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Biological response of osteoblasts in contact with porous zirconia structures for bone repair.

Cláudia Inês Resende Gonçalves

Dissertação conducente ao Grau de Mestre em
Medicina Dentária (Ciclo Integrado)

Gandra, 5 de junho de 2020

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Trabalho realizado sob a Orientação do Prof Doutor Júlio C. M. Souza (PhD, MSc) e Co-Orientação do Prof Nuno Sampaio (MSc)

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Eu, **Júlio César Matias de Souza**, com a categoria profissional de **Professor auxiliar** do Instituto Universitário de Ciências da Saúde, tendo assumido o papel de Orientador da Dissertação intitulada ***Biological response of osteoblasts in contact with porous zirconia structures for bone repair,*** do Aluno do Mestrado Integrado em Medicina Dentária, **Cláudia Inês Resende Gonçalves**, declaro que sou de parecer favorável para que a Dissertação possa ser depositada para análise do Arguente do Júri nomeado para o efeito para Admissão a provas públicas conducentes à obtenção do Grau de Mestre.

Gandra PRD, 2 de junho de 2020

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O Orientador

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RESUMO

O objetivo deste trabalho foi realizar uma revisão sistemática integrativa sobre os efeitos biológicos e mecânicos de *scaffolds* ou implantes porosos de zircónia para reparo de perda óssea severa. Uma busca eletrónica foi realizada na base de dados PubMed utilizando uma combinação dos seguintes termos científicos: *porous OR scaffold OR foam AND zirconia AND bone regeneration OR bone repair OR bone healing.* A pesquisa identificou 145 estudos, dos quais 23 foram considerados relevantes tendo em consideração resultados sobre métodos de proessamento, porosidade, a interconectividade dos poros, a bioatividade, formação óssea e a resistência mecânica dos *scaffolds* de zircónia.

A alta percentagem, as dimensões e a interconectividade dos poros são fatores-chave para a migração, adesão, proliferação e diferenciação celular. Além disso, a interconectividade dos poros permite a transferência de nutrientes entre as células e a formação de vasos sanguíneos. No entanto, observou-se uma diminuição da resistência mecânica nos *scaffolds* com o aumento do número e dimensão dos poros. Zircónia policristalina tetragonal estabilizada com ítria (Y-TZP) possui propriedades mecânicas apropriadas para a fabricação de *scaffolds* ou implantes com alta porosidade para reparo de perda óssea severa. Além disso, os *scaffolds* podem ser revestidos com cerâmica bioativa para melhorar a resposta celular, angiogênese e o crescimento ósseo sem comprometer interconectividade dos poros. *Scaffolds* e implantes porosos de zircónia tornam-se uma estratégia para reparo de perda ósseo extensiva, uma vez que composição química e a rede de poros favorecem a resposta biológica desejada e manutenção do volume ósseo.

PALAVRAS-CHAVE

Poros, *Scaffolds*, Implantes, Zircónia, Reparo ósseo.

ABSTRACT

The aim of this study was to conduct a scoping review on the on the biological and mechanical effects of porous zirconia scaffolds or implants for extensive bone repair. An electronic search was performed in the PUBMED database using a combination of the following scientific terms: *porous OR scaffold OR foam AND zirconia AND bone regeneration OR bone repair OR bone healing.* The research identified 145 studies, of which 23 were considered relevant. These studies provided important data taking into account the porosity, pore interconnectivity, biocompatibility, the mechanical strength of the material and the production methods of the scaffolds.

The high percentage of porosity, the size and interconnectivity of the pores are key factors for cell migration, attachment, proliferation, and differentiation. Also the pores' interconnectivity allows the exchange of nutrients between cells and formation of blood vessels. However, a decrease of mechanical strength in the scaffolds was noted with the increase of number and size of pores. Therefore, yttria stabilized zirconia tetragonal polycrystal (Y-TZP) has mechanical properties proper to manufacturing a high porous scaffold or implants for extensive bone repair. Additionally, the scaffolds can be coated with bioactive ceramics to enhance the cell response and bone ingrowth without compromising the pores' networking. Scaffolds and porous implants composed of zirconia become a strategy for extensive bone repair since the material and pores' network provide desired biological response and bone volume maintenance.

KEYWORDS

Porous, Scaffold, Implants, Zirconia, Bone repair.

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1. INTRODUCTION

Although many biomedical materials have been suggested for bone repair and tissue engineering applications, bioactivity, design, and physical properties are still clinical limitations of current scaffolds and porous implants.(1,2) Scaffolds and porous implants should reveal the following criteria: high cytocompatibility for cell migration and differentiation, appropriate mechanical properties to support loading, and interconnected porosity to allow cell migration and nutrients exchange.(3) Bioactive ceramics are the first choice as a source material for bone reconstructive scaffolds although their mechanical properties are not proper for extensive bone repair.(4–9) The compressive strength of pure hydroxyapatite porous blocks is lower (~0.3 MPa) when compared to trabecular bone (~12 MPa), cortical bone (~200 MPa), and zirconia scaffolds (5-10 MPa).(8,9)

