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3D Titanium Scaffold Properties and Osteogenesis of Stem Cells
(Sifat Perancah Titanium 3D dan Osteogenesis Sel Stem)

ROHAYA MEGAT ABDUL WAHAB*, MANAL NABIL HAGAR, NUR ATMALIYA LUCHMAN, FARINAWATI YAZID & SHAHRUL HISHAM ZAINAL ARIFFIN

ABSTRACT

Studies on porous titanium for use in dental applications have been growing due to their excellent properties such as low elastic modulus, biocompatibility and excellent strength. The porosity and pore size of titanium scaffold play an important role in bone formation. Thus, this paper reviews the properties of titanium scaffold and the relationship between the porosity and pore size of titanium with the osteogenesis of stem cells in respect of its mechanical properties and biological assessment. From this review, it was found that a pore size of less than 300 μm allows for good vascularization that can lead to direct osteogenesis without an interphase of cartilage formation. The minimum requirement for pore size is approximately 100 μm to assist in the migration requirement, cell size and transport, as a smaller pore size causes a hypoxic condition and induces osteochondral formation before osteogenesis, while a pore size from 500 to 1000 μm affects the differentiation of the stem cells. In addition, it was found that high porosity induces osteogenesis. The average porosity of the scaffold for cell proliferation was between 25-50 μm. In conclusion, highly porous titanium is a useful modern material for creating 3D structures for bone regeneration and implant fixation.

Keywords: Bone regeneration; pore size; porosity; stem cell; titanium scaffold

ABSTRAK

Kajian mengenai titanium berliang telah berkembang pesat dalam aplikasi pergigian disebabkan oleh ciri-ciri istimewanya seperti modulus kenyal, bioserasi dan kekuatan bahan. Keliangan dan saiz liang perancah titanium memainkan peranan yang penting dalam proses pembentukan tulang. Ciri-ciri perancah titanium dan hubungan antara keliangan dan saiz liang titanium dengan osteogenesis sel stem serta perkaitannya dengan sifat mekanik dan penilaian biologi diulas dalam artikel ini. Daripada ulasan ini, didapati bahawa saiz liang yang kurang daripada 300 μm membenarkan vaskularisasi yang baik yang boleh menyebabkan osteogenesis secara langsung tanpa interfasa daripada pembentukan rawan. Saiz liang minimum yang diperlukan adalah lebih kurang 100 μm dan ia sesuai bagi saiz sel dan pengangkutan serta membantu dalam migrasi sel kerana saiz liang yang lebih kecil boleh menyebabkan keadaan hipoksia dan merangsang pembentukan osteokondral sebelum osteogenesis. Saiz liang antara 500 hingga 1000 μm pula memberi kesan kepada pembezaan sel stem. Selain daripada itu, didapati bahawa peningkatan keliangan juga dapat merangsang osteogenesis. Purata keliangan bagi perancah untuk membantu proliferasi sel adalah antara 25-50 μm. Kesimpulannya, titanium yang mempunyai keliangan yang tinggi sesuai menjadi bahan moden untuk pembentukan struktur 3D bagi penjanaan semula tulang dan penetapan implan.

Kata kunci: Keliangan; penjanaan semula tulang; perancah titanium; saiz liang; sel stem

INTRODUCTION

Titanium and titanium alloys, based on their physical and chemical properties, appear to be especially suitable for dental implants, prostheses and many load-bearing orthopaedic applications. For the construction of endosseous implant devices, titanium and its alloys have become well-accepted and can be considered the materials of choice (Dabrowski et al. 2010). Titanium scaffold is considered to offer a new opportunity in dental science to enhance tissue formation due to its porosity that accommodates cell proliferation and differentiation (Rakhmatia et al. 2013). As compared to other types of scaffold (Table 1), the titanium scaffold fulfils the purposes of scaffolds such as biocompatibility, the ability to be sterilized, mechanical stability, and porosity, and also promotes osteogenic differentiation (Tamaddon et al. 2017). Titanium is highly recommended for use in dental applications because of its excellent mechanical properties that help to stabilize the bone graft under the membrane. Its elasticity prevents mucosal compression; its stability inhibits graft displacement; its rigidity prevents contour collapse, and its porosity accommodates cell proliferation and differentiation, which will eventually enhance tissue formation. Furthermore, its plasticity permits bending and adaptation to any bony defect (Rakhmatia et al. 2013). Stem cells are required in most research that involves cell differentiation because they have the unique ability to self-renew and differentiate into many cell types (Yazid et al. 2011). Porous surfaces are important in bone formation because they permit the mesenchymal stem cells to proliferate, provide greater mechanical stability and improve the mechanical interlocking between the titanium and surrounding bone. Large pores allow good vascularization that leads to direct osteogenesis, while small pores induce osteochondral formation before osteogenesis. The porosity and pore size of titanium scaffold play a significant role in bone regeneration (Karageorgiou & Kaplan 2005).

