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Special Issue Reprint

Zirconia and Innovative Biomaterials for Dental and Biomedical Applications

Edited by
Sangwon Park and John G. Fisher

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Zirconia and Innovative Biomaterials for Dental and Biomedical Applications

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About the Editors

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Preface to "Zirconia and Innovative Biomaterials for Dental and Biomedical Applications"

The use of zirconia in the biomedical field was initiated in the early 1970s, and the application of zirconia as a prosthetic material in dental-related applications was unlocked in the 1990s. Very recently, many scholars established the use of zirconia in the dental and biomedical field as an implant and as a scaffold. Thus, zirconia and zirconia-based materials have a wide range of applications in the biomedical and dentistry fields owing to their excellent mechanical properties, aesthetics, and biocompatibility. This Special Issue critically explores the art and state of zirconia surface treatments (mechanical/chemical/physical), which are a significant challenge in implantology in designing implant biomaterial using advanced technologies which have evolved rapidly to enrich the biological and osteointegration process of dental implants. The surface characteristics are proposed to improve the capacity of anchorage into the bone which determines the long-term clinical success rate. In addition, digital technology (CAD/CAM and 3D printing) in dentistry plays a crucial role in the fabrication of dental restorations and prostheses because of its efficient manufacturing process with high accuracy in a short time. The perfectibility of technology and the suitability of materials are considered the ultimate requisite for the future.

With an increase in age-related pathologies and associated illnesses worldwide, there is a necessity for the advancement of biomaterials to substitute tissue loss and boost regenerative mechanisms, as well as to ensure the healing process. Bioceramics, polymers, and metals are such materials that have been used to repair and restore bone and dental defects, especially for the reconstruction/regeneration of hard tissues. To achieve three-dimensional tissue regeneration, the porous scaffold with multifunctional properties was fabricated using additive manufacturing and conventional techniques.

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Review

Three-Dimensional Zirconia-Based Scaffolds for Load-Bearing Bone-Regeneration Applications: Prospects and Challenges

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Abstract: The design of zirconia-based scaffolds using conventional techniques for bone-regeneration applications has been studied extensively. Similar to dental applications, the use of three-dimensional (3D) zirconia-based ceramics for bone tissue engineering (BTE) has recently attracted considerable attention because of their high mechanical strength and biocompatibility. However, techniques to fabricate zirconia-based scaffolds for bone regeneration are in a stage of infancy. Hence, the biological activities of zirconia-based ceramics for bone-regeneration applications have not been fully investigated, in contrast to the well-established calcium phosphate-based ceramics for bone-regeneration applications. This paper outlines recent research developments and challenges concerning numerous three-dimensional (3D) zirconia-based scaffolds and reviews the associated fundamental fabrication techniques, key 3D fabrication developments and practical encounters to identify the optimal 3D fabrication technique for obtaining 3D zirconia-based scaffolds suitable for real-world applications. This review mainly summarized the articles that focused on in vitro and in vivo studies along with the fundamental mechanical characterizations on the 3D zirconia-based scaffolds.

Keywords: 3D zirconia-based scaffold; bone-regeneration applications; composite; coating; fabrication techniques; bioceramics

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1. Introduction

1.1. General Overview of Zirconia Bioceramics

Zirconium dioxide (zirconia) was first discovered by the German chemist Martin Heinrich Klaproth in 1789 [1]. The use of zirconia in the biomedical field emerged in 1969, as it is a promising alternative to alumina and metal for the construction of hip prosthesis in orthopaedic applications [2]. Zirconia is an oxide form of zirconium (strong transition metal), which does not exist in pure form in the Earth's crust. It is found in the minerals baddeleyite and zircon. Zirconia is a polycrystalline ceramic that exhibits three different crystallographic phases depending on the temperature and pressure: monoclinic (M), tetragonal (T) and cubic (C) [3]. Pure zirconia with a monoclinic structure is stable at room temperature and up to 1170 °C and has inferior mechanical properties to the other two phases. The transition from the monoclinic phase to the tetragonal phase occurs between 1170 °C and 2370 °C, accompanied by approximately 4–5 vol% reduction. Zirconia shrinks to the cubic phase above 2370 °C and up to the melting point (2680 °C) [4]. The transformation of the tetragonal zirconia lattice into the monoclinic phase occurs with approximately 3–4% volume expansion upon cooling. This phase-transformation behaviour of zirconia results in crack propagation over time, because of the internal stress

produced during cooling. The aforementioned phenomena can be inhibited by the addition of a relative amount of metallic oxides (also known as 'dopants' or 'stabilising oxides') such as Y_2O_3, MgO, CaO and CeO_2 [5]. This doped zirconia exhibits a unique property known as 'transformation toughening', and it is considered a key advantage for biomedical applications in orthopaedics and dentistry [5,6].

1.2. Inevitability of Widespread Use of Zirconia Bioceramics in Biomedical Applications

It is well known that zirconia is available in various chemical forms; however, in the field of biomedical research, only three types have primarily been used: yttrium-stabilised tetragonal zirconia polycrystals (3Y-TZP), magnesium-doped partially stabilised zirconia (Mg-PSZ) and zirconia-toughened alumina (ZTA) are the major contributors in biomedical and dental applications [7]. The use of zirconia bioceramics in dental applications in the form of dental prostheses started in the early 1980s and has attracted considerable attention in the dental community [5,8]. In the early days, wide variety metal alloys were employed in dental applications; titanium alloys exhibited clinical success rates of 92–98% yet had minor shortcomings corrosion induced metal ion dissolution into body fluids. [9,10]. The arrival of zirconia bioceramics was a blessing to dentistry owing to their tooth-like colour, high fracture toughness and low-temperature conductance. Owing to its high flexural strength (900 MPa) and non-reactivity with body fluids, researchers successfully employed zirconia for crown and bridge applications [11,12]. Extensive research attempts have been made to utilise the mechanical and biological advantages of zirconia in the form of dental posts as a synthetic tooth root to replace missing teeth [13,14]. Numerous reviews of the use of zirconia for implant applications have been conducted and served as a potential reference for the dental and orthopedic research community [3,7,9,10].

More importantly, after the effectual utilisation of zirconia ceramics for the construction of tooth reinforced repairs, clinicians extended the use of the valuable features of zirconia ceramics (that is, the lower elastic modulus and higher toughness for implant-reinforced renovations) [15]. After the commercialisation of zirconia dental implants in 1987 by Sigma Implants (Sandhause, Incermed, Lausanne, Switzerland), zirconia implants became widely accessible. However, the use of zirconia scaffolds for comprehensive load-bearing applications is commercially nonviable owing to various challenges, such as scaffold fabrication and surface modification. The promising development of zirconia implants has been documented worldwide, and several reviews have been conducted in recent years. For instance, Soon et al., and Yin et al., summarised the recent advances in fabrication techniques for zirconia implants [16,17]. Cionca et al., and Amleh et al., compared the clinical advantages and difficulties between zirconia and titanium implants in the previous review [18,19]. These reviews provide a basic understanding of the origin and evolution of zirconia bioceramics from dental prostheses to biomedical implant applications.

