue testing.

4.1. Morphological properties
The biggest pore size was found in the primitive scaffolds. The results from the micro-CT images show that the pore size in general increase with increasing porosity and a reduction in sheet thickness. The pore size is determined by BoneJ, which computes the size by fitting spheres into the pores of the scaffolds. Unit cells other than the primitive unit cell do not contain spherical pores, resulting in multiple small spheres fitted into one pore (Figure 5), which gives a wrong indication of the actual pore size. Therefore, micro-CT may not be the most suited method to accurately determine the pore size of non-spherical pores.

The porosities obtained in this study with micro-CT and dry weighing showed small differences. Although differences between these methods were found, they both give a good indication of the porosities. Where all pores are taken into account (the macro pores and the micro pores within the material) during the dry weighing method, only the macro pores are included in the micro-CT measurements. On the other hand, the measurements from the micro-CT depend on spatial image resolution [38], and the manually chosen brightness threshold, the algorithm (Bernsen) used for the auto local threshold, and its radius value. Comparison of the porosities of the scaffolds with their design porosity shows that selective laser melting is an accurate manufacturing technique to produce complex geometries when the process parameters are optimized.

The design of biomimetic materials elicits specific cellular responses and guides new tissue formation mediated by specific interactions, which can be manipulated by altering the design parameters such as the pore size, porosity, and the pore shape. Bigger pores and a higher porosity reduce the seeding efficiency and cell aggregation [20, 39-43]. The lower cell aggregation in these scaffolds enables more cell migration and a better oxygen and nutrient perfusion to the inner regions of the scaffold [41]. This improves the cell viability and the proliferation rate in scaffolds with bigger pores[1, 41, 42, 44], and eventually the formation of new bone.

The average pore sizes obtained in this study lie between 360µm and 896µm. Although it was found that scaffolds with pores bigger than 162µm showed a high escape level during seeding [43], the complex geometries and big surface area of the scaffolds used in this study would enable the retention of cells during seeding. Due to the complex geometries of the I-WP, gyroid, and diamond scaffolds, cell suspension is forced along the big surface area to which the cell can adhere (Figure 6). The primitive scaffolds would have the highest cell escape level, due to the mainly vertical flow paths in these scaffolds (Figure 6). During the bone regeneration process, the formation of blood
vessels (angiogenesis) is important for the supply of nutrients, oxygen and stem cells. These vessels grow from existing vessels from the outside to the inside of the scaffolds, and are guided by the shape of the scaffold architecture [45]. Therefore, the growth of blood vessels will follow the direction of the flow presented in Figure 6, but can also form branches in all directions. Because the void pathways within the primitive unit cell are the least complicated, the most efficient vascularization would take place within these scaffolds. In the other scaffolds, the vessels would probably grow many branches to reach the centre. It was found that scaffolds with pore sizes between 400-590µm are preferable for angiogenesis. Since the pores in this study cover this range and are interconnected, angiogenesis, bone formation, and bone ingrowth are likely to occur.

4.2. Experimental and computational approaches for permeability

The graphs of the permeability values show a discrepancy in the values for the apparent density of the scaffolds (experimental data) and the unit cells (CFD simulations). This mismatch was caused by the manner in which the scaffolds were assembled before building. For the assemblage of the scaffolds, the sides of the unit cells needed to be flat to make a good connection between two neighboring unit cells. Therefore, 'material' was removed from the unit cells. The apparent density was computed by the volume of a the unit cell divided by the volume of a solid cube with the same outer dimensions of the unit cell. By removing material from the sides of the unit cell, the size of the unit cell and the solid cube reduced, resulting in a higher apparent density. Because the I-WP and primitive scaffolds have relatively flat sides compared to the gyroid and diamond unit cells, the most material was removed from latter two. This led to a higher discrepancy in the apparent densities of these unit cells and scaffolds.

The pore size derived from the micro-CT data was used to determine the Reynolds number. The Reynolds number indicates whether the flow is laminar, turbulent or transitional. Because the Reynolds numbers 1<Re>10 were assumed to define the laminar regime of the flow, the permeability values were derived in this region. However, different methods are available to determine the pore size, resulting in different Reynolds numbers for the same fluid velocity. Therefore, the chosen method to determine the pore size indirectly affects the retrieved permeability values.

It was found that the permeability depends on both the porosity of the scaffolds as well as on the geometry of the unit cells. A higher porosity (lower apparent density) was associated with a higher permeability. This relation was also found by a study on the permeability of human vertebral trabecular bone [46] and Ti6Al4V scaffolds [38].

