Extrusion Bioprinting of β-TCP Scaffolds: A Platform for Dental Pulp Stem Cell Osteogenesis

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Abstract. Autologous bone grafting is a widely used method for the bone defect repair. However, there are still several clinical limitations, such as insufficient donor sites and the requirements for secondary surgery to harvest bone material. Allogeneic bone grafting may induce host immune rejection. Extrusion-based 3D printing has the capacity of precise manufacturing, controllable scaffold structures, providing highly customized repair solutions for bone tissue engineering and significantly advancing personalized medicine. Biomaterial scaffolds play a crucial supporting role in bone tissue engineering. For instance, β-tricalcium phosphate (β-TCP) not only exhibits Oste inductive properties and excellent biocompatibility but also creates a suitable microenvironment for cell adhesion and osseointegration. Moreover, due to their multipotent differentiation potential, particularly their exceptional osteogenic differentiation capacity, dental pulp stem cells (DPSCs) have worked as one of the most extensively studied cell sources. The integration of extrusion-based 3D printing with β-TCP scaffolds enables the behavior of DPSCs to be directed in a controlled manner, generating a multi-level synergistic enhancement effect during bone defect repair.

Keywords: Extrusion-based 3D printing, β-TCP biostructure, DPSCs.

1. Introduction

Among the challenges in bone defect repair, clinical practice must not only address the limited availability of donor tissue sources but also overcome the significant limitations of synthetic bone substitutes in terms of bioactivity and osteogenic efficacy [1]. β-TCP bio ceramics are a superior biomaterial widely used in bone defect repair due to their chemical composition, which closely resembles that of natural bone, their degradation rate supporting bioactivity and osteogenic efficacy, and their excellent bone-inducing properties. By employing extrusion-based bioprinting technology, it is possible to construct biomimetic multi-layered porous structures with high precision within β-TCP scaffolds. With these advantages combined, DPSCs have emerged as a promising cell choice in bone tissue engineering due to their significant multidirectional differentiation potential, particularly their unique advantage in osteogenic differentiation [2]. Particular attention should be paid to the fact that the initiation of the intrinsic mechanism precisely inducing osteogenic differentiation of DPSCs through the construction of a synergistic microenvironment utilizing the physicochemical properties of scaffold materials remains a key scientific question that has not yet been fully elucidated in this field.

Existing research indicates that β-TCP scaffolds produced via 3D printing technology, which leverages the synergistic effects between materials and structure, can significantly enhance the bone regeneration capacity. For example, Jiao et al. (2022) found that β-TCP scaffolds enhance the stability of RUNX2 mRNA by increasing its N6-methyladenosine (m6A) modification levels through upregulating METTL3 expression, thereby promoting the bone marrow mesenchymal stem cells' (BMSCs) osteogenic differentiation [3]. In terms of immune microenvironment regulation, Miao Qiu-Ju (2022) demonstrated that three-dimensionally printed β-TCP scaffolds can modulate macrophage polarization toward the M2 phenotype, thereby enhancing PDGF-bb-mediated angiogenesis and promoting bone repair. Additionally, to optimize this effect, a larger pore size (400 μm) and strontium doping (10-20 mol%) can be employed [1].

Regarding DPSCs, Fahimipour et al. (2018) found that a heterogeneous hybrid scaffold combining 3D-printed β-TCP with a freeze-dried collagen matrix significantly enhanced the proliferation and

osteogenic differentiation of dental pulp cells [4]. Another study by CAO et al. (2020) compared 3D-printed PLGA/TCP and β-TCP scaffolds, demonstrating that both materials promote proliferation, migration, and osteogenic differentiation of human dental pulp stem cells (hDPSCs), with β-TCP exhibiting superior mechanical properties [5]. Extrusion-based 3D printing technology offers unique advantages in fabricating biphasic calcium phosphate (BCP) scaffolds with complex geometries and high porosity, which play a crucial role in nutrient diffusion and cellular infiltration [6].Additionally, the macro-micro hierarchical structure of the scaffold—such as highly interconnected fiber microchannels—can accelerate bone regeneration by enhancing protein adsorption, ion release, and cell adhesion while promoting osteogenesis and angiogenesis [7]. These findings confirm that β-TCP scaffolds exhibit multiple biological functions in bone tissue regeneration by modulating the immune microenvironment, promoting angiogenesis, and directly facilitating bone integration. Despite significant advances in osteogenesis research, there remains a lack of systematic reviews and analyses regarding the directed osteogenic differentiation of DPSCs on extruded 3D-printed β-TCP scaffolds. The current literature on this research direction remains relatively limited, with most studies focusing on other cell types such as bone marrow-derived mesenchymal stem cells (BMSCs) or emphasizing different biomanufacturing techniques. Therefore, this review aims to fill this gap for bone regeneration based on DPSCs. By systematically evaluating and analyzing existing research findings, the review provides critical theoretical support and technical pathways for developing functionalized, customized scaffolds for pulp regeneration and maxillofacial bone repair.