1.3. Commencement of Zirconia over Calcium-Phosphate Scaffolds in Bone-Regeneration Applications

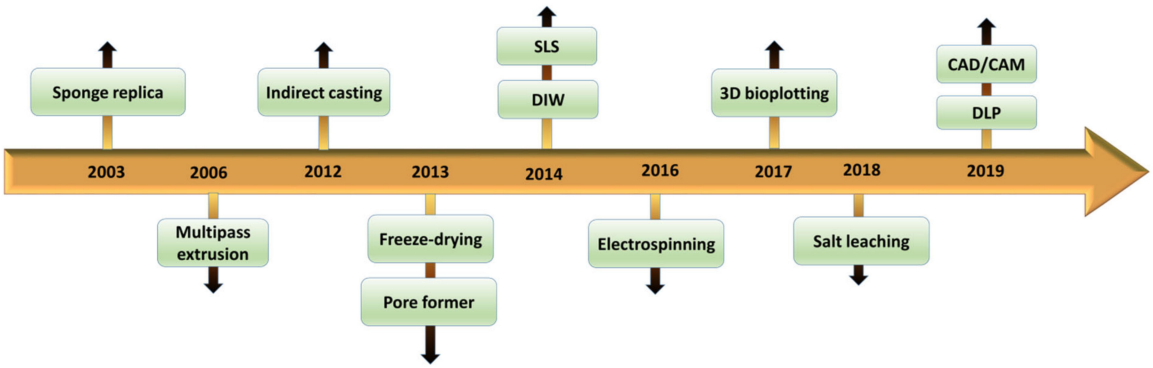
Bone-tissue regeneration using synthetic biomaterials with an identical chemical composition to human bone has developed as a pioneering and favourable strategy for the restoration of bone defects [20]. Calcium phosphate (CP) has chemical and biological behaviours identical to those of natural bone, and calcium phosphate-based materials have been extensively studied by various research groups worldwide [21–23]. However, they have their own faintness in terms of mechanical properties, which is a major concern for load-bearing applications [24]. More importantly, CP-based materials are biodegradable in nature when subjected to the human body and fail to support the reconstruction process because they do not maintain their original shapes [25]. To overcome this calamity, researchers used a composite formation strategy, blending calcium phosphate with mechanically strong and biologically inert zirconia [26]. It is well known that zirconia has an elastic modulus, fracture toughness and osseointegration properties similar to those of human bones [27]. Since traditional two-dimensional (2D) biomaterials cannot retain

three-dimensional (3D) architectures, 2D designs have a limited ability to mimic the multi-dimensional extracellular background, which is essential for promoting cell feasibility and functionality [28]. The design of synthetic bone-graft materials in the form of 3D porous scaffolds loaded with tissue-activating features or precise cells to launch bone restoration is an innovative approach [29,30].

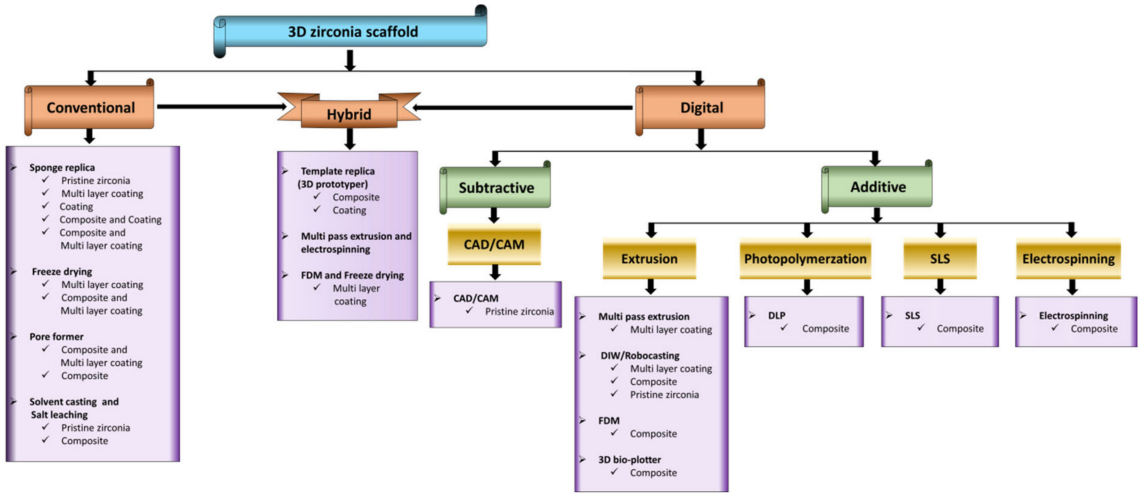
In recent times, considerable research attention has been focused on the progress of engineering techniques for developing CP biomaterials in the form of 3D scaffolds [31,32]. In a recent review, Ryan et al., reported that the available 3D-printing techniques have matured sufficiently to formulate 3D porous CP scaffolds [33]. Additionally, they stated that 3D-printed CP scaffolds must reach the level 1 preclinical stage to confirm effectiveness in a large animal before bone-regeneration checks are performed for human trials. However, the synthesis of 3D zirconia-based scaffolds remains in a stage of infancy because of the lack of availability of 3D fabrication techniques [34].

Few 3D fabrication techniques have been successfully investigated for fabricating 3D zirconia-based scaffolds.

The evolution of the fabrication techniques for the fabrication of 3D zirconia-based scaffolds over time is presented in Scheme 1. As shown, 3D zirconia-based scaffold fabrication via the sponge replica technique for bone-regeneration application started in the late 2000s. However, the use of modern 3D fabrication techniques for 3D zirconia-based scaffolds started very recently. According to the types of 3D fabrication techniques, this article can be divided into three main sections: conventional, hybrid and digital (Scheme 2).

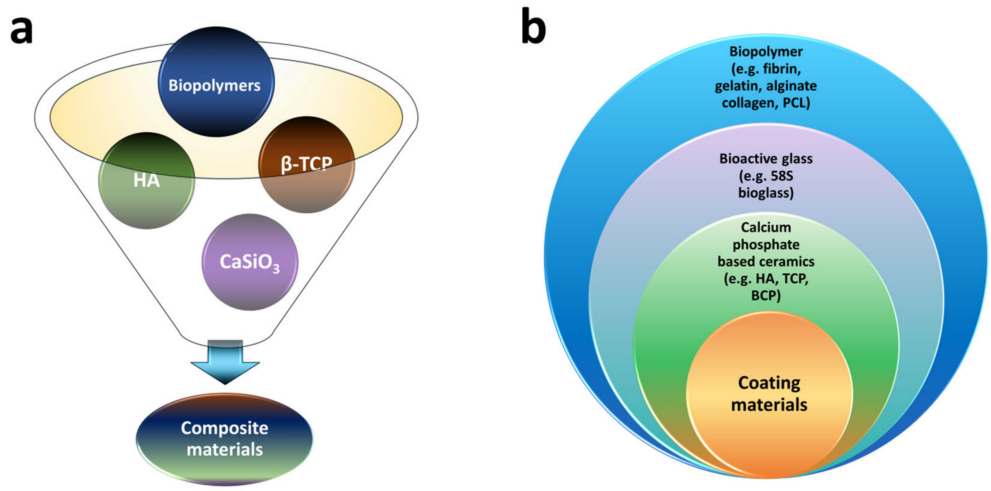


Scheme 1. Evolution of 3D zirconia-based scaffolds (with regard to the scaffold fabrication technique) for bone-regeneration applications.