TABLE 1. Comparison of the properties of different scaffolds

		Types of scaffold	Examples	Advantages	Disadvantages	Properties
1	Inorganic material (Li & Wozney 2011)					
	a.	Ceramic	Hydroxyapatite (HA), tricalcium phosphate (TCP)	Widely used because the chemical nature is identical to the bone	Brittle and slow biodegradation (O'brien 2011)	Excellent biocompatibility, high mechanical properties, very low elasticity, promotes cell adhesion and enhances osteogenic differentiation (O'brien 2011)
	b.	Non-ceramic	Calcium phosphate - based cement (CPCs)	CPCs are injectable so can be handled by surgeon and set in the bone site (O'brien 2011)	Exothermic reaction, lack of macroporosity, and their intrinsic radioopaque nature cause difficulty in detecting the healing of bone (Li & Wozney 2011)	High solubility *in vivo* which increased the bone formation
	c.	Calcium sulphate ($CaSO_4$)	$CaSO_4$	Cost reduction, and rapid resorption (Asadi-Eydivand et al. 2018)	Poor mechanical properties (Asadi-Eydivand et al. 2018)	Good stability, degradation, and poor mechanical properties
	d.	Metals	Titanium scaffold	Non-toxicity, excellent biocompatibility, high porosity and surface area	Not biodegradable (Chocholata et al. 2019)	Good biocompatibility, sterilized, promotes osteogenic differentiation, mechanical stability, strength, along with permeability and corrosion resistance (Dabrowski et al. 2010)

e.	Bioglasses (silicate and borate compositions)	Biogran (BIOMET 3i, Palm Beach Gardens, FL), Novabone (Nova-Bone Products LLC)	High solubility which increases the bone regeneration (Hoppe & Boca 2014)	Lack of porosity	Good mechanical properties, high solubility, and non-cytotoxic degradation (Fernando et al. 2016)
2	Natural polymers	Fibrin, hyaluronic acid, proteoglycan, alginate, chitosan and collagen (decalcified bone matrix (DBM), fibrillar collagen, and gelatin)	Excellent biocompatibility, rapid resorption, and identified in the early stages of wound healing (Alaribe et al. 2019)	Disease transmission and sterilization	Excellent biocompatibility, high porosity which increases cell infiltration and vascularization, poor mechanical properties (O'brien 2011), biodegradable with low toxicity, supports cell adhesion and growth (Thavornyutikarn et al. 2014)
3	Synthetic polymers	Polystyrene, polypropylene fumarate, polyethylene glycol, poly-l-lactic acid (PLLA), polyanhydride, polyphosphazenes (Li & Wozney 2011), polyglycolic acid (PGA) and poly-dl-lactic-co-glycolic acid (PLGA) and poloxamers (O'brien 2011)	Sterilization is easy, reproducible manufacture, and tailored control	Chronic inflammatory response, complex architecture, toxic degradation (O'brien 2011) and poor clearance particularly for the high molecular weight polymer (Li & Wozney 2011)	Poor biocompatibility, potential toxic degradation and good stability (Alaribe et al. 2019)
4	Composite (combination of ceramic with polymers or combination of natural polymers with synthetic polymers)	Hyaluronic acid-impregnated PLA sponge	Takes the benefits from different materials that are exploited	Poor mechanical properties and immune-response difficulties (Chocholata et al. 2019)	Depend on the materials that are exploited

PROPERTIES OF TITANIUM SCAFFOLD AND ITS ROLE IN REGENERATION

Animal studies have reported that titanium (Ti) is a safe metal for an implant and the bone directly fused to it (Dabrowski et al. 2010). Porous titanium scaffold has many properties such as biocompatibility, the ability to be sterilized, promoting osteogenic differentiation, mechanical stability, strength, Young's modulus, along with permeability and corrosion resistance (Dabrowski et al. 2010). Both bulk material of titanium and sponge-like titanium have been used for tissue engineering purposes. Sponge-like titanium consists of titanium fibres that are sintered together to create a mesh structure. The mesh structures are advantageous due to their flexibility, strength, biocompatibility, porosity, and interconnectivity. Titanium's strength is useful for bone replacement as its flexibility distributes the stresses between the implant and tissue. In addition, the porosity of Ti allows tissue ingrowth and stabilization, in addition to which the design of titanium can be varied by varying the density and diameter of the fibres.