2. Characteristics and Advantages of β-TCP-Based Biostructure

2.1. Characteristics of β-TCP Biostructure

β-TCP bio-scaffolds hold great promise as materials for bone defect repair, with their application potential primarily stemming from the following characteristics: First, this material exhibits exceptional biological activity, possessing not only outstanding osteoconductive properties—effectively supporting osteoblast adhesion, proliferation, and extracellular matrix synthesis—but also demonstrating certain Oste inductive capabilities. Simultaneously, the β-TCP scaffold undergoes gradual biodegradation, with its degradation products (calcium and phosphate ions) not only supplying raw materials for new bone formation but also exhibiting extremely low cytotoxicity [7, 8]. Secondly, modern fabrication techniques such as 3D printing and gel casting-foaming methods enable the construction of scaffold systems featuring high porosity (typically exceeding 80%) and interconnected macropores (pore sizes approximately 200–500 μm). These structures not only exhibit highly interconnected pores but also support ordered design at both macro- and micro-scales, achieving multi-level structural regulation. This structure provides a favorable environment for cell migration, nutrient transport, and vascular ingrowth. In particular, the fibrous microporous architecture significantly increases the specific surface area and surface roughness, thereby promoting cell adhesion and inducing the upregulation of osteogenic and angiogenic genes, ultimately accelerating bone tissue regeneration [7, 9]. Furthermore, although pure β-TCP scaffolds often exhibit mechanical limitations, their compressive and flexural strengths can be effectively enhanced through polymer composites (e.g. PCL, PLGA) or surface modifications (e.g., PLGA coating). These methods also enhance the scaffold's toughness, enabling it to meet the demands of weight-bearing bone repair applications [10, 11]. Therefore, β-TCP bio ceramics possess significant application value and broad development prospects in the field of bone tissue engineering due to their tunable physicochemical properties, excellent biocompatibility, and recognized osteogenic activity.

2.2. Advantages over Ordinary Brackets

Through controlled degradation kinetics, β-TCP scaffolds achieve optimal temporal alignment with the rate of new bone formation. This effectively avoids the stress shielding effect and non-biodegradability issues associated with long-term retention of inert metal implants, which are difficult to overcome [12]. Calcium and phosphate ions released from β-TCP can directly participate in the

biomineralization process during degradation, inducing osteogenic differentiation. In contrast, traditional inert scaffolds lack this bioactive function [13]. Its degradation kinetics are highly synchronized with the process of new bone regeneration, establishing a biologically active adaptive repair mechanism rather than the mechanical support strategy employed by traditional scaffolds [7]. The table 1 compares key performance differences between β-TCP bioactive scaffolds and traditional scaffolds such as metal/polymer in osteogenic repair, highlighting their comprehensive advantages in mechanical properties and biological responses (cell behavior and osteogenic differentiation).

Table 1. Comparison of β-TCP stents with conventional inert stents

Comparison Dimensions	Advantages of β-TCP Stents	Common defects in stents (e.g., metal, pure polymer)
Mechanical Properties	Research indicates that β-TCP scaffolds fabricated via 3D printing exhibit higher compressive strength. Concurrently, increasing the proportion of β-TCP in the material further enhances its Young's modulus [5, 10].	Research indicates that pure polymer scaffolds, such as those made from PLGA, often lack sufficient mechanical properties to provide the structural stability required for bone tissue engineering [5].
Biological Response (Cell Behavior and Osteogenic Differentiation)	Research indicates that scaffolds constructed using 3D printing technology from β-TCP and its collagen composite effectively promote cell proliferation, adhesion, and osteogenic differentiation, while significantly enhancing alkaline phosphatase (ALP) activity [4, 5].	Pure polymeric materials (e.g., PLGA) tend to form localized acidic microenvironments during degradation, which may adversely affect cell adhesion capacity [5].

2.3. Collaboration with DPSCs

β-TCP provides suitable physicochemical anchorage sites for DPSCs through its sustained ion release and multi-level microporous structure, thereby promoting effective cell adhesion and colonization. The phosphate ions (PO_4^{3-}) released from β-TCP activate the expression of key osteogenic genes such as Runx2, thereby driving the early osteogenic differentiation process [3]. The aforementioned properties provide the necessary theoretical basis for in-depth exploration of its molecular mechanisms.