Zirconia has been developed for biomedical applications regarding desired properties such as: strength, fracture toughness, chemical stability, and high biocompatibility.(7–11) Zirconium dioxide (ZrO_2), known as zirconia, adopts a tetragonal structure at high temperature between 1170°C - 2370°C and a monoclinic crystal at room temperature. *In vitro* and *in vivo* studies have consistently shown that zirconia, in its various physical forms (monoclinic, cubic, and tetragonal), induced no toxic, immune, or carcinogenetic effects on cells, connective, or bone tissues.(11) Zirconium dioxide is doped with metal oxides such as yttria (Y_2O_3), magnesium or calcium, to stabilize tetragonal phase. Yttria stabilized zirconia polycrystals (Y-TZP) has an elastic modulus at 240-270 GPa, flexural strength at 1200 MPa, fracture toughness at 8 $MPa.m^{1/2}$, and a high biocompatibility.(2,5,12) The load-bearing capability of zirconia is proper to maintain bone volume and avoid continuous tissue remodeling after placement of the scaffold. Additionally, bioactive ceramics are used to cover the porous scaffolds for enhanced cell stimulation although maintaining the porosity.(7,8,10)

A scaffold or porous implant should withstand loading during handling and surgical procedures for periodontal and peri-implant therapies. In cases of extensive bone loss, the endosseous porous structure must possess mechanical properties to avoid fractures from occlusal stresses that could compromise the bone ingrowth process.(2) Porosity, pore size, and even pores' interconnectivity significantly affect cell behavior, angiogenesis, and bone ingrowth in porous ceramics(4). Introduction of interconnected pores allows for cell migration, proliferation as well as vascularization. Effective circulation of fluid and transportation of nutrients through the pores, enables bone tissue growth whereas the increased surface area of porous structures leads to better bonding with host tissues.(12,13) However, a high porosity and pore size of the scaffold or porous implant affect their mechanical properties. A balance between the desired mechanical and biological functions can be established by controlling the porosity and selection of biocompatible ceramics.(13) That is accomplishable by manufacturing the porous structures with zirconia covered or not with bioactive ceramics such as calcium phosphate-based ceramics.(11) In an *in vivo* study, porous zirconia was used as a substrate for hydroxyapatite (Hap) coating, resulting in a strong and bioactive scaffold to stimulate bone repair. In fact, zirconia enhanced the overall osteoconductivity of the scaffold and improved its mechanical properties while the bioactive coating improved the bone ingrowth.(12) Hydroxyapatite coatings or scaffolds forms an apatite outer layer which chemically react with proteins, blood platelet, and osteogenic cells.(14) Proteins and minerals also interact on zirconia leading to the activation of blood platelets and migration of osteogenic cells. The osteoconductive process follows with the attachment, proliferation, and differentiation of osteoblasts.(11)

The main aim of this study was to perform a scoping review on the beneficial effects of porous zirconia scaffolds or implants for extensive bone repair. It was hypothesized that a balanced porosity and pore size in porous zirconia structures enhance the osteogenic cell behavior, angiogenesis, and bone formation.

2. METHOD

A literature search was performed on PubMed (via National Library of Medicine) using the following combination of search terms: "porous" OR "scaffold" OR "foam" AND "zirconia" OR "bone regeneration" OR "bone repair" OR "bone healing". A manual search of the reference lists in the selected articles was also performed. The inclusion criteria encompassed articles published in the English language, until January 16th, 2020, reporting effect of porous zirconia structures on the osteoblast growth and bone repair. The eligibility inclusion criteria used for article searches also involved: articles written in English; *in vitro* testing; meta-analyses; randomized controlled trials; animal assays; and prospective cohort studies. The total of articles was compiled for each combination of key terms and therefore the duplicates were removed using Mendeley citation manager. Selected articles were individually read and analyzed concerning the purpose of this study. The following variables were collected for this review: authors' names, journal, publication year, objectives, zirconia preparation, zirconia type, porosity, pores' size and interconnectivity, bone growth, osteoblast proliferation and osteoblast viability.

3. RESULTS

The literature search identified a total of 145 articles in PubMed, as shown in Fig. 1. After reading the titles and abstracts of the articles, 92 were excluded because they did not assemble the inclusion criteria. The remnant 27 potentially relevant studies were then evaluated (Fig. 1). Of those articles, 4 studies were excluded because they did not afford comprehensive data considering the purpose of the present study. Thus, 23 studies were included in this review.

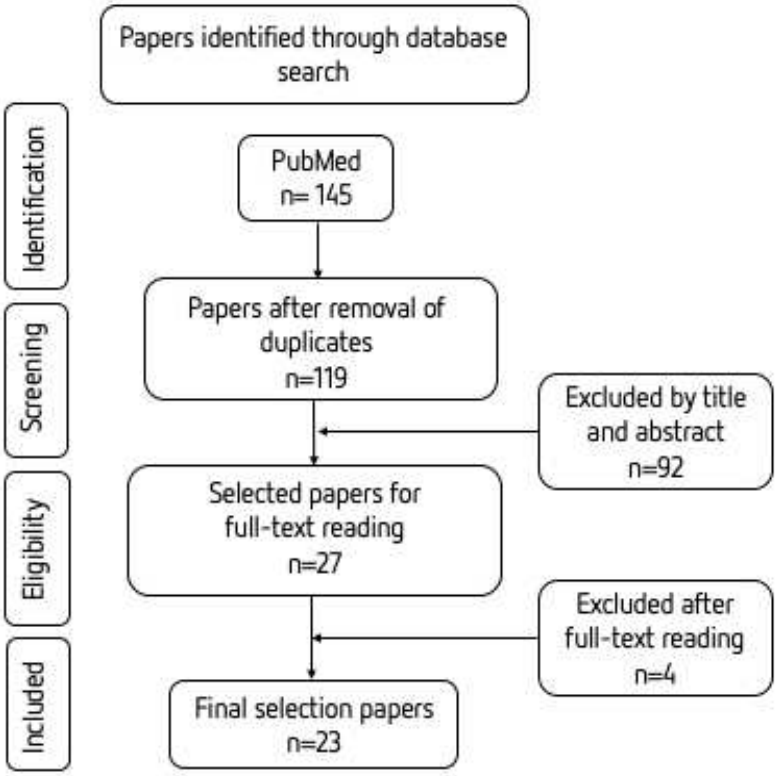

Figure 1. *Flow diagram of the search strategy used in this study.*

Of the 23 selected studies, 3 (13%) articles investigated the surface topography, 11 (47.8%) evaluated the biocompatibility, 7 articles (30.4%) evaluated the effect of porosity, and 2 (8.7%) articles investigated the environmental degradation and its influence on mechanical properties. The main outcomes of the selected studies can be drawn as follow:

- Most of the studies assessed YTZP containing 3% Y_2O_3 known as YTZP which has a high strength and biocompatibility (5,11,13,15–18). YTZP have been used as implants although there a few studies on the effect of the porosity on the mechanical properties (12,19)
- A porosity above 70% is beneficial to osteogenic differentiation and therefore a macro-scale pore size ranging from 100 up to 400 μm promotes cell ingrowth into the scaffold and angiogenesis (1,2,5–8,10,11,13,14,20,21). Also, the size of the pores enhances the transferring of nutrients and oxygen among the cells. Pores at macro-scale (1-50 μm) provide an increase in the wettability of the scaffolds as well as the adhesion of proteins and cells (6,10). (22)
- Blood fluid flow, cell migration, and angiogenesis also depend also on the interconnectivity among the pores at different macro- and micro-scale thereby establishing a 3D-vascular network. (13,16,21) The results have reported that a high interconnectivity rate is linked to a high porosity.(9,11) On the osteoblast growth, cells adhere to surfaces and the spread into the interconnected pores.(3,5,12,23)
- Viability, proliferation, and differentiation of cells increase when the porosity and pore size increases.(4,5,11–13) Some studies showed that cell adhesion changed depending on the chemical composition of the zirconia scaffold. Scaffolds containing more than 80% ZrO_2 showed less affinity to cells than those containing less ZrO_2. (9) Zirconia scaffolds with 30% Hap enhanced cell proliferation. (8,13,17)
- *In vivo* studies reported a faster bone formation into scaffolds with a higher porosity and enriched with Hap.(2,4,5) New bone ingrowth started by lining the surfaces and gradually filling the entire pore volume from the periphery of the scaffold towards to the core. (1,2,20) Radiographic examination showed clear boundaries of surrounding bone to zirconia scaffold interfaces. After healing time, the bone to zirconia scaffolds region reveal a transition zone due to the gradual deposition and ingrowth of bone tissue. (10,20)

Table 1. Data from the selected articles.

Author (year)	Purpose	Study design	Methods	Zirconia types	Porosity	Main outcomes
Malmstrom et al., (2009)(6)	Evaluation of the effect of material and microporosity on bone ingrowth and osseointegration of zirconia and hydroxyapatite scaffolds.	*In vivo* 12 patients (6 men and 6 women, 48–72 years old) subjected to dental implant placemet in the maxilla	Biopsies SEM x-ray diffraction (XRD) Photography (Nikon digital camera) Histomorphometry ((Eclipse E600 light microscope) and connected computer software) CAD	3% Y_2O_3 and 97% Z_rO_2 (YTZP), (Tosoh, Japan)	Interconnected pore channels with 350 μm diameter. 40% porosity	Microporous HA revealed times larger bone ingrowt and seven times larger bo contact as compared with YTZP scaffolds.
Kim, et al., (2008)(2)	Investigation the in vivo performance of the engineered bioceramic scaffolds using a rabbit calvarial defect model.	*InVivo* Eighteen male New Zealand white rabbits weighing 2.5–3 kg	SEM Compressive strength test (Instron (Model 3344, USA))	(3 mol % Y_2O_3, Cerac Inc., WI)	45 ppi large pore; 60 ppi small pore; ~~ 84-87% high porosity ~~75% low porosity	The scaffold with relativel high porosity exhibited be bone regeneration ratio, b the pore size of the scaffo did not have any significa influence on their bone regeneration ability.

Malmstrom et al., (2008)(1)	Evaluation of the effects of material composition and surface topography on bone ingrowth and bone contact	*InVivo* Eight female adult New Zealand white rabbits, weighing 4.4–5.6 kg	CAD XRD SEM Optical Interferometry (MicroXam™, PhaseShift, Tucson, USA) Morphometry ((Eclipse E600 light microscope) and connected computer software)	3% Y_2O_3 and 97% Z_rO_2 (YTZP) (Tosoh, Japan)	Interconnected pore channels with a size around 350 µm 50% porosity	The bone contact in scaffo of zirconia and hydroxyap was not found to be influe by the two different surfa topographies.
Grandfield et al., (2010)(4)	Evaluation of the bone-bonding abilities of HA and Z_rO_2 scaffolds produced by free-form fabrication in the human maxilla at 3 months and 7 months.	*InVivo* Patients between the ages of 20 years	CAD tool (Solid Works, Concord, MA, USA) Biopsies SEM TEM	3% Y_2O_3 and 97% Z_rO_2 (YTZP) (Tosoh, Japan)	Interconnected channels (approximately 350 µm) 0,7% porosity	In HA scaffolds implanted months, images reveal the vivo formation of an inter apatite layer that exhibits intimate contact with bon along the interface region
Song et al., (2014)(5)	Evaluation of the properties of a porous zirconia scaffold coated with bioactive materials and compare the *in vitro* cellular behavior of MC3T3-E1 preosteoblastic cells to titanium and zirconia disks and porous zirconia scaffolds	*InVitro*	SEM XRD Energy disperse x-ray spectrometer (XFlash Detector 5010, Brunker, Fitchburg, WI, USA)	3% Y_2O_3 and 97% Z_rO_2 (LAVA™ Zirconia Block, 3M ESPE, Neuss, Germany)	200-500 µm	Zirconia had greater osteo cell activity than titanium interconnecting pores of t zirconia scaffolds showed enhanced proliferation an differentiation. The activit osteoblast was more affe by microstructure than by coating materials.