Differences between the experimental permeability values determined for a pressure of 9000Pa and the laminar regime (1<Re>10) with increasing apparent density can be explained by the applicability of Darcy's law. Darcy's law shows a linear relationship between the flow rate and pressure difference. Therefore, the permeability remains constant in the laminar region. However, when the flow becomes turbulent, energy dissipates due to inertial effects, which reduce the flow rate and thus the permeability. This can be seen in Figure 8, where the permeability for the pressure difference of
9000Pa is much lower than the value determined for the laminar region. The big differences in the graphs of the primitive and gyroid scaffolds for the lower apparent densities indicate that the flow is turbulent at 9000Pa. The small difference between these values in the graphs of the primitive and I-WP scaffolds with the highest apparent density suggest that the flow is almost laminar at a pressure difference of 9000Pa.

It was observed that the permeability values of the laminar CFD simulations were higher than the values derived from the laminar regime of the experiments. This may suggest that the limit of the laminar regime of the unit cells may lay at a higher Reynolds number than 10. In this case, the permeability determined for this laminar model is relatively lower in the laminar regime, resulting in a higher value for the permeability. The opposite holds for the values derived for the pressure drop of 9000Pa. Where the Reynolds number for the experiments lay between Re=10 and Re=167, the Reynolds number of the simulations lay between Re=356 and Re=8039. At 9000Pa, the flow in the simulations may therefore be relatively more turbulent, resulting in the overall lower values compared to the experimental values.

The permeability values obtained in this study experimentally, varied between 5.48\cdot10^{-11} and 6.10\cdot10^{-9}\,\text{m}^2. These results are within the range of the permeability of 1\cdot10^{-11}-4.7\cdot10^{-9}\,\text{m}^2 in trabecular bone samples of the human proximal femur [18]. The overall lower permeability values of the diamond structure can be explained the tortuosity of the geometry. Tortuosity describes the relationship between the length of the flow path and the length or thickness of a sample/unit cell along the pressure gradient [47]. A higher tortuosity indicates that the streamlines in the porous medium are not completely straight and parallel to each other [48]. In the primitive, I-WP, and gyroid scaffolds, part of the fluid can flow straight through the geometry, while this is not possible in the diamond structure. Therefore, the highest tortuosity is found in these scaffolds, followed by the I-WP, gyroid and primitive scaffolds.

The permeability of a scaffold determines the ease with which cell suspension and body fluids can flow through the scaffold. A lower permeability is associated with a lower fluid velocity, giving the cells more time to adhere to the surface of the pore walls during seeding. In the primitive scaffolds, where the permeability is the highest, i.e. the flow velocity is the highest, a low cell seeding efficiency is likely. The lower permeability in the diamond scaffolds makes these scaffolds the most suited for cell seeding. On the contrary, a lower permeability limits the perfusion of nutrients and oxygen [38], resulting in a low cell viability and proliferation [1, 41, 42, 44], which will limit bone regeneration.

4.3. Mechanical behavior - failure modes

Different failure modes were found during the static mechanical tests with and without lubricant. Barreling was observed for the primitive and gyroid scaffolds, where the cross section in the vertical plane of the sample becomes barrel-shaped. This failure mode is partly caused by the friction at the interface between the platens and the end surfaces of the sample [49]. During shortening of the sample, the friction at the platen-
implant surface interfaces prevents deformation at the ends of the scaffold compared to the mid of its height, where the material is free to deform [50, 51]. The increased deformation at the mid-height of the sample generates high circumferential strain, which causes vertical cracks in the hoop plane. The I-WP and diamond scaffolds showed more brittle fracture, dividing the samples in several pieces, caused by shear. This happened with little yielding prior to failure, which can be observed in the stress-strain curves form the static compression tests (Figure 11, Figure 12).

4.4. Mechanical behavior – characteristic values
Assessment of the mechanical properties of scaffolds with novel designs, manufactured with selective laser melting, is necessary to ensure that the scaffold properties are within the range of human trabecular bone. In the early stages of implantation, the mechanical properties of the scaffold matching those of trabecular bone are important for mechanical support [52]. The quasi-elastic modulus is a measure for the stiffness of a material, i.e. the greater the modulus, the smaller the deformation resulting from the application of a given stress.