Scheme 2. Overview of the 3D fabrication techniques for zirconia-based scaffolds, along with the surface optimisation tactics using coating and composite strategies.

Originally, researchers investigated zirconia-based scaffolds using conventional moulding approaches, including sponge replicas, freeze-drying, pore-formers and salt leaching. Even though the conventional techniques for fabricating zirconia-based scaffolds are economical, the precise formation of complex structures is difficult [34]. Thus, in recent years, researchers have used 3D digital techniques as alternative methods for fabricating zirconia-based scaffolds, as for other biomaterials. In the following sections, we discuss the roles of previously reported composite/coating approaches in enhancing the mechanical and biological properties of zirconia-based scaffolds. The most common composite and coating materials used for the bioengineering of zirconia-based scaffolds are presented in Scheme 3.



Scheme 3. (**a**) Composite materials and (**b**) coating materials for bioengineered zirconia-based scaffolds.

Thus, this review systematically summarises the challenges and advancements in the development of 3D fabrication techniques for 3D zirconia scaffolds and their role in bone-regeneration applications for the first time as per the author's knowledge. We focused on studies in which the biological activity of zirconia scaffolds was successfully demonstrated in vitro and in vivo. Finally, guidance for future research directions for formulating optimal zirconia scaffolds via conventional and modern 3D digital techniques is provided. More importantly, we comprehensively addressed the gap between the academic reliability and clinical reliability of various 3D zirconia scaffold techniques for bone regeneration. We hope that this review will draw attention to the production of 3D zirconia scaffolds and promote their clinical implementation.

2. 3D Zirconia-Based Scaffolds via Conventional Technique

2.1. Sponge Replica Technique

The polymeric sponge replica method is the most popular technique for producing interconnected porous bioceramics. This method is known for its simplicity. It involves the coating of open-cell polymeric foam with the desired bioceramic slurry and subsequent thermal treatment to burn-out the polymeric foam and obtains a bioceramic with a 3D structure similar to that of the original polymeric foam [35]. The composite/coating design mixture of biocompatible materials and bioinert porous zirconia-based scaffolds have been studied extensively by multiple research teams [36]. By fixing bioinert zirconia scaffolds as a mainstream loadbearing framework, several scholars have fabricated advanced coating/composite formations using biocompatible and osteoinductive bioceramics.

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH_2)$, HA) is a well-known material in the calcium-phosphate family owing to its analogous properties to human bone components and has been employed for bone replacement [37]. Tricalcium phosphate (β-TCP), which is commonly referred to as bone ash, has a chemical formula of $Ca_3(PO_4)_2$. Similar to HA, TCP is

rich in calcium and phosphorus, which can induce new-bone construction [38]. β-TCP has been formulated directly in the form of scaffolds and tested for bone-tissue regeneration applications [39]. Similarly, the biphasic calcium phosphate (BCP) bioceramic—a mixture of TCP ($Ca_3(PO_4)_2$) and HA ($Ca_{10}(PO_4)_6(OH_2)$—has been used as a bone-craft material because it has a good chemical bone-bonding property and a greater bioresorption capability than HA and TCP individually [40]. BCP promotes the growth of osteoblasts/osteoclasts in a more habitual fashion than pristine HA and TCP [41]. Our literature assessment indicated that HA, TCP and BCP are the calcium-phosphate ceramics that are predominantly employed to formulate bioengineered zirconia scaffolds. In the following sections, we comprehensively discuss the approaches and scientific advancements of calcium-phosphate incorporation into zirconia scaffolds and zirconia incorporation into calcium-phosphate scaffolds, which have been investigated by numerous bone-tissue engineers.

Initiation of the application of CP-altered zirconia-based scaffolds for bioactivity, an enhancement was started in 2003. Kim et al., proposed a biocompatible HA coating (dip-coating) on the surface of a zirconia scaffold (synthesised via the sponge replication technique) [42]. In particular, to minimise the chemical conversion reaction between HA and zirconia, a fluorapatite (FA) layer was adopted as an intermediate. The authors found that the strength of the HA-coated zirconia scaffolds increased by a factor of 7 (compared with the HA), which is encouraging for load-bearing applications. Furthermore, the in vitro results confirmed that the surface-treated porous zirconia scaffolds significantly promoted the growth and proliferation of the osteoblast cells compared with untreated zirconia scaffolds. It is known that FA-inserted HA surface-treated zirconia scaffolds provide enriched bone-tissue regeneration properties. In 2004, Kim et al., varied the external coating with different calcium-phosphate coatings via the powder slurry method (TCP, HA, FA) or in the form of composites including TCP + HA and HA + FA on sponge replication technique-derived zirconia scaffolds [43]. Before the direct CP coating was formed, the zirconia scaffolds were coated with FA as an intermediate layer to minimise the interaction between the CP and the zirconia scaffolds. The authors found that the cell evolutions depended on the coating environment. For example, from the cell-differentiation output of MG63 cells, the alkaline phosphate establishment was found to be enriched in surface-treated scaffolds HA (HA, HA + FA and HA + TCP) compared with pure TCP- and FA-coated scaffolds. In 2004, the same group studied the effects of coating zirconia with HA via the sol-gel and slurry methods [44]. The dissolution rate in the case of the sol-gel-slurry coating was higher than that for the slurry coating. Additionally, the authors controlled the dissolution rate by adjusting the annealing temperature of the sol-gel HA layer. Osteoblast proliferation was confirmed by biological assessments using human osteoblast-like cells (MG63 cells).

After the successful formulation of zirconia scaffolds into strong and bioactive scaffolds via apatite dual-layers inner FA layer and an HA outer layer on the zirconia scaffolds, Kim et al., further authorised the clinical prospects of bioengineered zirconia scaffolds by performing in vivo studies on a rabbit calvarial defect model in 2008 [45]. To recognize the geometrical impacts on the bone-regeneration activities, the authors varied the porosity and pore size of the scaffolds. Remarkably, the bioengineered scaffold exhibited a higher porosity (~84–87%) and compressive strength (~7–8 MPa) than pure apatite-based scaffolds (~74% and ~2 MPa, respectively). Furthermore, according to the in vivo studies on the rabbit calvarial defect model, they proposed that the new-bone construction ensued effectively within the pore channels of all the apatite-engineered zirconia scaffolds, where the bone regeneration phases were similar to those of the pristine HA scaffold. Thus, Kim et al., confirmed the capability of apatite-engineered zirconia scaffolds for bone-regeneration applications by succeeding fundamental studies from in vitro to in vivo amendments, which is crucial for realising any academic research activities persevering further to real-world standards.

