Comparison of titanium dioxide scaffold with commercial bone graft materials through micro-finite element modelling in flow perfusion

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ABSTRACT

TiO_2 scaffolds have previously shown to have promising osteoconductive properties in previous *in vivo* experiments. Appropriate mechanical stimuli can further promote this osteoconductive behaviour. However, the complex mechanical environment and the mechanical stimuli enhancing bone regeneration for porous bioceramics have not yet been fully elucidated. This paper aims to compare and evaluate mechanical environment of TiO_2 scaffold with three commercial CaP biomaterials i.e. Bio-Oss, Cerabone, Maxresorb under simulated perfusion culture conditions. The solid phase and fluid phase were modelled as linear elastic material and Newtonian fluid, respectively. The mechanical stimulus was analysed within these porous scaffolds quantitatively. The results showed that the TiO_2 had nearly heterogeneous stress distributions, however lower effective Young's modulus than Cerabone and Maxresorb. The permeability and wall shear stress (WSS) for the TiO_2 scaffold was significantly higher than other commercial bone substitute materials. Maxresorb and Bio-Oss showed lowest permeability and local areas of very high WSS. The detailed description of the mechanical performance of these scaffolds, which could help researchers to predict cell behaviour and to select the most appropriate scaffold for different *in vitro* and *in vivo* performances.

Keywords: Scaffold, Finite element method, Titanium dioxide, Micro-CT, CFD.

1 Introduction

In oral and orthopaedic surgery, large bone defects caused by trauma, tumours or bone resorption usually do not heal naturally. The defect cannot be self-healing if bone defect was larger than a critical size [33]. The diameter varies from species to species and varies upon skeletal defect. The defect sites can be repaired and reconstructed by bone tissue engineering principles [50,16]. The main reasons to apply bone scaffolds are to provide an environment for bone formation, maintain the space and at the same time supply mechanical support to the skeleton during the healing process [16,50]. From natural and synthetic materials, a variety of bone graft substitutes were developed [18]. Synthetic materials can be made on demand, mass-produced and with tailored pore structure. There are many important features for synthetic bone substitutes; one is to withstand the mechanical load on the defect once implanted. Additionally, the fluid flow through the porous scaffold is known to influence osteogenesis through mechanical stimulation of bone precursor cells [27].

Real geometry of scaffold can be acquired and reconstructed non-destructively based on micro-computed tomography images (μCT), and fluid velocity, fluid pressure and fluid shear stress generated by fluid flow within pores can be analysed quantitatively using computational fluid dynamics (CFD) [6,44,41,29]. In addition, the tensile or compressive strain sensed by the cells under load in a body can be evaluated by μFEM [31,41,36]. Previous studies have shown that parameters of pores, such as porosity, size and shape, play an important role on mechanical stimuli on the scaffold surface [4,12,11]. Considering the influence of inlet velocity and viscosity, Sandino et al. investigated mechanical environment of calcium phosphate bone cement and porous phosphate glass with irregular morphology quantitatively based on FE models obtain from µCT [41]. However, the study on accurate micro-mechanical environment for a variety of porous bioceramics by taking into account the structural parameters, materials and loading conditions *in vitro* has been insufficiently investigated in the current literature.

TiO_2 scaffold has shown excellent biocompatibility, high porosity with interconnective pores and sufficient compression strength [13,46,39] which is very suitable as an ideal bone graft substitute material. As a novel scaffold material, TiO_2 scaffold has exhibited excellent bone healing in several *in vitro* and *in vivo* experiments [34,49,16]. Commercial scaffolds such as Bio-Oss® (Geistlich Pharma AG, Switzerland) and BoneCeramic® (Institut Straumann AG, Switzerland) exhibit different morphology [40]. However, the influence of mechanical stimuli and fluid flow in these synthetic biomaterials has not yet been thoroughly investigated. As there seems to be a correlation between *in vivo* performance and morphology of the porous structure for bone graft material [23,14,26], it is evidently important to simulate and compare the new developed bone graft materials, such as the investigated TiO_2 scaffold with other commercial bone graft materials.