The material used in this study, Ti6Al4V, has an elastic modulus of approximately 109-120GPa [53, 54], which is higher than the elastic modulus of human trabecular bone (0.1–5GPa) [55]. The elastic modulus of the Ti6Al4V implants used in this study was reduced by increasing the porosity, as well as by the shape of the unit cells. The quasi-elastic moduli found in this study lie between 3.2GPa and 6.4GPa. The lowest value of 3.2GPa was found for the scaffolds based on the primitive unit cell, with a porosity of 71%, an average pore size of 896µm, and a strut thickness of 243µm. This result is similar to the homogenized elastic modulus of 3.5GPa found by Yavari et al., 2015 for Ti6Al4V scaffolds based on dodecahedron unit cells with a strut thickness of 230µm, a pore size of 580µm, and a porosity of 68%. Although the quasi-elastic gradient, pore size and strut thickness lie close to each other, smaller cylindrical implants (a diameter of 1mm and a height of 15mm) were used. This indicates that not only the porosity, but also the geometry of the unit cell affects the implant stiffness. The geometry of the unit cells affect the mechanical properties by the amount of struts oriented in the direction of the applied load.

The diamond and I-WP scaffolds are characterized by struts in the vertical and horizontal direction, while the gyroid and primitive scaffolds have a more wave-like and rounded structure. In these latter geometries, stresses are more likely to distribute evenly, while stresses can more easily build up in the I-WP and diamond structures. The difference in these geometries could explain the smooth stress-strain curves of the primitive and gyroid scaffolds. The smooth stress-strain curves of the gyroid, I500, I600, and primitive scaffolds can be assigned to their failure mode (Figure 10b). In this mode, the layers of the scaffolds are compressed gradually on top of each other without the occurrence of visible fractures or shear band formation. The high amount of peaks and valleys in the stress-strain curves of the diamond and I-WP scaffolds is related to the formation of shear bands (Figure 10a,c). These shear bands were formed after
stresses were built up within the struts. The stress-strain curves show peaks at the point where the stress concentration within the struts is the highest. When shear band formation or shear fracture occurs (Figure 10c), stresses are released and a valley becomes visible in the stress-strain curves (Figure 11, Figure 12). The stress-strain curves show that the plateau region becomes shorter when the apparent density increases. This was also found by a study on Ti6Al4V foams manufactured by a magnesium space holder technique [56]. The length of the plateau region is thought to be related to the porosity of the structure [56].

The quasi-elastic modulus of some scaffolds was higher than 5GPa, which is the maximum elastic modulus found for trabecular bone [55]. Therefore, only the P500, P600, I500, G500, G600 and the D500 would be suitable as bone implant when stress shielding needs to be avoided.

4.5. Effect of MoS2 on mechanical properties

Some differences in mechanical properties of the scaffolds were found when MoS2 lubricant was applied to the scaffolds during the static compression tests. Slightly higher values for the plateau stress, plateau end stress, and elastic gradient were observed, while lower values for the quasi-elastic gradient were found. The shapes of the compressed samples of the tests with and without lubrication did not show clear differences. Therefore, it can be concluded that the use of MoS2 lubrication did not significantly reduce the friction between the platens and the samples. This may be explained by 1) the lubricant got entrapped between the struts of the end surfaces of the scaffold and 2) different platens were used in these tests compared to the non-lubricated tests. The latter may also have had effect on the different values of the mechanical properties. Because the platens were loose steel plates, the measured deformation and stresses were of the combination of the scaffold and the plates. It was found that up to a load of 30kN, no deformation of the platens was present. However, when the load increased to 50kN, plastic deformation of the platens was observed (Appendix B). The damage of the platens increased the surface roughness, which in turn increased the friction at the interface of the platens and the sample surfaces. The amount of friction due to the anchoring of the end surfaces of the scaffold into the platens depended on the surface roughness of the unit cells. The lowest surface roughness of the gyroid unit cell led to the least difference in the mechanical properties of the static mechanical tests with and without lubricant. The highest differences were found for the primitive unit cell, which has the highest surface roughness. To determine if the application of lubricant can reduce the friction at the interface, damage to the platens should be prevented by the use of platens with a high hardness which are part of the machine.

4.6. Fatigue tests

The different types of scaffolds showed different fatigue lives when they were loaded with a sinusoidal load between 6% and 60% of their yield strength (Figure 17, Figure 20). It was found that all primitive scaffolds were fractured before $10^6$ cycles, while the
fatigue life of the I-WP, diamond and gyroid scaffolds varied between $10^4$ and $7 \cdot 10^5$ cycles. Samples of the I800 and D500 were still intact after $10^6$ cycles. The shorter fatigue life of the primitive scaffolds could be related to the clear difference of the geometry and the connection between adjacent primitive unit cells and the other unit cells (Figure 1). Where the I-WP, diamond and gyroid scaffolds form a complex structure in which unit cells are hard to distinguish, the primitive scaffolds do not. These scaffolds have only a small circular connecting area at every side of the unit cell, while the other geometries have a larger connecting area with adjacent unit cells.