The biocompatibility of titanium mesh is determined by the response of the tissue and the absence of allergic reaction to titanium. A study that compared the tissue responses of three mesh materials showed that the titanium mesh induced a better tissue response than Fecralloy and stainless steel 316L (van den Dolder & Jansen 2007). In addition, this study found that titanium does not cause allergies. Pattanayak et al. (2011) showed how new bone penetrated into the pores and directly bonded to the walls after implantation of bioactive Ti into the femur of white rabbit. This scaffold was fabricated by selective laser melting and was treated chemically by NaOH and HCL to provide bioactivity. In an orthopaedic implant application, porous titanium showed a relatively high permeability but had low resistance to corrosion when compared to a cast titanium (Dabrowski et al. 2010). Pores play an important role in bone tissue formation because they allow the migration and proliferation of osteoblast and mesenchymal cells. Porosity facilitates the circulation of body fluids and encourages the growth of cells (Arifin et al. 2017). According to Karageorgiou and Kaplan (2005), direct osteogenesis was noticed in the porous scaffold, while there was no new bone formation in the non-porous scaffold, which indicated the necessity for porosity.

A study that investigated the influence of hierarchical hybrid micro/nano-textured titanium surface features on osteoblast differentiation found that this surface can promote bone formation and accelerates bone growth (Meng et al. 2013). Another type of Ti scaffold is titanium web (TW), which is flammable, unstable and difficult to prepare, and it was demonstrated that TW with a diameter of 8 mm is essential for ectopic bone formation. Also, it was determined that a higher porosity and higher pore size of TW showed greater bone formation (Amemiya et al. 2012). The scaffold for bone defect regeneration should show a high mechanical property and have open porosity to ensure bone ingrowth such as in metal. It was determined that open porosity is an important factor in osteogenesis (Otsuki et al. 2006). Salt leaching, gas foaming, phase separation, freeze-drying, and sintering are the most common techniques that are used to create porosity in biomaterials (Karageorgiou & Kaplan 2005). The pores of the structure must be interconnected in order to ensure bone growth. A porous structure can be manufactured by freeze-casting, the space holder technique, rapid prototyping and laser processing. Rapid prototyping and laser processing are able to generate porous titanium with a simple pore architecture, while freeze-casting and the space holder technique form a porous material with randomly distributed pores (Barbas et al. 2012). According to Fujibayashi et al. (2004), the macroporous structure of porous titanium block with approximately 40% porosity was more complex and effective for osteogenesis than titanium fibre mesh cylinders with approximately 40-60% porosity. This was concluded by implanting them into the dorsal muscle of a mature beagle dog after undergoing specific chemical and thermal treatments.

The minimum requirement for pore size is 100 μm due to the cell size, but a pore size greater than 300 μm was recommended, as it enhances new bone formation (Karageorgiou & Kaplan 2005). A pore size below 100 μm in an *in vivo* study showed fibrous tissue formation, while a bigger pore size (150 μm) favoured bone tissue formation (Wysocki et al. 2016). The highest cell proliferation was shown in Ti-6AL-4V alloy scaffold with 200 μm pore size (Wysocki et al. 2016). In addition, Stangl et al. (2001) showed that a 200 μm pore size is the most preferred (Wysocki et al. 2016). On the other hand, pore sizes from 500 to 1000 μm can affect the differentiation of cells (Van Bael et al. 2012). A study proved that Ti-6AL-4V alloy scaffold with pore sizes of 500, 600, 700, and 900 μm was filled by cells after 6 weeks in 100%, 44%, 10%, and 0% of the scaffold, respectively (Wysocki et al. 2016). Chen et al. (2009) demonstrated that when reducing the particle size of the titanium, the surface energy increased on the alkali-heated treated porous and non-porous titanium, which will lead to a higher apatite-inducing ability. A study showed that more bone formation was seen in rats that received two titanium scaffolds than rats with empty defects. These scaffolds have different structures (Ti-120, Ti-230) due to their different strut size. Reducing the strut scaffold size by 50% in the Ti-120 structure resulted in a large decrease of the elastic modulus and showed more bone ingrowth (Wysocki et al. 2016).