The purpose of this study was to compare mechanical environment and fluid flow within a novel TiO_2 scaffold with the other three commercial bone graft materials (Bio-Oss®, BoneCeramic® and Maxresorb®) by finite element analysis (FEA). The four scaffolds were analysed quantitatively using FEA in combination with computational fluid dynamics (CFD) based on micro-CT images. The morphologies of the four bone graft substitutes were observed by scanning electron microscopy (SEM), and pore morphological parameters were quantified by micro-CT. The influence of fluid flow direction, the influence of fluid viscosity, and the influence of inlet velocity on hydrodynamic environment were investigated.

2 Materials and Methods

2.1 Preparation of scaffolds

Three commercial bone graft substitutes and a custom-made titanium dioxide (TiO_2) bone graft substitute were used in this study as listed in Table 1. The TiO_2 bone graft substitute was prepared from commercial TiO_2 powder (Kronos Titan GmbH, Germany) using polymer foam replication method as previously described [46]. The TiO_2 scaffolds have been optimized for many years and it has been shown in previous publications that the standard deviation between batches are not significant [46,13,39]. Geistlich Bio-Oss® (Geistlich Pharma AG, Switzerland) is made from natural bovine bone. The structure is similar to human bone. Purification and sterilization were performed by placing it in a high temperature for 15 hours for Cerabone® (AAP Biomaterials GmbH, Germany), all the organic compounds, proteins in bovine bone were removed by high-temperature sintering, and potential immune response was eliminated. Maxresorb® (Botiss Dental GmbH, Germany) is a synthetic bone graft substitute, and its component is 60% hydroxyapatite and 40% beta-tricalcium phosphate.

2.2 Characterization of pore morphologies

The pore architecture of the four scaffolds was analysed from the reconstructed 3D datasets processed with the software CTAn 1.14 (Bruker microCT, Belgium). Porosity and interconnectivity of the three-dimensional non-destructive bone substitute materials were determined as previously described [46,47]. A parametric study of pore structures was performed in Mimics 14 (Materialise, Belgium) to do the Boolean operation and to evaluate the morphologies of the four scaffolds.

2.3 Reconstruction of biomaterial scaffold

Four scaffolds of 5 mm diameter and 2.5 mm height with different morphologies were used: TiO_2, Cerabone, Bio-Oss, Maxresorb (Table 1). The samples were scanned on 5.98 μm voxel size resolution using a table top microCT system (Skyscan 1172, Bruker microCT, Belgium). The three-dimensional samples were reconstructed using Mimics 14 (Materialise, Belgium), and four 1.5 mm^3 scaffold structures with different morphologies were obtained (Figure 1). Threshold was segmented and three-dimensional solid models were established using Mimics 14 (Materialise, Belgium). The pore part of fluid domain was obtained by Boolean operation with a cubic model of 1.5 mm^3 in Mimics [24]. The fluid and solid models were remeshed in 3-Matic 6.0 (Materialise, Belgium), and then volume mesh was created in ANSYS ICEM 16.2 (ANSYS Inc, USA). The solid and fluid mesh models were created for the finite element analyses. Nodal interconnections were maintained at the interface of the two phases. The solid phase and fluid phase represent the scaffold material and the pore volume, respectively. Solid mesh was used to simulate uniaxial compression test in a bioreactor, and pore mesh was used to simulate fluid flow under perfusion culture condition. Four-nodal three-dimensional tetrahedral elements for the material and the interconnected pores were made in ANSYS ICEM 16.2 (ANSYS Inc, USA). In addition, grid independence test was performed for each of the scaffold by different grid size, the calculation carried out for solid and fluid phases was performed in ANSYS Mechanical 16.2 (ANSYS Inc, USA) and ANSYS Fluent 16.2 (ANSYS Inc, USA) for each grid, and relatively static value in outlet was observed. It was found that when it reaches the number of grid in Table 1, the accuracy will not be significantly improved when increase the number of grid. Furthermore it can be judged, grid has already meet the calculation requirements to a certain extent.