Balagangadharan et al., (2018)(23)	Synthesizion and characterizion biocomposite scaffolds containing chitosan (CS), nano-hydroxyapatite (nHAp) and nano zirconium dioxide (nZ_rO_2) along with microRNA (miRNA) for BTE applications.	*InVitro*	Biocomposite scaffolds were fabricated using freeze-drying method. SEM XRD	Sigma Aldrich, MO, USA.	Interconnected 55–65µm	The prepared CS/nHAp/n biocomposite scaffolds sh osteoinductive property, a the addition of bioactive molecule such as miR-590 to the scaffolds further increased osteoblast differentiation.
Shao et al., (2016)(7)	Evaluation of the effects of porous gradient composites with hydroxyapatite/zirconia and autologous iliac in repair of lumbar vertebra body defects in dogs.	*InVivo* 18 adult beagle dogs, aged five to eight months and weighted 10–13 kg.	X-ray Biopsies	Produced by School of Materials Science and Enginnering, Shanghai University	150 and 300 µm	Histomorphologic study showed that the amount o bone within pores of the porous gradient hydroxyapatite/zirconia composites increased continuously with a prolon implantation time, and tha partial composites were degradated and replaced new-bone trabeculae.
Kim et al., (2003)(24)	Fabrication of various calcium phosphate coatings of single phases (HA, FA, TCP) and their mixtures (HA+ FA, HA + TCP) over Z_rO_2 porous scaffolds using the powder slurry method and	*InVitro*	XRD SEM	(3 mol % Y_2O_3, Cerac Inc., WI)	500–700 µm ~90%	For all coated scaffolds, th cells spread well and migr deep into the pore channe suggesting the osteoconducting characteristics of the poro scaffolds.

	investigation of their *in vitro* dissolution behaviors and the cellular responses to them.					
An et al., (2012)(8)	Evaluation of the usefulness of the porous ZrO_2/HAp composite material for bone tissue repair, in this study was investigated physical properties and cellular compatibility of the material. Moreover, it was also implanted cell-loaded porous ZrO_2 /HAp scaffolds in critical-size bone defects to evaluate the effect of the material for bone tissue repair.	*InVivo* Eight-week-old male SD rats (320−360g)	SEM TEM XRD	3% Y_2O_3 and 97% ZrO_2, (Tosoh, Japan)	70-90 µm Interconnected pores channels 2,5-2,8% porosity	Scaffolds containing more 80% ZrO_2 showed less aff to cells than did scaffolds containing less ZrO_2. Cell proliferation study indicat that higher contents of HA (≤30%) in the composite enhanced cell proliferatio

Matsumoto et al., (2011)(17)	Fabrication of a composite material that has mechanical properties similar to biocortical bone and high bioaffinity by compounding hydroxyapatite (HAp) with the base material zirconia (ZrO_2), which possesses high mechanical properties and low toxicity toward living organisms. The material characteristics including the cellular and tissue affinity of the fabricated material were investigated in this study.	*InVivo* SD rats (6-week-old, male) ($n = 5$)	TEM SEM XRD Compression test (AGS-500D, crosshead speed = 1mm/s, Shimadzu, Japan)	3% Y_2O_3 and 97% ZrO_2, (Tosoh, Japan)	10μm	In this study, while ZrO_2 showed poor cell adhesion HAp and the ZrO_2 /HAp composite specimens sho favorable cell adhesions.
Hadjicharalambous et al., (2015)	Fabrication and characterization the mechanical properties of medium porosity (50–60%) and bimodal pore size scaffolds: alumina (A-61), yttria-stabilized zirconia (Z-50) and zirconia–alumina composite (ZA-60) (80 wt% Zr(Y)O –20 wt% Al O), and comparing the proliferation, morphology and	*InVitro*	SEM XRD Compression tests Instron-1185 Universal Testing Machine with 100kN capacity at a strain rate of $3x10^{-4}s^{-1}$.	ZrO_2 (3 mol. % Y_2O_3) Siberian Enterprise Chemical Group	100μm 50% porosity	This study demonstrates t suitability of all three poro ceramic materials for osteoblast proliferation, differentiation and matrix mineralization, with the zirconia-containing mater Z-50 and ZA-60 displayin better cellular response.