Continuous growth was noted in a random pore size scaffold, while discontinuous growth was observed in the scaffold with pores of the same size and a solid wall. Faster healing was detected in the whole space of the defect (Simon et al. 2003). Many studies have proved that high porosity induces osteogenesis. According to Luthringer et al. (2013), the optimum porosity of scaffold for cell proliferation was 25-50 μm. Porous titanium scaffold provided mechanical support in an early phase and it facilitated bone formation, resulting in a high mechanical strength of the large bone defects treated (Van der Stok et al. 2013).

COATING OF 3D TITANIUM SCAFFOLD

Fabricated porous Ti/HA may be an appropriate material for future bio-medical applications (Raza et al. 2015). Although hydroxyapatite coating is beneficial for bone formation, a study hypothesized that the titanium scaffold has an intrinsic potential to induce bone formation without the need for a hydroxyapatite coating. This was concluded by comparing titanium scaffold coated with hydroxyapatite and non-coated titanium *in vitro* (Tamaddon et al. 2017). Coating the titanium alloy surface with sintered titanium beads in the tibiae of sheep increased the strength of the implant. However, coating with hydroxyapatite did not result in a significant osseointegration (Karageorgiou & Kaplan 2005).

Acid-etched coating (higher surface roughness) for a Ti implant demonstrated a higher osseointegration when compared with grit-blasted and fibre mesh coating (lower surface roughness) in the femurs of rabbit (Karageorgiou & Kaplan 2005). Coating the titanium alloy implant of a dog mandible with 50 μm of porous hydroxyapatite did not increase the osseointegration. However, there was more bone formation in the maxilla. Therefore, it was indicated that coating with hydroxyapatite had a useful affect for areas with poor bone (Karageorgiou & Kaplan 2005). The sintering behaviour of titanium-blended 316L SS powder compacts showed a higher rate of shrinkage in titanium-blended 316L SS as compared to non-blended compacts because of the faster annihilation of porosity, which promotes osteogenesis (Aslam et al. 2014). Jemat et al. (2018) demonstrated that titanium alloys coated with yttria-stabilized zirconia (YZP/TiO2) had a higher porosity and surface roughness when compared with the pure YZP coating, which is beneficial for cell growth and attachment.

Polyether ether ketone (PEEK) is a favorable material for interbody spinal fusion. Titanium-coated PEEK had significantly greater shear strength than uncoated PEEK. The shear strength was greater in deeply porous titanium due to greater osseointegration, which is not available in the uncoated PEEK. This combination reduced the problem that results from the fibrous tissue that forms along the PEEK and it is beneficial for spinal fusion cages (Guyer et al. 2016). Smooth titanium stimulated a greater response than PEEK. However, the rough titanium alloy stimulated cells to create an osteogenic-angiogenic microenvironment. Thereby, the response was greater than with the smooth titanium (Guyer et al. 2016). Seeding bone marrow stromal cells onto titanium web (TW) indicated the efficacy of TW as a bone scaffold material (Vehof et al. 2001). A few studies of titanium web in animal experiments reported that apatite coating of TW provided better bone formation (Hayakawa et al. 2008).

Hibi et al. (2006) used tissue-engineered osteogenic material (TEOM) comprising autologous mesenchymal stem cells (MSCs), platelet-rich plasma (PRP) and calcium chloride solution with thrombin for the alveolar cleft osteoplasty of a 9-year old female patient. The alveolar cleft was supported with a titanium mesh plate to provide space without disturbing the blood supply. After nine months, post-operatively the case showed 79.1% of regenerated bone. A similar study with titanium indicated that TiO2 coating aids in bone remodelling and the growth of osteoblasts. TiO2 has a compressive strength and it secreted higher bone markers when compared with silica and calcium phosphate (Mediaswanti et al. 2013). The application of a thicker porous oxide film on the surface of titanium screws in a tibia defect showed more bone formation when compared to thinner non-porous oxide layers (Sul et al. 2002).