A frequency of 15Hz was used for the fatigue tests, which is higher than the average frequency for a walking speed of 1.4m/s (approximately 2.0Hz) [57, 58]. While the applied frequency is higher than the walking frequency, it can be assumed that frequency effects up to 200Hz can be neglected in fatigue design and testing when temperature effects and corrosion can be neglected [59].

The fatigue life of scaffolds for bone regeneration is important, because the scaffold should provide support until the newly formed bone is developed well enough to take over the loads applied to the implant. Although the bone regeneration process depends on different parameters that describe the architecture of the scaffold, within a few months the bone should be supportive enough and the implant will only be present to guide new bone and blood vessel formation. Approximately $2 \cdot 10^6$ loading cycles per year is would be applied on the implant in case a patient moves 3 hours a day [51]. Although the fatigue life of the primitive scaffolds is lower than $2 \cdot 10^6$ cycles per year, the load applied to the scaffold within the body will be much lower than used in this study. Moreover, the load on the scaffold decreases with further progress of bone formation [60]. In a study by Yavari et al., 2015, different percentages of the yield stress were applied to see the effect on the fatigue life of the samples. It was found that the fatigue life increases when a lower percentage of the yield stress is applied to the scaffolds [61]. Considering the maximum load in the vertical direction during a walking speed of 5km/h, the load on the knee and hip joint are around 3 and 4.5 times the bodyweight, respectively [62]. With this assumption, the maximum load applied to the scaffold would be about 4500N, which is half of the maximum load used in the fatigue tests for the primitive unit cells with the lowest apparent density.
5. Conclusion
This study showed that the type of TPMS unit cell and its apparent density affect the permeability and mechanical properties of scaffolds for bone regeneration. A higher apparent density is related to a lower surface area, a lower permeability, and higher values for mechanical properties such as quasi-elastic gradient, plateau stress, plateau end stress, and yield stress. Scaffolds with more vertical struts, such as the I-WP and diamond geometries, showed more brittle behavior during static compression. The primitive and gyroid scaffolds behaved more ductile, resulting in smooth stress-strain curves. The use of MoS\(_2\) lubricant did not significantly reduce the friction at the interface of the platens and the end surfaces of the scaffolds. This might be explained by the use of platens with a lower hardness compared to the platens used during the static compression tests without lubrication. The scaffolds based on the primitive unit cells showed the shortest fatigue life, which may be caused by the relatively low surface area with which these unit cells are connected to each other. It could be concluded that the P500, P600, I500, G500, G600 and D500 scaffolds would be suitable as scaffolds for bone regeneration, based on their mechanical properties. Of these scaffolds, the P500 is the most promising for bone regeneration, because this scaffold has the most space for oxygen and nutrient perfusion, cell proliferation, angiogenesis, and eventually bone formation.
References


Appendix A - static mechanical tests, shape of scaffolds after compression
In the following pages, the shapes of the scaffolds are displayed. The scaffolds after the static compression test without and lubricant, are presented on the left and right side, respectively. Similar failure modes were observed for both tests.

**Primitive**

*P500*

*P600*
Gyroid

G500

G600
Diamond

D500

D600
Appendix B – Failure modes of static compression tests with lubrication

Different failure modes were observed during the static compression tests. The pictures below give an overview of the failure modes for the different types of TPMS scaffolds. Damage to the platens occurred during the test, which may be one of the reasons why different mechanical values were found for the scaffolds tested with and without lubricant.

**Primitive**

P500  
P600  
P700  
P800

**I-WP**

I500  
I700  
I800

**Gyroid**

G500  
G600  
G700  
G800

**Diamond**

D500  
D600  
D700  
D800

*Damage of the platens used during compression tests with MoS2*
Appendix C – Fatigue tests - Stress-cycles to failure graphs

The graphs show the strain as a function of the cycles to failure. It was found that the primitive scaffolds had the shortest fatigue life. The D500 and the I800 were still intact after $10^6$ cycles.

Figure 20: Strain-cycles to failure curves for the four different types of unit cells with the four different porosities performed with 60% of the yield stress and a frequency of 15Hz. a) Primitive, b) I-WP, c) Gyroid, d) Diamond
Fatigue tests - failure modes

Primitive

I-WP