	differentiation of MC3T3 pre-osteoblasts on these materials.					
Aboushelib et al., (2017)(20)	Evaluation of osteogenesis ability of CAD/CAM porous zirconia scaffolds enriched with hydroxy apatite used to augment large boney defects in a dog model.	*InVivo* 2-year-old healthy male Beagle dogs (weighing 10–12 kg)	Cone beam CT radiographic imaging CAD/CAM Pore Sizer (Porosimeter, Micromeritics 9320, USA) Energy dispersive X-ray (EDX) XRD	3% Y_2O_3 and 97% Z_rO_2, (Tosoh, Japan)	Interconnected pores 85 ± 24 µm	HA enriched zirconia scaf revealed significantly high volume of new bone form (33% ± 14) compared to t controls (21% ± 11). New b deposition started by coat the pore cavity walls and proceeded by filling the e pore volume. Bone ingrow started from the surface o scaffold and propagated towards the scaffold core. Islands of entrapped hydr apatite particles were observed in mineralized b matrix.
Zhu et al., (2015)(11)	Evaluation of the relationship between porosity, pore size, mechanical strength, cell adhesion, and cell proliferation in the zirconia scaffolds.	*InVitro*	SEM XRD Micro-CT Compression tests computer-controlled Universal Testing Machine (Instron-3365,	3% Y_2O_3 and 97% Z_rO_2, (Tosoh, Japan)	830–577 µm 92,7-68,0% porosity	The zirconia scaffold with porosity of 75.2% posses favorable mechanical and biological properties for fu applications in bone tissu engineering.

			USA)			
Shao et al., (2018)(10)	Evaluation the effects of porous gradient composites with hydroxyapatite/zirconia and autologous iliac in repair of lumbar vertebra body defects in dogs.	*InVivo* Twenty-four healthy rhesus monkeys (5-7 years old, 5-8 kg)	Micro-CT Histomorphometry Biomechanical testing X-ray	Produced by School of Materials Science and Enginnering, Shanghai University	150-300 μm	The results of biomechani testing indicated that the vertebral body compressio strength of the PGHC impl was lower than the other implants. RT-PCR and wes blot analyses showed that expression of bone-relate proteins in the BGS implar significantly higher than ir PGHC implant. The BGS displayed reparative effect similar to autologous bone Therefore, BGS use in vert bone defect repair appear promising.

4. DISCUSSION

4.1 Zirconia

Zirconia is a ceramic which has been introduced in the biomedical field for replacing metallic materials mainly due its high biocompatibility and mechanical properties.(25) In the last decade, yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) has emerged in dentistry as a promising material for various applications such as single- and multi-unit restorative structures due to aesthetic outcomes such as color and opacity that mimic a natural teeth appearance.(26) The flexural strength of YTZP is around 900–1200 MPa(11) while the fracture toughness is at approximately 6 MPa (15) Nevertheless, monolithic zirconia has some limitations for osseointegrated implants linked to its high elastic modulus (at about 240–260 GPa) and ultralow chemical reactivity for osteogenic cell stimulation. The elastic modulus of zirconia is significantly higher than that recorded on cortical bone (10–20 GPa) that can result in stress shielding and peri-implant bone loss.(27)

Zirconia can be found in three polymorphic forms at ambient pressure: monoclinic up to 1170 °C; tetragonal in between 1170 and 2370 °C, and cubic in between 2370 and 2706°C. (7) Zirconium dioxide (ZrO_2) adopts a tetragonal structure at usual sintering temperature and a monoclinic crystal after cooling down to the room temperature. The volume expansion caused by cooling from high temperature resulted in cracking. Then, yttrium oxide (Y_2O_3), magnesium oxide (MgO), or calcium oxide (CaO) was added to the ZrO_2 to retain the tetragonal phase and synthesize the YTZP.(5,6,12) YTZP has superior biocompatibility and mechanical properties in comparison to other TZPs.(5,6) Therefore, MgO-stabilized zirconia also exhibits high mechanical strength, excellent chemical stability, and adequate biocompatibility.(12)

The control of the elastic modulus and wettability of zirconia can be achieved with the manufacturing of zirconia scaffolds. Thus, a high porous zirconia (~85%) still maintain and high compressive strength of around 5–10MPa.(2,8) Biocompatible ceramic scaffolds support the *in vitro* and *in vivo* cell growth although the mechanical properties for extensive bone repair is still

a challenge. Another challenge deals with the modelling of complex structures is to accurately construct their microstructural geometries.(28) Bioactive scaffolds composed of calcium-based structures (e.g. hydroxyapatite) provide very limited control over the inner architecture of the material and possess consequent low strength. (1) Apart from a bioactive scaffold material, scaffolds are further required to have a suitable design that will promote the entire infiltration of bone tissue, bone marrow, and blood vessels as take place when autograft and allografts are used.(1)

The 3-D open cell structures show the most interesting design for bone tissue engineering applications due to their similar structure to the trabecular bone. For instance, the spongy shape of the trabecular bone can be acquired via the replica method to manufacture ZrO_2 foams with different porous design.(28) Recent developments in computer-aid design (CAD) and rapid prototyping methods have become a feasible solution since the 3D-design can be carefully planned at macro- and micro-scale prior to the manufacturing of the scaffold.(1,4,28) Free form fabrication, which uses a 3D-printing principle, is an effective method to control pore architecture (size, shape, and interconnectivity) of the scaffolds for specific clinical applications.(4) Previous experimental studies on the bone response to different ceramic materials have presented results revealing not only the material composition importance but also the effect of the 3-design on the biological and mechanical response of the scaffolds.(1)

4.2 Manufacturing of Zirconia Scaffolds

Porous zirconia can be produced by different methods such as powder sintering, CAD-CAM and replica method, as illustrated in Figure 2. On the replica method, a polyurethane foam template with proper dimensions is impregnated with the ceramic slurry.(2,11) The zirconia slurry mixture is often prepared by ultrasonic dispersion into distilled water. Then, the polyurethane template is immersed in the slurry and centrifuged to remove excessive ceramic slurry. After drying at 80 °C for 12 h, zirconia thermally treated at 800 °C for 1 h and then at 1350 °C for 5 h at a heating rate of 2 °C min^{-1} before cooling down to the room temperature. A previous study evaluated the effect of several thermal treatment (1, 3, 5, and 7) on the morphological aspects of the zirconia scaffolds.