Likewise, in 2012, An et al., performed in vivo studies on a fabricated zirconia-HA composite [20]. The authors constructed zirconia/HA scaffolds via the conventional replication (polyurethane foam-scaffold) technique by dipping polyurethane sponge in the zirconia/HA

slurries. The authors demonstrated that the compressive strength of the zirconia/HA scaffold increased from 2.5 to 13.8 MPa with an increase in the zirconia content from 50 to 100 wt%. Additionally, the authors found that the biological activity of the zirconia/HA scaffold was superior to that of pristine zirconia alone. More importantly, in vivo examination using fibrin gel comprising bone marrow-derived stromal cells (BMSCs) loaded with a zirconia/HA bioceramic scaffold offered a promising 3D surrounding for BMSC persistence and enriched the bone restoration near the implanted scaffold. The definite fabrication of the zirconia/HA scaffold via the polyurethane foam-scaffold technique and its successful bone regeneration in eight-week-old male SD rats are presented in Figure 1. Thus, An et al., demonstrated that bioinert zirconia can be used as an effective bone-generation material for larger bone defects with the aid of bioactive HA composite formation.

Interestingly, platelet-rich plasma (PRP) was introduced as a bone-growth-supporting agent to HA/zirconia scaffolds by Latifi et al., and Shahsavari-pour et al. [46,47]. In particular, zirconia scaffolds were produced via the polyurethane foam-replication technique and subjected to FA coating and HA coating via the slurry technique, followed by PRP/heparin sulfate (HS) impregnation to obtain HA/zirconia/PRP scaffolds. Scanning electron microscopy (SEM) of the HA/zirconia scaffolds revealed their high porosity, where the pores were impregnated with PRP gel, and the high-resolution SEM outputs of the PRP-impregnated HA/zirconia frameworks revealed nanoscale porosity (Figure 1b). Enriched osteoblastic proliferation and mineralisation of MG-63 cells were observed for the PRP/HS-impregnated scaffold. To examine the bone-restoration capability of the HA/zirconia scaffold with and without PRP, the authors performed an in vivo experiment, creating a rectangular bone defect in a rabbit mandible and replacing the defect using custom designed rectangular scaffolds (Figure 1c). The in vivo studies were conducted for a period of 8 weeks, and the HA/zirconia/PRP scaffolds were found to repair the artificial bone defects. The authors reported that the HA-zirconia scaffolds with and without PRP accurately imitated the bone mandibular properties in the short-term studies. Nonetheless, long-term observations revealed that PRP played no role in enhancing the synergic regenerative properties. It has been proposed that PRP has osteoinduction and antimicrobial activities.

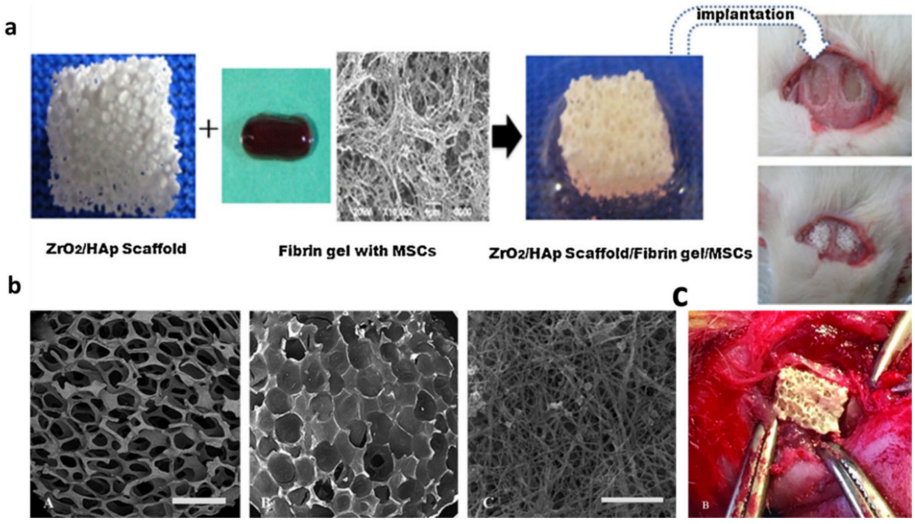


Figure 1. (**a**) Fabrication of the zirconia/HA scaffold via polyurethane foam-scaffold technique and its successful bone-regeneration in eight-week-old male Sprague dawley (SD) rats. Image is used from An et al., reprinted with permission from Elsevier [20], copyright© 2012. (**b**) PRP-impregnated HA/zirconia frameworks: (**A**–**C**) high-resolution SEM outputs of the PRP-impregnated HA/zirconia frameworks. (**c**) In vivo experiment for PRP-impregnated HA/zirconia frameworks; (**B**) in vivo demonstrations via a rectangular bone defect in the rabbit mandible and replacement of the defect using the deliberately designed rectangular scaffolds. Image used from Shahsavari-pour et al. [47], reprinted with permission from Elsevier, copyright© 2018.

Utilisation of β-TCP as a bioactive composite for tailoring the structural properties of zirconia scaffolds was proposed by Alizadeh et al., in 2014 [48]. In detail, the 3D β-TCP/zirconia (yttrium-stabilised) scaffold composite formation is favoured by external slurry mixing of β-TCP/zirconia at different wt% ratios (zirconia.Y_2O_3/β-TCP: A1:50/50, A2:40/60 and A3:30/70) and subsequent sponge formation using a polyurethane sponge (polyurethane foam-replica technique). The authors studied the physical and mechanical outputs with diverse β-TCP/zirconia ratios. They established that the porosity of the β-TCP/zirconia scaffolds can be changed from 65% to 85%. Subsequently, the authors confirmed that the compressive strength varied from 4.95 to 6.25 MPa with an increase in the zirconia content from 30% to 50%. The in vitro biological activity of the β-TCP/zirconia scaffolds was characterised using human endometrial stem cells, and it was found that the cell attachment and proliferation were enriched for the β-TCP/zirconia scaffolds with a ratio of zirconia.Y_2O_3/β-TCP 30/70. Conversely, Mohammad et al., reported that reinforcing the HA (75 wt%) matrix with 25 wt% zirconia enhanced the apatite-layer formation on the surface of the porous scaffold and increased the compressive strength to approximately 13.2 MPa [49].

A porous monolithic functionally gradient FG/BCP/zirconia scaffold with a cancellous bone structure was designed via the conventional polyurethane foam-replication technique by Lee et al. [50]. To recover the bioactive properties and reduce the amount of microdefects, the BCP/zirconia and BCP slurries were treated on the monolithic zirconia scaffold as an in-between layer and an external layer, respectively. The dimensions of the knitted pores and supports were approximately 100–250 μm and 110–300 μm after multilayer addition, which were appropriate for bone-renewal. The biological activity of FG/BCP/zirconia was evidenced by the rapid proliferation and cell attachment of the osteoblast-like MG-63 cells.

The use of dual bioceramic β-TCP/HA coatings on a polyurethane foam-replication technique-derived zirconia composite scaffold was described by Song et al. [51], and in vitro cellular-behaviour measurements provided evidence for the MC3T3-E1 pre-osteoblastic cell activity. Compared with titanium, the osteoblast activity on the surface-activated β-TCP/HA/zirconia composite scaffold was higher. Additionally, the osteoblast activity was mainly affected by the microstructure rather than the coating group. The short duration of the cellular-level investigation and the partial distribution of the coating materials in the interconnecting pores were limitations of this study that must be addressed in the future.