EFFECT OF POROSITY AND COATING IN OSTEOGENESIS

The role of porosity and interconnectivity in scaffolds is to facilitate cell migration within the porous structure so that cell growth is enabled while overcrowding is avoided. In addition, the porosity enhanced the cell proliferation rate, which might be due to the transport of nutrients and oxygen *in vitro* (Loh & Choong 2013). Many studies have proved that high porosity induces osteogenesis. Dental implants coated with structured titanium with 44% and 48% porosity. These implants were grafted in a canine mandible and femoral defect and the results showed more bone formation for the higher porosity at the initial timepoints in the mandible (2 and 4 weeks) and at all time points in the femur (14 weeks) (Karageorgiou & Kaplan 2005). More bone formation was observed in rat calvarial defects when the pore size was increased from 35.4 to 47.7%. However, when nickel-titanium implants with 66% and 47% porosity were implanted in rat femoral defects, no statistical differences could be detected in bone formation, as the porosity was between 40-60% (Kujala et al. 2003).

Many studies have suggested that the minimum requirement for pore size is above 100 μm. A study in the distal femoral cortex of rabbit showed that bone formation was delayed in laser-textured titanium alloy (Ti6Al4V) with 100 μm pore size when compared with 200 μm and 300 μm (Götz et al. 2004). This study demonstrated that 200 μm was the optimal pore size for laser-textured titanium alloy (Ti6Al4V) because the osseointegration was faster than for the other pore sizes (Götz et al. 2004). A study by Liu et al. (1999) noted that, as long as the pore size is between 100 and 300 μm and the porosity between 40-60%, no differences in bone formation can be shown. For example, nitinol implants with the same thickness and different pore sizes (353, 218, and 179 μm) with respective porosities of 43%, 54% and 51% were placed in cranial defects in rabbits and showed no differences in bone formation. Another study indicated no statistical differences in bone ingrowth, but more fibrosis was detected in nitinol implants with

505 μm pore size compared to 209 μm pore size that were implanted in rat femoral defects (Kujala et al. 2003).

The advantage of larger compared to smaller diameter porosity is that chondrogenesis occurred before osteogenesis in small diameter tunnels, while in large diameter tunnels the bone was formed directly. The reason for direct osteogenesis in the large diameter was due to the higher oxygen tension and supply of nutrients (Karageorgiou & Kaplan 2005).

THE EFFECT OF PORE SIZE AND POROSITY ON MECHANICAL PROPERTIES AND BIOLOGICAL RESPONSE OF POROUS TITANIUM SCAFFOLDS

For a successful grafting, a balance between the mechanical properties (stiffness and strength) and microarchitecture (porosity and pore size) should be achieved. Porous titanium scaffold was prepared in two types of porosities, therefore, their mechanical properties mimic the cortical and trabecular bone. Unlike the open porosity ranges that fitted well with the cortical bone, the trabecular ones were lower (Torres-Sanchez et al. 2017).

In the biological aspect, scaffolds with the lowest pore ranges (45-106 μm) showed the highest number of cell attachments in the early stage. Furthermore, the proliferation of cells is slower than the larger pore size. The optimal pore microarchitecture (i.e. pore size and porosity) for scaffolds to be used in bone grafting for cortical bone was set to < 212 μm with volumetric porosity values of 27-37%, and for trabecular tissues to 300-500 μm with volumetric porosity values of 54-58% (Torres-Sanchez et al. 2017).

CONCLUSION

Titanium has become a new class of material that is used for various dental engineering applications. Titanium and titanium alloy can be considered the materials of choice for dental implants, prostheses and many load-bearing orthopaedic applications. This review provides an overview that facilitates the understanding of the effect of porosity and pore size on the cellular behaviour and mechanical properties of a titanium scaffold. Interconnected porous scaffold networks that enable the transport of nutrients, removal of wastes, as well as facilitating the proliferation and migration of cells, are essential. The porosity and pore size influence the cell behaviour as well as determining the final mechanical property of the scaffold. Porosity is an important factor in osteogenesis, where it plays a significant role. Chondrogenesis occurred before osteogenesis in small diameter tunnels, while in large diameter tunnels the bone was formed directly. The appropriate pore size that enhances new bone formation is between 100 and 300 μm, while the average porosity of scaffold for cell proliferation is 25-50%.

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Rohaya Megat Abdul Wahab*, Manal Nabil Hagar, Nur Atmaliya Luchman & Farinawati Yazid
Centre for Family Oral Health
Universiti Kebangsaan Malaysia
Jalan Raja Muda Aziz
50300 Kuala Lumpur, Federal Territory
Malaysia

Shahrul Hisham Zainal Ariffin
Centre for Biotechnology and Functional Food
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor Darul Ehsan
Malaysia

*Corresponding author; email: rohaya_megat@ukm.edu.my

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