The shaped scaffolds were finally sintered at 1400 °C for 2.5 h at 10 °C min^{-1}, and then cooled down to 800 °C at 5 °C min^{-1}, followed by a furnace cooling down to the room temperature.(11) The porous structure and porosity of the scaffolds were affected by the sintering cycles. As the number of sintering cycles increased, the pore size and porosity decreased. Specifically, the scaffold's porosity decreased from 92.7 ± 0.3% to 68.0 ± 0.3% when the sintering cycles increased from 1 to 7, at an average rate of decrease in porosity of about 4.1% per sintering cycle. Scaffolds with a higher porosity had well inter-connected pores, whereas scaffolds with low porosity had pores with only limited interconnectivity. Blocked pores appeared in the scaffold treated with 5 sintering cycles and were apparent in scaffolds treated with 7 sintering cycles. The compressive strength of the zirconia scaffolds increased from 0.6 to 4.4 MPa when the sintering cycle was increased from 1 to 7 and, correspondingly, the porosity of the scaffolds decreased from 92.7% to 68.0%.(11)

In another study, the pore size was controlled by utilizing two types of polyurethane foam templates: 45 ppi for the large pores and 60 ppi for the small pore. In addition, the porosity was controlled by adjusting at approximately 75 or 85% by the replication cycle. Spherical pores with pore sizes of 500-700 μm developed well. For the counterpart scaffolds with a small pore size 150−200 μm pores also developed well. The pores were observed to be well-interconnected, without showing any pore-blockings. However, in the scaffolds with low porosities, some pores were severely clogged. The compressive strength was lower in scaffolds with higher porosity (2)

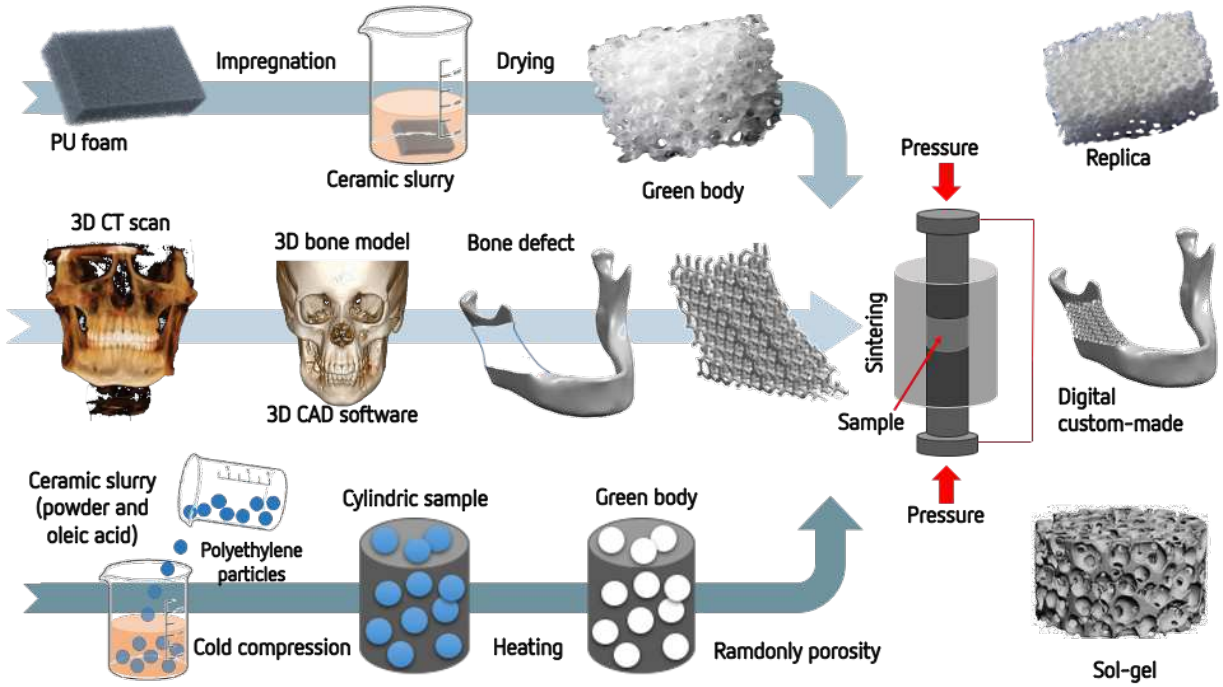

Figure 2. Manufacturing of Zirconia Scaffolds

The zirconia powder can be milled to produce different size of zirconia particles or mixture of zirconia and other oxides. A previous study assessed the effect of mixture of zirconia and alumina on the porosity and mechanical properties of zirconia- and alumina-based scaffolds. The ceramic powders were submitted to ball milling for 25 h to reduce agglomeration and heterogeneity of the powder. Ceramic slurries were produced from the different ceramic powders mixed with oleic acid as dispersant. Macro-particles with 90–250 mm size of 20 vol%. polyethylene particles were placed into the ceramic slurries as macro-pore formers during the thermal treatment.(13) Polyethylene particles were eliminated by thermal treatment at 300 °C in an air furnace for 3 h (heating rate of 200 °C/h). The pores were maintained after thermal treatment and cooling down at room temperature(12,13) The mixtures were pressed on a hydraulic press under 100 MPa pressure in steel die molds to obtain cylindrical (15 mm in diameter, 5 mm in height) samples. (12,13) Polyethylene beads were removed by burning in an air furnace at 300 °C for 3 h (heating rate of 200 °C h–1). During thermal treatment the organic material was extracted, generating the desired pores within the microstructure. Sintering was performed in air at a temperature of 1400°C in LHT 02/17 High-Temperature Furnaces (Nabertherm) with an isothermal exposure time

of 2 h. (13) On the sintering manufacturing, the percentage of shrinkage cannot be controlled and therefore small dimensional variations can take place.(13)