2.4 Simulation of fluid environment within pores

The fluid analysis simulated a perfusion bioreactor as previously implemented [44,43]. Simulation of interstitial fluid flow was performed in ANSYS Fluent 16.2 (ANSYS Inc, USA). Inlet fluid velocity was 34 μm/s [24], and no-slip conditions were assumed for the wall. Fluid pressure of the outlet side was set as zero. The inlet velocity 34 μm/s was between 0.01 mm/s (lowest) and 1 mm/s (highest) in previous study [51], and bone tissue differentiation can be sufficiently promoted when the inlet fluid velocity of 0.01 mm/s, cartilage differentiation results was better when the inlet velocity was 0.1 mm/s. However, which may cause unexpected cell activity (growth or death) by too high or additionally low fluid shear stress, therefore, 34 μm/s was chosen as inlet velocity to obtain suitable stimuli for cells.

The influence of viscosity and inertia force were compared by calculating Reynolds number (Re) (Equation 2), where the density of the culture medium was assumed to be ρ=1000 kg/m^3, d is the trabecular spacing, and V is the average fluid velocity. The viscosity of the culture medium was set to η=0.851×10^{-3} Pa s [24].

$$\mathrm{Re} = \frac{\rho V d}{\eta} \tag{2}$$

Since the Reynolds number was less than 1, laminar flow was assumed and subsequently laminar flow analysis was performed for the simulated perfusion fluid flow system. Distributions of fluid velocity, static pressure, fluid shear stress in cross-section were evaluated using Matlab R2012b (The Mathworks, USA).

By comparing with the permeability of cancellous bone (or scaffold) measured by experiment, the modelling of the scaffold was verified [15,35]. The permeability for macro-porous models was calculated according to Darcy's law defined as

$$Q = K(-\frac{\mathrm{d}P}{\mathrm{d}x}) \tag{3}$$

where Q is the volume flow rate, K is the coefficient of permeability, and $\mathrm{d}P/\mathrm{d}x$ is the gradient of pressure. If the same section is modelled as a Newtonian fluid flow, the permeability can be defined as

$$K = \frac{Q\eta\Delta x}{\Delta P} \tag{4}$$

where η is the fluid viscosity, Δx is the length across which fluid flows through the scaffold, and ΔP is pressure difference from inlet to outlet [10].

Fluid shear stress and fluid pressure acting on the wall of the scaffolds combined with fluid velocity streamlines were analysed for the fluid analyses. The influence of fluid flow direction, the influence of fluid viscosity, and the influence of inlet velocity on hydrodynamic environment were investigated. For each material, three levels of viscosity, three levels of inlet velocity, and two levels of inlet fluid flow directions were used as shown in Table 2 [35].

2.5 Analysis of the solid models

The solid phase was modelled as linear elastic material according to compression test (Table 3). Uniaxial strain of 0.5% was applied on the nodes of upper side of mesh, and the nodes of the lower side were fixed to simulate an unconfined compression test. According to Zhang et al. [51], 0.5 % uniaxial strain was most conducive to generate bone tissue; therefore 0.5% uniaxial strain was adopted. The principal strain on the surface of the scaffolds was calculated using ANSYS Mechanical 16.0 (ANSYS Inc, Pittsburgh, USA). The mechanical properties of scaffolds are described by the effective Young's modulus E_f (Equation 1), where R is the reaction forces on the bottom, A is the total cross-sectional area of scaffold model, $\varepsilon = (\Delta l / l)$ is axial strain.