Furthermore, Lee et al., extended the BCP/zirconia scaffold study to design a unique multilayer BCl/zirconia scaffold with immobilised collagen surface modification (Col-BCP/zirconia) via a polyurethane foam-replication technique [52]. The average pore size of the scaffolds was in the range of 160–500 μm, which was sufficient for inducing new-bone growth [53]. In vitro cell proliferation and differentiation studies (MC3T3-E1 pre-osteoblast cell) revealed that the collagen-modified Col-BCP/zirconia scaffold was superior to the unmodified scaffolds. Collagen inclusion improved the cytocompatibility of the BCP/zirconia scaffold without affecting the bulk properties. More importantly, in vivo examinations of the Col-BCP/zirconia scaffold implanted into rabbit femurs after 1 and 5 months indicated that they have considerable promise for new-bone formation compared with the BCP/zirconia scaffold (Figure 2). Thus, the pioneering research outcomes highlight the importance of the Col-BCP/zirconia scaffold as an artificial bone material.

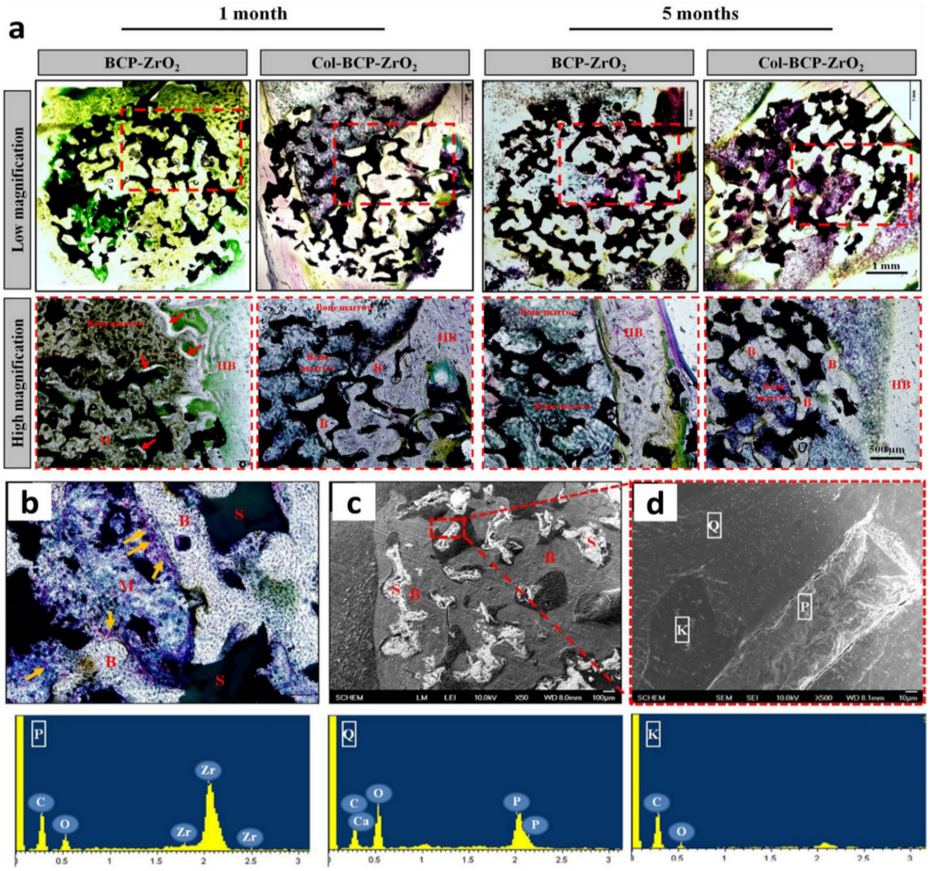


Figure 2. (**a**) Histological analysis of BCP/zirconia and Col-BCP/zirconia scaffolds 1 and 5 months after implantation in the femurs of rabbits, observed via Villanueva bone stains at low and high magnifications. HB: host bone; M: bone marrow; B: new-bone formation. (**b**–**d**) Histological analysis of Col-BCP-zirconia scaffolds after 5 months of implantation in the femurs of rabbits, observed via different methods. (**b**) Villanueva bone stain: HB, host bone; M, bone marrow; B, new-bone formation; S, scaffold. The SEM image in (**c**) is magnified in (**d**). The energy-dispersive X-ray spectroscopy profiles of P, Q and K were taken from (**c**). Images used from Lee et al. [52] reprinted with permission from Elsevier, copyright© 2015.

Additionally, 58S bioactive glass (BG58S)—a combination of calcium/phosphate units and silicate units—was reported to quickly bond with bone and stimulate bone formation, and it has been widely studied for dental implant applications [54]. This highly advantageous feature of BG58S was employed to modify the surface activity of zirconia scaffolds for the first time by Guimaraes et al., in 2019 [55]. Initially, zirconia scaffolds were designed using the conventional polyurethane foam-replication technique, with average pore diameters of 318, 423 and 564 μm, validating the 3D open-cell constructions. The BG58S coating was applied by immersing the zirconia scaffolds in a BG58S sol-gel solution. The coating thickness was controlled by optimising the viscosity of the sol-gel solution and altering the immersion rate, as shown in Figure 3a–f. The authors verified the in vitro biocompatibility of the zirconia scaffolds with and without the BG58S coating by employing MG-63 osteoblast-like cells (Figure 3g–i). The cell feasibility and proliferation were enhanced with a reduction in the pore size. Additionally, the scaffolds with the BG58S coating enhanced the cell viability and promoted cell proliferation, highlighting the importance of the chemical composition on the surface. The results of this study emphasise the significance of the chemical configuration on the surface, aperture diameter and microporosity in the utilisation of zirconia scaffolds as bone grafts.