Regarding the CAD-CAM method, zirconia scaffolds can be designed by CAD (e.g. STL file) for further manufacturing process. In this way, the scaffold is manufactured at different dimensions, porosity, pores' networking, and size of pores.(1,4,6) The percentage of shrinkage can be estimated to control the dimensions of scaffolds and pores after thermal treatment.(6) The manufacturing process can involve a 3D-printing or micro-machining. A previous study performed the scaffold manufacturing by using an inkjet printing corresponding to the designed macropores.(1,4,6) A thermoplastic polymeric material was used as a the moulding structure, surrounded by a holding wax-based material. The holding material was separately detached from the mould leading to the building up of the struts and pores of the scaffold.(1,4) The molds were infiltrated with 50 vol%. zirconia slurries prepared by ball milling process. (1,4,6) The ceramic suspensions were accomplished by using slip casting (colloidal filtration) in which the excess of water was drained from the suspension on a plaster plate.(1,4) The use of colloidal slip casting processes provides a variety of zirconia structures from a high down to a low porosity depending on the percentage and size of pores.(6) In a previous study, zirconia/hydroxiaptite(Hap) assemblies were heated up to 600°C at 1°C/min to burn away the mold and additives. Further heating was performed on Hap up to 1,200°C at 5°C/min and of zirconia up to 1,500°C at 5°C/min for 2 h. The bulk porosity of the sintered materials was recorded at approximately 1.2 mm and 390 nm for HA and Zr_2O_2 scaffolds, respectively (4) and macroporosity of approximately 40 vol% consisting of squarely shaped and interconnected pore channels with a size approximately 350 μm. (1,6)

4.3 Biological effects of the porous zirconia scaffolds

Size, percentage and the interconnectivity of pores are critical morphological properties influencing the scaffolds' biological efficiency, as shown in Table 1. Previous studies reported that

high porosity and proper large pore size at macro-scale (100-400 um) induce migration, adhesion, and differentiation of osteogenic cells as well as angiogenesis, nutrient exchange, and bone formation.(2,4,6,11,12) However, the size of pores can decrease the strength of the scaffolds. Interconnectivity, which is related to both pore size and the extent of porosity is required to promote body fluid circulation(11) and cell migration to the core of the implant, as illustrated in Figure 3. That promotes the formation of a vascular network (angiogenesis), a previous process to bone ingrowth.(4,23) Several reports in the literature emphasizes the importance and benefits of pore interconnectivity in bone growth and implant fixation.(13,16,21) A recent study attributed enhanced cell viability to the internal structure of the scaffold rather than to the type of struts and bioactive coating material.(20) Other study reported that the coated scaffold was partially obturated as compared to the non-coated zirconia scaffold in SEM micrographs. Scaffolds with a