$$E_f = (R / A) / (\Delta l / l) \tag{1}$$

3 Results

3.1 Characterization of the scaffold structure

The morphology of each bone graft substitute was visualized by SEM and quantified by micro-CT analysis. The SEM images of the four different biomaterials are shown in Figure 2, and Figure 3 (A) shows the calculated pore diameter distributions for the four samples provided by the micro-CT analysis. Figure 2 (A) shows Bio-Oss bone graft substitute, which exhibits large pieces of parallel plate-like structure. Figure 2 (B) shows Cerabone bone graft substitute, the morphology of which is composed of trabeculae with different pore sizes. Figure 2 (C) shows TiO_2 bone graft substitute, which consists of a highly interconnected porous network. Figure 2 (D) shows Maxresorb bone graft substitute, whose structure consists of spherical pores of different sizes, and many small pores in scaffold were not interconnected. In this case, the fluid cannot reach very small pores of the scaffold. Compared with the three commercial bone graft substitutes, the fabricated TiO_2 bone graft substitute showed the highest porosity and highest number of interconnected pores. The pore diameters for the four different scaffolds were mainly between 50 and 750 µm (Figure 3 A). Furthermore, the mean pore diameters for Maxresorb (140 µm) was smaller than for the three other materials. Maxresorb had also wider pore size distribution than the other tested materials. The most narrow pore size distribution was found for TiO_2 scaffold with a range of 50-550 µm. The highest porosity was found for TiO_2 scaffold (86.0%), followed by Cerabone (69.0%), Maxresorb (67.5%) and Bio-Oss (60.0%) (Table 4). The mean strut thickness was lowest for TiO_2 scaffold (50.4 µm) and highest for Bio-Oss (158.5 µm). The specific surface area was similar for three of the tested materials, apart from Maxresorb, which had slightly higher values (7.23 mm). The interconnective pore sizes were much higher for Cerabone and TiO_2 scaffold (Figure 3 B). The connective pores were smaller for Bio-Oss and Maxresorb, where only 50% of the porous volume was accessible with connection size less than 100 µm for Bio-Oss and 250 µm for Maxresorb.

3.2 Distribution of fluid velocity, fluid pressure, and fluid shear stress

Considering the fluid dynamics inside the porous materials, three properties were simulated, namely fluid velocity, fluid pressure and fluid shear stress. The streamlines of velocities revealed a highly tortuous behaviour, which can be seen in Figure 4. The highest value of average pressure over the whole surface was observed in the Bio-Oss and Maxresorb scaffolds (Figure 5 andTable 5). These two materials had also the lowest permeability rate. This can explain that the highest value of static pressure appeared due to the smaller channels or interconnection, which is typical for the Bio-Oss pore structure

(Figure 2 A, Figure 4 A). A reduced change of static pressure was observed for the TiO_2 scaffold, and this value was lower when compared to the other scaffolds. The fluid pressure declined gradually from inlet to outlet in the TiO_2, Cerabone and Maxresorb scaffolds, whereas abrupt changes took place in the Bio-Oss scaffold (Figure 4). A fluctuation of static pressure in a higher range was observed in the Maxresorb scaffold. Fluid shear stress at inlet was consistent with fluid velocity profile for Cerabone and TiO_2 scaffold (Figure 6). The higher values of fluid shear stress appeared between 0-1.5 mPa. The fluid simulations showed that the fluid flowed through smaller cross-sectional areas for Bio-Oss and Maxresorb. These two bone graft materials also exhibited wider range of fluid shear stress, fluid pressure and fluid velocity, for which particularly Maxresorb had high values (Figure 4, 5 and 6).

3.3 Influence of inlet fluid flow direction, fluid viscosity and inlet velocity on hydrodynamic environment

The influence of the different inlet velocities can be seen by examining fluid shear stress (Maximum) while the viscosity was set to 1.45×10^{-3} Pa s as the same setting in previous study (Figure 6 E) [36]. Changing inlet fluid flow side was found to have significant effect on hydrostatic pressure (Table 6). The values of fluid shear stress increased as the inlet velocity increased proportionally. The highest hydrodynamic pressure was seen for Bio-Oss, which also had the lowest fluid velocity. The effect of different viscosities on maximum fluid shear stress was also considered when the inlet velocity was 10 μm/s (Figure 6 F). In order to compare with Sandino et al. [42], the inlet velocity was set to 10 μm/s. In our study, the resulting WSS (Figure 6 F) showed the value of fluid shear stress increase as the viscosity increased, and was highest for

Maxresorb (Table 6). TiO_2 scaffold exhibited highest fluid flow but moderate fluid shear stress. Cerabone showed lowest fluid flow and yet also lowest shear rates.