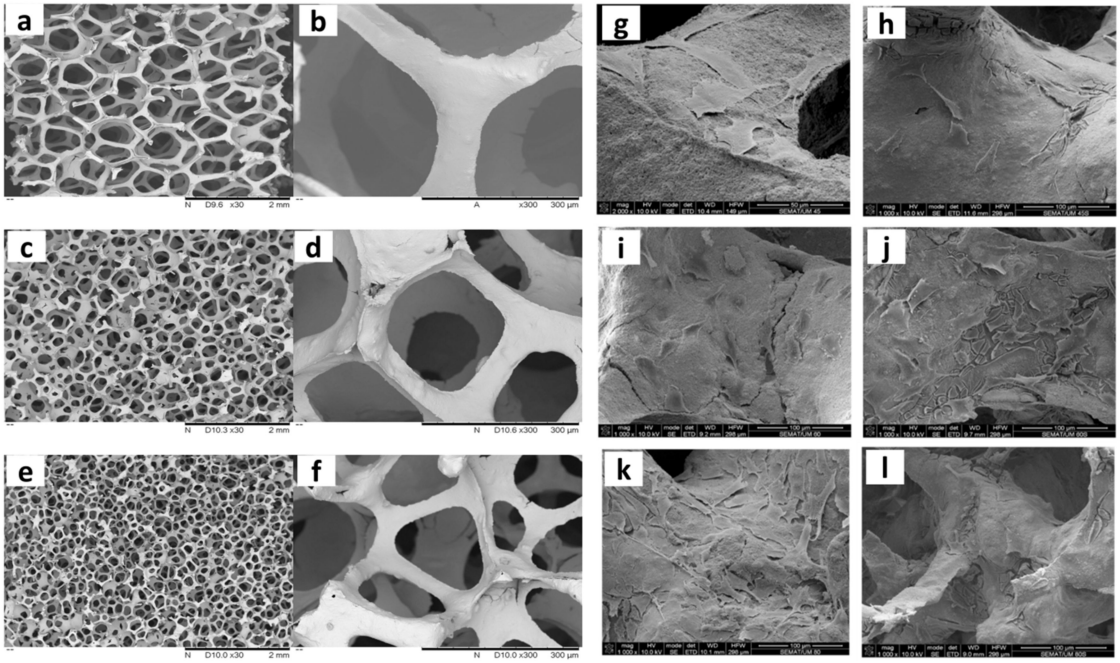


Figure 3. SEM images of 3D zirconia scaffolds (**a**,**b**) 564-Z, (**c**,**d**) 423-Z and (**e**,**f**) 318-Z at low (left) and high (right) magnifications. SEM images of MG-63 osteoblast-like cells on the uncoated 564-Z, 423-Z and 318-Z (**g**,**i**,**k**) and coated 564-Z.BG, 423-Z.BG and 318-Z.BG (**h**,**j**,**l**) scaffolds after 2 d of cultivation. Images used from Guimaraes et al. [55] reprinted with permission from Elsevier, copyright© 2019.

As mentioned previously, in 2020, Gouveia et al., used the protocol reported by Guimaraes et al., in 2019 for the synthesis of 58S bioactive glass and the fabrication of porous zirconia scaffolds reinforced with 58S bioactive glass [56]. Additionally, polyurethane sponges with 4, 60 and 80 pores per inch were fabricated. Moreover, the authors described an additional processing route that involved coating the pre-sintered zirconia scaffold template (1150 °C) with bioactive glass, followed by sintering at 1500 °C. The infiltration process occurred. Along with this processing step, an additional process was conducted, which involved BG58S coating and heat treatment at 600 °C.

Pristine zirconia grafts were studied for dental implant applications in the early 1980s because of their excellent aesthetic appearance and bioinert properties. However, in the available literature, there are limited research articles on the use of pristine zirconia grafts for bone-repair applications. Zhu et al., (2015) reported the optimisation of porous nano-sized zirconia scaffolds via a replica technique with a sufficient porous structure as that of a cancellous bone [57]. The porosity and pore size of the scaffold were controlled by changing the sintering conditions. The tremendous cell adhesion and enhanced proliferation of BMSC cells were observed after 14 d of incubation for a scaffold with 75.2% porosity. Additionally, the scaffold exhibited suitable mechanical properties for load-bearing applications.

Recently, Kim et al., (2018) studied the bone-formation abilities of pristine zirconia grafts engineered using the polyurethane-based sponge replication technique [27]. The designed grafts were subjected to in vivo studies to replace the bone defects in rabbit calvaria and compared with commercial graft materials including Osteon II (Os) and Tigran PTG (Ti). Even though the experimental groups achieved a great extent of new-bone development than compared with the defect group, the differences in the results among the experimental designs were insignificant owing to the similar granule sizes, shapes and porosities of the graft materials. Hence, it is important to design highly porous zirconia scaffolds with a definite bone size and shape using advanced techniques or to alter the surface of zirconia for enhancing the bioactivity.

Askari et al. [58] targeted the advancement of a computational framework (together with experimental confirmation) to regulate the mechanical characteristics of zirconia foams with different pore sizes (manufactured using the foam replica method) for bone-tissue reestablishment applications. Micro-computed tomography (CT) images were filtered to separate noise and smooth margins before fabricating 3D zirconia foams with an adaptive body-centred cubic background framework. The authors verified and scrutinised the stress distributions and magnitudes, scaffold deformation, stresses and plastic strains using the developed micro-CT-based finite-element model. The model was capable of depicting the mechanical stimuli on cells and the confined stress effort in the scaffolds.

2.2. Freeze-Drying Technique

Rather than fabricating zirconia in the form of a 3D structure, the 3D bioactive coating layer can be targeted over the zirconia surface. For example, the development of a thick, scaffold-like HA coating on durable zirconia substrates via a freeze-drying-assisted technique for fabricating porous scaffold-like HA/zirconia composites was proposed by Jiang et al. [59]. In vitro tests confirmed that the porous scaffold-like HA/zirconia composites were bioactive. Additionally, the 3D-HA coated onto the core zirconia retained sufficient mechanical properties for load bearing.

The foregoing studies mainly involved the use of calcium phosphate-based bioceramics with bone-like properties to reproduce the bone-graft activities of zirconia scaffolds. However, Teimouri et al., in 2015 established the use of inorganic polyoxometalates (POMs) with a unique biological property to promote the biological activity of zirconia by constructing a POM/zirconia/silk fibroin nanocomposite framework via the freeze-drying method [60]. The in vitro bioactive behaviour of the POM/zirconia/silk fibroin in simulated body fluid (SBF) was examined, and a uniform distribution of fibroblast cells on the POM/zirconia/silk fibroin composite scaffold was observed. The authors introduced a unique method for enhancing the bioactivity of bioinert zirconia; however, in vivo studies must be conducted to observe the effects of inorganic POMs in the human biological environment. The authors performed another study based on silk fibrin (SF) and nano-zirconia; however, the POM polymer was replaced by chitosan (2%) to fabricate a biocomposite scaffold with an interconnected porous structure using a freeze-drying technique [61]. The compression strength and modulus of the composite scaffold were twofold higher than those of the polymer scaffold (SF/CS) owing to the existence of zirconia in the polymer matrix. Moreover, in an evaluation of human gingival fibroblast (HGF) cells, the composite scaffold exhibited higher biocompatibility.

Recently, a comparative investigation of the physical and biological properties of chitosan-nano-HA (CS-nHA), chitosan-nano-zirconia (CS-nZrO) and chitosan-nano-calcium zirconate (CS-nCZ) porous composite scaffolds (Figure 4a) fabricated via freeze-drying for bone-reestablishment applications was performed by Gaihre et al. [62]. The in vitro activity of the OB-6 pre-osteoblast cells was superior to that of the extended filopodia on CS-nHA and CS-nZrO compared with the CS-nZrO composite scaffolds (Figure 4). The authors expected to investigate further studies on the osteogenic capability of CS-nZrO composite scaffolds.

2.3. Pore Former/Space Holder Technique

The vacuum slip casting technique was used to construct an alumina/zirconia composite scaffold with the aid of expanded polystyrene (EPS) beads (acting as pore formers) by Liu et al. [63]. The designed scaffold exhibited homogeneously circulated interconnected pores. The alumina/zirconia composite scaffold was further coated with a thick bioactive glass (58S33C) layer. The bioactive glass-coated alumina/zirconia scaffolds exhibited optimal porosities (60–66%), high strength (5.42–7.52 MPa) and enhanced bioactivity (apatite-layer formation in the SBF solution after 24 h). The authors showcased multiple bioactive glass-coated macropores as permanent scaffolds for bone-tissue restoration.