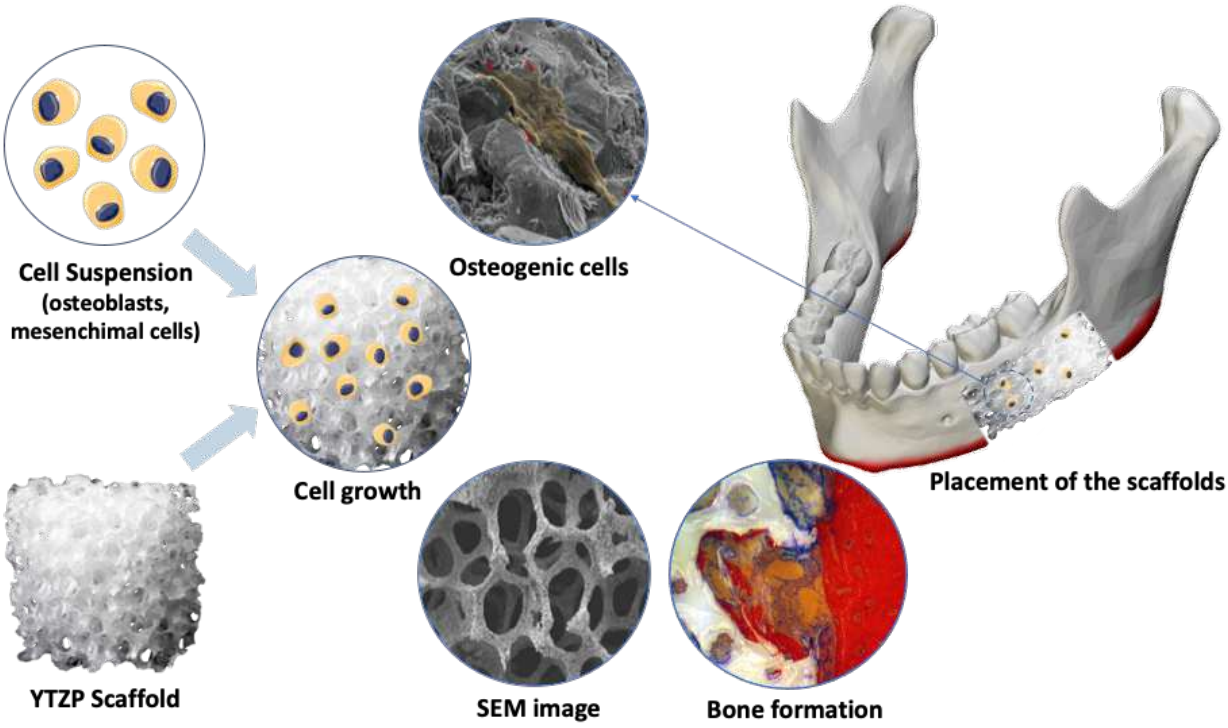

Figure 3. Schematics of cell culture on zirconia scaffolds.

higher porosity should also reveal well interconnected pores although the strength and surgical handling of the scaffolds must be preserved.(9,11)

Although the scaffold with a higher porosity is suggested to be more favorable in terms of bone formation capability, the mechanical benefits resulting from the reduction in porosity should not be ignored. (2) The mechanical performance of scaffolds is crucial for bone repair in the case of extensive bone loss such as at jaw body or ramus.(2,11) Thus, the balance of porosity and strength must be accomplished for enhanced bone healing.(2,11,13,21) For the growth of osteogenic cells, porous surfaces support the adhesion and spreading of the cells from the outer region throughout the pores' network.(3,5,12) A larger number of cells can proliferate into pores' size between 100-400 um due to the surface area of attachment and space for nutrients exchange.(21) A recent study reported optimum conditions for cell growth, proliferation, and extracellular cell matrix (ECM) when the scaffold porosity was approximately 90%.(8) A higher number of cells was detected into scaffolds with higher porosity (~93%) when compared to scaffolds with a 68.0% porosity.(11) Previous studies had validated that pores with diameters larger than 100 um can provide a proper framework for the proliferation, differentiation, and migration of osteoblast, chondrocyte, and vascular endothelial cells.(7) Additionally, the cell behavior inside an implanted scaffold will also be influenced by the effect of the material chemistry as occurs on zirconia-coated scaffolds with bioactive ceramics.(1) New bone growth starts by lining and gradually filling the entire pore volume.(1,2,20)

In vivo studies have also shown the beneficial effects of the higher porosity on the osseointegration events, as illustrated in Figure 3. A previous study in rabbit calvaria bone defects reported that scaffolds with high porosity percentages (84-87%) and pore interconnectivity exhibit significantly higher bone formation when compared to the scaffold with a lower porosity (75%). Scaffolds with low porosity percentage, the interconnectivity of pores was severely decreased.(2) A previous study in tibia and femur of rabbits reported that the areas inside the scaffolds were filled with irregular woven bone. Blood vessels were detected in the newly formed bone inside the macro-scale pores. Irregular woven bone was also seen as bone trabeculae reaching the scaffolds from the surrounding bone. Bone tissue was often in intimate contact with

the scaffold surfaces both on the outer and inner regions.(1) Other studies had found that new bone tissue grown into the porous scaffold material of 2–3 mm in depth within three months post surgical placement.(7) The pores of the scaffolds were entirely filed with new bone tissue for 12 months without any clinical complications.(7) In addition, these findings also showed that the porous scaffold materials had improved mechanical properties and high compatibility.(7)

5. CONCLUSIONS

In the present review, relevant articles reported significant biological and physical evidences on the effect of zirconia scaffolds for enhanced bone healing. The main outcomes of the selected studies can be drawn as follow:

- Most studies described the manufacturing of zirconia scaffolds by using CAD/CAM, replica method, and powder sintering. The control of the size and percentage of pores can be achieved by design the models with the desired macroporosity and manufacture them using free form fabricated equipment using an inkjet printing principle, select a polymeric sponge according to density, since this factor is directly related to the final structure of the scaffolds produced by the replica method or adding polyethylene particles that disappear during the thermal treatment, creating pores in the case of the power sintering method.
- Considering the mechanical properties of zirconia, a high porosity at approximately 85% and large pores ranging from 100 up to 400 μm can be accomplished without compromising the application of zirconia scaffolds in extensive surgical bone sites.
- The porosity percentage and size of pores of the scaffold as well as the pore interconnectivity are fundamental features for the cell migration and differentiation, angiogenesis, and exchange of among osteogenic cells. In fact, macro-scale pores size ranging from 100 up to 400 μm allows cell ingrowth into the scaffold and angiogenesis, while pores at micro-scale (1-50 μm) provide an increase in the wettability, protein adsorption, and cell adhesion.
- Mesenchymal stem cells are adult, multipotent CTs, capable of differentiating into cells such as osteoblasts. When grown in the scaffolds were found to attach, proliferate, and differentiate, later forming new bone growth started by lining for cavity and propagated to gradually fill the entire pore and volume, growing from the periphery of the scaffold to the center.
- Most *in vivo* studies reported increased bone growth by contact and distance osteogenesis into the porous zirconia compared to zirconia at high density. Porous zirconia showed

significantly improved new bone formation into the pore channels for both 4 and 12 weeks of scaffold placement in rabbits and about 3 months in humans.

- Further studies should be carried out concerning the optimum balance between the porosity and the mechanical strength of the zirconia scaffolds. Also, the hybrid bioceramic containing zirconia and modified surfaces could be more explored once the surface of the porous structure play a key role on the adsorption of proteins and osteogenic cells.

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