3.4 Distribution of strain

In order to compare with Sandino et al., the compressive load was set to 0.5% [42]. When the four scaffolds were under a 0.5% compressive load, there were less changes of von Mises strain on Bio-Oss and Cerabone scaffolds than on the other models (Figure 7). Strains on the surface were higher at thin rod-shaped struts for the TiO_2 scaffold. More heterogeneous values of strains were observed on the Maxresorb scaffold (Figure 7 D). The statistical distribution of major principle strain reveals that compressive strain areas were larger than tensile strain areas under compressive loads (Figure 7 E). Most compressive strains were between 0% and 0.05%. For Bio-Oss scaffold, the compressive strain area between -0.05% and 0% was larger than the others tested bone graft materials.

3.5 Validation study for permeability

Indeed the literature range is very large and it includes both experimental and numerical simulation results (Table 5). As it can be seen from the results that cancellous bone structure [35,10,15,45] has large range of permeability, while the permeability of idealized structure [2,36] and the current study was similar, and in the lower range of values. Permeability of the scaffolds was calculated according to Darcy's law (Table 5). The highest permeability was seen for TiO_2 scaffold and lowest for Maxresorb (Figure 8). Our permeability values obtained computationally were comparable to cancellous bone as described by Grimm and Williams [15] and Nauman et al. [35] and also similar to other studied bone graft materials using computational studies [3,10,36,45].

4 Discussion

One of aim was to quantify the mechanical properties of three different commercial bone graft materials under compressive loading and fluid flow and compare them with those of a novel TiO_2 bone graft substitute. Mechanical stimulus acting on cells at the initial bone formation stage was estimated based on finite element analysis. In this study, uncoupled solid and fluid mechanical models were investigated considering neither complex chemical and biological reactions nor cell migration and proliferation processes.

Pore structures and morphology play a crucial role on cell growth, vascular ingrowth and mechanical stimuli transferred in scaffold [25,23,28,37,21]. Similar to real bone structure, pore shape, size and distribution of the biomaterials were irregular (Figure 3); however, the pores were not completely interconnected for Maxresorb (Figure 3 B). Compared with the scaffolds consisting of idealized unit cell, this irregular structure can provide more real physiological environment for cells [51]. Where, the mean pore diameters of Bio-Oss and Cerabone scaffold were larger than mean pore size of the TiO_2 (Figure 3 A). The mean pore diameters were more consistent with favourable range of pore size (300-400 μm) for cell growth and Harversian osteoid formation [48].

Considering scaffold as a porous structure, the ability of material exchange can be characterized by interconnectivity and permeability. The predicted permeability of these scaffolds in present study was within range of cancellous bone by comparative analysis, which may affect the rate of cell migration and bone ingrowth for bone regeneration [22]. TiO_2 had higher pore interconnectivity and higher permeability (1.678×10^{-9} m^2) (Table 5) which was more conducive to nutrient transport and metabolic product excretion, and furthermore to improve *in vivo* bone ingrowth [32]. While Maxresorb had lowest permeability (0.031×10^{-9} m^2) (Table 5), which may enhance cell seeding efficiency [19], whereas induce more formation of cartilage instead of bone [20]. The value of permeability for Maxresorb was similar to the value (0.03×10^{-9} m^2) obtained by Hui et al. [17], which may be favourable for vascularisation and mineralisation within the implant. Maxresorb and Bio-Oss had fewer and smaller interconnections (Figure 3 B), which explains the low permeability rate and the higher observed shear rate. These small interconnective pore sizes functions a restriction (such as the throat of a convergent-divergent nozzle or a valve in a pipe) into a lower pressure environment and thus the fluid velocity increases.

Mechanical stability of scaffolds is essential to provide the necessary mechanical support for the recruited cells during the healing of bone defects. The compressive strength of TiO_2 scaffold was higher than 3.4 MPa, which is within the range of human jaw trabecular bone [16]. The E_f of Maxresorb was the highest (25972.1 MPa) (Table 5), which may be related to the complex morphology, smaller overall pore size and lower porosity. The E_f of TiO_2 was lower (4899.1 MPa), which may be resulted due to higher porosity. In fact, the TiO_2 scaffold had 17-25% higher porosity than the other materials (Table 4). When compared to other studies, the calculated Young's modulus of the present scaffolds was higher than the other studies [17]. Strain is an effective mechanical stimulus to stimulate MSCs. Appropriate compression and tensile strain can be beneficial to bone formation when compressive load is applied [30]. One limitation of this study was the values of the strains within scaffold might be affected because deformation generated by fluid was ignored. Compared with the regular models [51], inhomogeneous structure formed heterogeneous distributions of stress and strain.