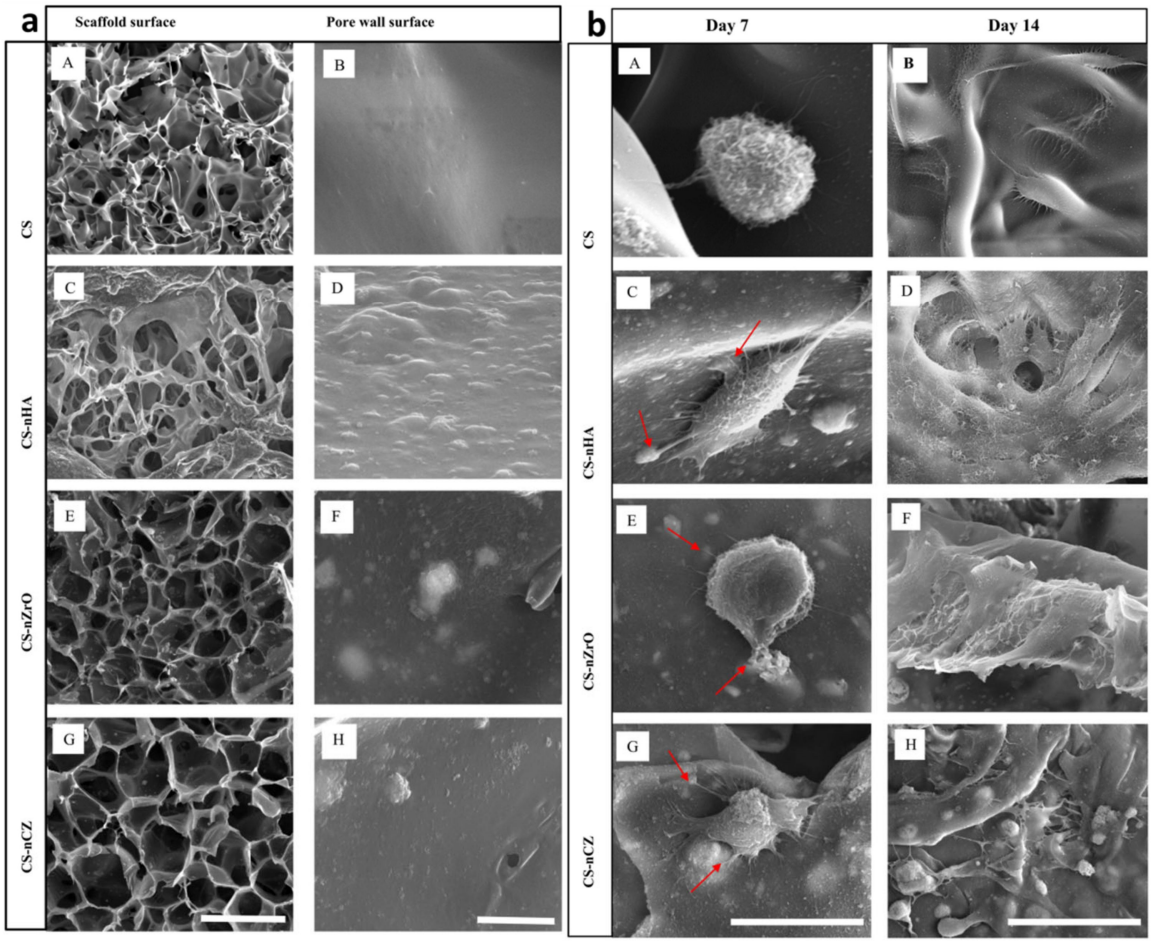


Figure 4. (**a**) SEM images showing the highly porous morphology of the scaffold surface (**A**,**C**,**E**,**G**) and magnified images showing the surface of the scaffold wall (**B**,**D**,**F**,**H**); the scale bars indicate 100 and 2 μm for the scaffold surface and the pore wall surface, respectively. (**b**) SEM images (**A**–**H**) showing the morphology of pre-osteoblast attached and proliferating along the surfaces of different scaffolds; the scale bars indicate 20 and 50 μm in the images taken on days 7 and 14, respectively. Images used from Gaihre et al. [62] reprinted with permission from Elsevier, copyright© 2019.

In one of the reported studies, the bone-growth activities of yttria-stabilised zirconia (YSZ) and magnesium-stabilised zirconia (MgSZ) porous scaffolds were compared (the scaffolds were formed using EPS beads) [64]. The MC3T3-E1 pre-osteoblast cell activity on the YSZ was enriched owing to the surface chemical dissimilarities between the YSZ and the MgSZ. However, the authors claimed that both YSZ and MgSZ porous ceramics can be effective scaffolding materials for cortical bone, because of their comparable mechanical and biological properties.

2.4. Solvent Casting and Salt Leaching

Solvent casting and particulate leaching are among the simple and time-effective techniques for producing bioceramic scaffolds with the desired porosity [65]. Unique PLA-HA-YSZ nanocomposite scaffolds with diverse compositions were fabricated via solvent casting and particulate leaching by Ziaee et al. [66]. Among all the ratios tested, a PLA15%—HA-15%YSZ nanocomposite scaffold exhibited the highest compressive strength and SBF activity. The compressive strengths of the scaffolds were reduced after they were soaked in SBF, and the scaffolds containing HA underwent larger strength reductions than those containing YSZ.

In 2018, Mokhtar et al., conducted a case study to assess the efficacy of a custom-made porous zirconia scaffold along with a buccal trapezoidal flap for closure of oroantral

fistula [67]. This study was performed on 10 patients suffering from oroantral defects due to extraction of the first and second premolars in a maxillary region with dimensions of approximately 6–9 mm. Initially, a virtual bone model with defects was constructed using the stereolithography photopolymer-based 3D-printing technique from cone-beam computed tomography (CBCT). The zirconia scaffold was prepared via solvent casting and the salt-leaching technique, and the fitness was assessed with the bone model before the sterilisation process. Clinical (extraoral and intraoral examinations) and radiological (CBCT) assessments were performed before and after surgery. Postoperative clinical follow-up evaluations were performed at 2 weeks, 1 month and 3 months. A radiographic examination was conducted after 2 weeks using panoramic radiography. CBCT was performed after 3 months to evaluate the bone formation; the bone density was compared with that during preoperative CBCT. The intensity of pain and frequency of minor complications (postoperative bleeding/edema) were significantly reduced over time, while the bone density increased by approximately 41.2%. The authors found that the zirconia scaffolds with interconnected porous structures enrich the formation of new bone in oroantral defects.