In this study, a small region (1.5 mm^3) was analysed. According to study of Maes et al., 2009, to use only a small portion may cut off channels of interconnected pores, which may lead to unrealistic BCs [24]. Perfusion improves mechanical environment within scaffold, while providing a higher seeding efficiency than static seeding or mechanically stirred bioreactor, and a better uniform distribution of cells [44]. Hydrodynamic environment of scaffold (fluid pressure, fluid velocity and fluid shear stress) under perfusion culture are affected by pore morphology parameters (pore size, porosity, interconnectivity, etc.) [42,5]. The average fluid shear stress in TiO_2 was significantly higher than other samples (Table 5), but also with higher fluid velocities. The result (1.46 mPa) obtained by Maes et al. (2009) [24] using hydroxyapatite was about half of our measured fluid shear stress (2.55 mPa), which may be due to the relatively low porosity of their samples. With the same average pore diameter, WSS of Bio-Oss and TiO_2 varied widely, showing the pore size is not the only factor for WSS. At the same time, with similar porosity and pore size, Bio-Oss and Cerabone have similar average WSS. *In vitro* studies have shown that the WSS can be the regulator for inducing osteogenic differentiation [9]. WSS lower than 0.05 mPa may cause cells to proliferate, while values higher than 56 mPa may lead to apoptosis or cells to be washed away [38]. According to the studies of Sandino et al., and Cartmell et al., 37-46 mPa shear stress can stimulate osteoblast differentiation into bone cells [42,8]. In this study, WSS of TiO_2 and Maxresorb were widely distributed and many high values appeared most likely due to the irregular pore structures in this material. Sandino et al., saw similar high values for velocity and shear stress, and Sandino et al observed that the distribution of the fluid pressure decreased from the inlet to the outlet [42]. However, it has been shown previously that differentiation of the adhered bone cells will be induced when exhibiting wall shear stress within the region that we observed (WSS: from 1.35 to 2.55 mPa, Table 5) [8].

Medium viscosity and inlet velocity are important boundary conditions in the perfusion fluid flow system. Consistent with previous studies [42,10,2], fluid shear stress increased linearly in proportion with viscosity increase under low fluid flow (Figure 6 F). Due to irregular shape of the analysed porous materials, one might expect small local regions with higher velocities and turbulence flow behaviour. In this study, the fluid shear stress increases linearly with increasing inlet fluid velocity (Figure 6 E). This was consistent with the observation by Sandino et al. [42]. A suitable WSS can be obtained on the surface of a scaffold by performing *in vitro* culture by adjusting inlet velocity to facilitate the differentiation and growth of MSCs [51,36].

The results showed that the examined bone graft materials could generate sufficient shear stress to stimulate osteogenic and differentiation of MSCs by adjusting inlet velocity. These results contribute to promote the formation of bone tissue by sensitivity of MSCs adhered to the surface of different materials to mechanical stimuli (fluid shear stress, fluid pressure, solid strain). In addition, repair of bone defects could be improved by selecting a bone graft substitute material with appropriate mechanical properties to transfer optimal mechanical stimulus to the adhered cells regenerating bone tissue. Different levels of stimulus were found amongst the investigated bone scaffolds. The WSS was found to be very sensitive to viscosity and boundary conditions. High WSS values were found for Bio-Oss, which may lead to undesirable cell behaviour inside the porous structures. Even though conflicting data exist on the outcome of placing Bio-

Oss® in e.g. extraction sockets in humans, several authors shows that Bio-Oss® particles placed in extraction sockets were, 3–7 months later, mainly surrounded by connective tissue [1,7]. The high WSS seen in our simulation could be one of the reasons why little bone is typically seen inside the Bio-Oss.

5 Conclusions

The use of computer simulations for the development of medical devices or for their use as a pre-clinical tool is novel and the subject of research of great interest. There is currently a strong drive amongst the politicians and the EU research council to try to reduce the number of *in vivo* experiments and develop better *in silico* tools for predicative behaviour of medical devices. This study provides detailed information regarding the influence of pore morphology, fluid flow and mechanical properties on mechanical environment of three commercial bone graft material (Cerabone, Bio-Oss, and Maxresorb) compared with a novel porous titanium dioxide scaffold intended for bone tissue engineering applications. The results showed that the TiO_2 has nearly heterogeneous stress distributions, whereas lower effective Young's modulus than both Cerabone and Maxresorb. The permeability and wall shear stress (WSS) for the TiO_2 scaffold was significantly higher than other commercial bone substitute materials. These findings favours the TiO_2 scaffold for further study in clinical trials. Maxresorb and Bio-Oss showed lowest permeability and very high WSS at local areas, which could predict inferior clinical performance.

Conflict of interest

Tiainen and Haugen holds patents behind the technology for the TiO_2 scaffolds (EP Patent 2,121,053, US Patent 9,629,941 US Patent App. 14/427,901, US Patent App. 14/427,683, US Patent App. 14/427,854). The rights for these patents are shared between University of Oslo and Corticalis AS. Haugen is a shareholder and board member of Corticalis AS.

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Figure Captions

Figure legend graphical abstract: Schematic representation of the establishment procedure. Take the establishment process of cerabone as example. Left shows a slice of Micro-CT image from cerabone, and 1.5 mm × 1.5 mm region of interest was shown in the red box. A 1.5 mm^3 cube was cut out by boolean operation in Mimics (Materialise, Belgium), and the cubic model was remeshed in 3-Matic 6.0 (Materialise, Belgium). The cubic model is shown in blue, and the empty space in red.

Fig. 1 Simulation procedure of scaffold with bone substitute material. Take the simulation process of TiO_2 as example. A 1.5 mm^3 cube was cut out by boolean operation in Mimics (Materialise, Belgium). The boundary conditions were imposed on the solid phase and the fluid phase, separately. The solid phase is shown in grey, and the fluid phase in blue. For the fluid flow model (right), side A or side B is used as inlet fluid flow.

Fig. 2 SEM micrographs of four bone graft substitutes with typical microscopic appearances of each bone graft substitute. A: Bio-Oss, B: Cerabone, C: TiO_2, and D: Maxresorb.

Fig. 3 (A) Pore size of the four different scaffolds; (B) Interconnectivity of the four different scaffolds through openings smaller than 350 mm in diameter.

Fig. 4 (A-D) The cross-sectional view of static pressure on the walls in combination with streamlines color-coded according to velocity magnitude when the inlet velocity was 34 μm/s. Flow is from top to bottom. (E, F) Distributions of fluid velocity, static pressure in cross-section of the four scaffolds. A: Bio-Oss, B: Cerabone, C: TiO_2, and D: Maxresorb.

Fig. 5 Static pressure distributions on scaffold wall when the inlet velocity was 34 μm/s. Flow is from top to bottom. A: Bio-Oss, B: Cerabone, C: TiO_2, and D: Maxresorb.

Fig. 6 (A-D) Wall shear stress distribution in combination with streamlines color-coded according to velocity magnitude when the inlet velocity was 34 μm/s. Flow is from top to bottom. The outer wall of the fluid was removed in order to visualize the internal fluid flow. (E, F) The influence of inlet velocity and viscosity on WSS was shown for 4 different scaffolds. (G) Distributions of fluid shear stress in cross-section of the four scaffolds. A: Bio-Oss, B: Cerabone, C: TiO_2, and D: Maxresorb.

Fig. 7 (A-D) Von mises strain contours of scaffolds with four different bone graft substitutes structures under 0.5% compressive strain. (E) Major principal strain distribution on scaffold surface under overall compressive strain of 0.5%. In the figure, the tensile strain region and the compressive strain region were divided by a vertical line.

Fig. 8 Comparison of permeability results for experimental studies, computational studies and current study.