3. Fabrication of 3D Zirconia-Based Scaffolds via the Digital Technique

3.1. Computer-Aided Design/Computer-Aided Milling (CAD/CAM) Technique

After the acclaimed applications of zirconia ceramics in dental crowns and implants, zirconia ceramics were expected to dominate the biomedical field. However, the direct utilisation of zirconia scaffolds to reconstruct bone is also initiated in the maxillofacial reconstruction area in the dental field. Aftan et al., developed an innovative method for mandibular reconstruction [68]. The authors designed a patient-specific zirconia prosthesis via the CAD/CAM technique using a zirconia block. They employed the zirconia prosthesis technique for the restoration of mandibular flaws in a human trial. This case study was performed on 20 patients for 62 months, with defects caused by mandibular trauma, tumours, and congenital abnormalities, which required surgical resection and reconstruction. A few patients underwent two surgeries; the Boweman Conroyd appliance was placed in the first surgery, and it was replaced by the predesigned zirconia prosthesis in the second surgery. The zirconia prosthesis was designed according to the patient defects and surrounding anatomical structures using a 3D CAD model. Milling was performed on the zirconia block, followed by sterilisation. For the placement of a dental implant, the authors voluntarily created a hole in the prosthesis. The results were encouraging, with a 95% success rate, and the reconstruction did not affect the mandibular function or aesthetics. Minor complications (pain and edema) were reported.

It is well known that CAD/CAM is a destructive technique that cannot produce zirconia scaffolds with high porosity. Recently, an interesting approach for designing zirconia scaffolds with ~40% porosity and a multilayer assembly via CAD/CAM was proposed by Marques et al. [69]. The distinctive design strategy involves 5-axis milling (XYZ axes and rotating axes (A and B axes)) that allows the part to move 360° in both directions and 20° towards the front and back [69]. A schematic of the zirconia scaffolds designed using the CAD/CAM model is presented in Figure 5a. The scheme comprehensively illustrates the process of designing complex zirconia scaffolds using a modified CAD/CAM model, followed by mechanical testing (compression testing), whereby the Young's modulus values were found to be associated with the host bone. The rapid diffusion behaviour of water inside the channels of the zirconia scaffolds was established using capillary testing. Likewise, to replicate the endosseous implant, the authors demonstrated implant insertion imitation practice, wherein the implant exhibited superior fixation at the early stage of implantation. Tribological tests revealed that the cavities/valleys inside the scaffolds were loaded by bone. Even though the authors mimicked the bone implantation of the zirconia scaffolds via tribological tests, it is essential to verify the cell activity of the scaffolds in the biological environment.

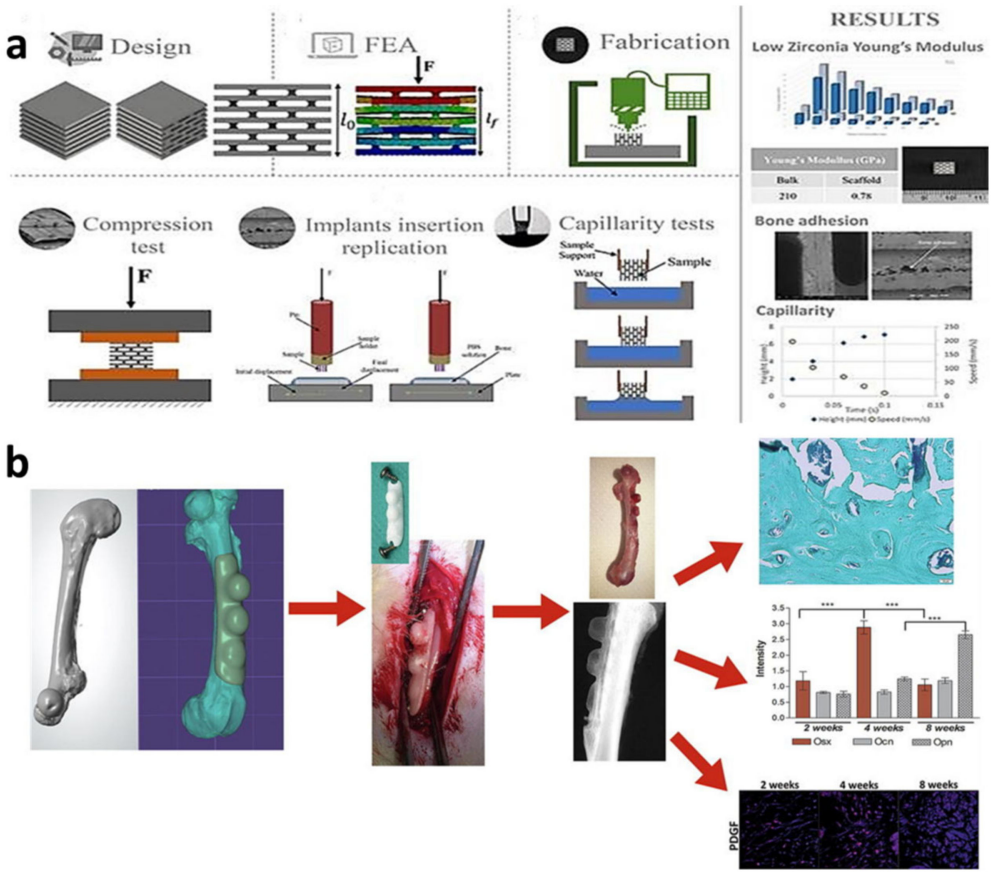


Figure 5. (**a**) Schematic of zirconia scaffolds designed via a CAD/CAM model. Image used from Marques et al. [69] reprinted with permission from Elsevier, copyright© 2019. (**b**) Detailed illustration of the design and milling of 3D-tailored CAD/CAM zirconia space-maintaining strategies for a rat femur. Image used from Tetre et al. [70] reprinted with permission from Elsevier, copyright© 2021.

To all the above, most recently, Tetre et al., revealed a unique method for evaluating bone reconstruction using zirconia scaffolds in the rat femur via a 3D-CAD/CAM approach [70]. A thorough illustration of the design and milling of 3D-tailored CAD/CAM zirconia space-maintaining strategies for a rat femur is presented in Figure 5b. As shown, a 3D-customised assembly was produced with different heights. The authors verified the guided bone regeneration (GBR) abilities of the rat femur via Gomori's trichrome histomorphometrical examination. The authors monitored GBR activation at different time intervals (2, 4 and 8 weeks) and found that the Haversian system was present in freshly grown bone. Thus, the tailored milled zirconia scaffold in this study provided a detailed understanding of progressive bone-tissue construction. The successful bone-reconstruction ability of the zirconia scaffold highlights the effectiveness of the 3D-tailored CAD/CAM technique for extending its application to various orthopaedic applications.

3.2. Extrusion-Based Techniques

3.2.1. Multi-Pass Extrusion Technique

Following the clinical success of an extrusion-based technique to fabricate zirconia scaffolds for dental applications, the design of 3D zirconia scaffolds for bone-regeneration applications has been widely studied [23]. Lee et al., analysed this technology even before the worldwide upsurge in zirconia scaffolds for dental applications [71]. They fabricated HA-coated micro-channelled fibrous Al_2O_3-(monoclinic, M-zirconia)/(tetragonal, t-zirconia) composite scaffolds via the multi-extrusion process in 2004 [72]. In a preliminary study, they developed a multi-extrusion printing technique for designing zirconia composite scaffolds with good mechanical properties. In another study, the research group