Tables

Table 1 Bone graft substitute materials used in current study. All materials have CE label and is available for the European market.

Abbreviation	Product name	Producer	Material
TiO_2	Titanium dioxide	Corticalis AS	TiO_2
Bio-Oss®	Bio-Oss® Spongiosa granules	GeistlichPharma AG	Natural bone mineral of bovine origin
Cerabone®	Cerabone®	Botiss dental GmbH	Bovine hydroxyapatite
Maxresorb®	Maxresorb®	Botiss dental GmbH	60% HA and 40% β-TCP

Table 2 Three levels of fluid viscosity, four levels of inlet fluid velocity, fluid density and two kind of inlet fluid flow side used for the parametric study of the fluid flow. For inlet fluid flow side see figure 1.

Viscosity (Pa s)	Inlet fluid velocity (μm/s)	Density (kg/m^3)	Inlet fluid flow side
	1		
0.7×10^{-3}			
	10		A
1.45×10^{-3}		1000	
	34		B
2.1×10^{-3}			
	100		

Table 3 Material properties imposed for the parametric study of the solid model.

Samples	Young's modulus (GPa)	Poisson's ratio
Bio-Oss	15[a]	0.3[a]
Cerabone	83[b]	0.28[b]
TiO_2	230[c]	0.29[c]
Maxresorb	102[c]	0.276[c]

[a]Miranda et al., 2008 [b]Birmingham et al., 2015 [c]Ebrahimian-Hosseinabadi et al., 2011

Table 4 Pore morphological parameters (mean strut thickness, mean pore diameter, interconnectivity, porosity, surface area-to-volume ratio (SA/V) and specific surface of area) and elements number of the solid and pores mesh with standard deviation.

Sample	Mean strut thickness (μm)	Mean pore diameter (μm)	Porosity (%)	SA/V (mm^2/ mm^3)	Specific surface of area(mm)	Solid mesh	Pores mesh
Bio-Oss	158.5 ± 15.3	320 ± 56.7	60.1 ± 3.4	14.02 ± 0.98	5.61 ± 0.36	1947058	2660664
Cerabone	117.4 ± 11.8	300 ± 43.2	69.0 ± 3.8	18.89 ± 1.3	5.88 ± 0.35	1631219	3127267
TiO_2	50.4 v 4.9	320 ± 35.1	86.0 ± 4.5	40.13 ± 2.8	5.59 ± 0.41	931903	4102310
Maxresorb	62.3 ± 5.6	140 ± 33.6	67.5 ± 3.6	22.22 ± 1.6	7.23 ± 0.43	2939265	2809344

Table 5 Effective Young's modulus (E_f), permeability (K), average values of fluid velocity, fluid pressure and fluid shear stress within pores of the samples.

Sample	E_f (MPa)	K ($10^{-9}m^2$)	Average Velocity (mm/s)	Average Pressure (mPa)	Average WSS (mPa)
Bio-Oss	4466.7	0.30	0.038	40.1	1.35
Cerabone	6834.2	0.60	0.041	22.7	1.44
TiO_2	4899.1	1.68	0.037	32.9	2.55
Maxresorb	25972.1	0.031	0.034	47.7	1.98

Cases with inlet velocity = 10 μm/s, viscosity = 1.45×10^{-3} Pa s are presented.

Table 6 Maximum fluid velocity, hydrostatic fluid pressure and wall shear stress within the samples.

Sample	Inlet fluid flow side	Max. velocity (mm/s)	Hydrostatic fluid pressure (Pa)	Wall shear stress (mPa)
Bio-Oss	A	5.61	66.75	25.02
	B	4.23	48.37	20.61
Cerabone	A	1.06	39.63	13.33
	B	1.02	12.65	8.44
TiO_2	A	24.53	28.41	25.36
	B	31.7	42.25	28.12
Maxresorb	A	9.98	36.64	40.12
	B	13.91	38.65	46.82

Cases with inlet velocity = 10 μm/s, viscosity = 1.45×10^{-3} Pa s are presented. Inlet fluid flow side (A & B) see figure legend graphical abstract.