3. Mechanism of Scaffolds on Osteogenic Differentiation of DPSCs

3.1. Regulatory Role of Physical Signals

By precisely controlling porosity (70%–80%) and pore size (200–500 μm), the 3D-printed β-TCP scaffold creates an optimal three-dimensional growth environment for DPSCs while providing essential mechanical stimulation. Research by Siqueira et al. indicates that scaffolds with pore sizes (from 200 to 500 μm) promote cell migration and tissue regeneration. Additionally, configurations with higher porosity (e.g., greater than 86%) typically exhibit superior cell compatibility [9]. A scaffold with a pore size of 300 μm enhances the migration capacity of DPSCs, though the specific magnitude of this enhancement remains to be determined in the study. The stiffness of the scaffold (e.g., 10–20 MPa) matches the mechanical properties of natural bone tissue, enabling it to support cellular spreading and differentiation processes [14]. Fig.1 illustrates the mechanism by which the β-TCP bio scaffold regulates cellular behavior through its physical signals: the microporous structure (pore size >100 μm) provides pathways for growth factors and MSCs to enter; the high porosity effectively expands the specific surface area, thereby enhancing interfacial biological activity; simultaneously, the increased surface roughness further mediates cell adhesion and proliferation [13].

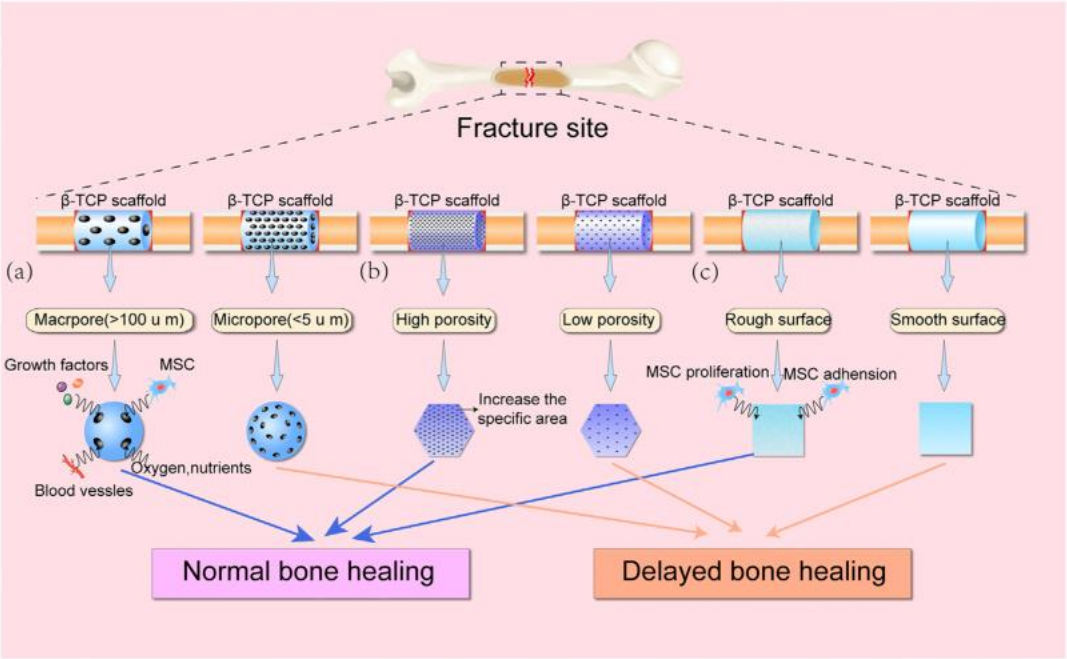

Figure 1. The influence of physical characteristics of β-TCP scaffolds on bone healing [13]

3.2. Regulatory Role of Chemical Signals

Calcium ions (Ca^{2+}) and phosphate ions (PO_4^{3-}) released from β-TCP during degradation can activate calcium-sensitive receptors (CaSR) and other signaling pathways (Fig. 2), thereby regulating the process of osteogenic differentiation. Research by Chang et al. indicates that, as a member of the G protein-coupled receptor (GPCR) family, CaSR is activated upon increases in extracellular Ca^{2+} concentration. This activation subsequently mediates downstream signaling events, including pathways such as phospholipase C (PLC) and mitogen-activated protein kinase (MAPK), ultimately upregulating the expression of osteogenesis-related genes such as Runx2 [15]. Upon activation, CaSR significantly upregulates alkaline phosphatase (ALP) activity and further enhances osteogenic efficiency. Studies indicate that by modulating Ca^{2+} concentration, ALP activity can be increased by approximately 30%, while the number of mineralized nodules rises by 50%, confirming a clear positive correlation between ion concentration and osteogenic efficiency [15]. On the other hand, degradation products of β-TCP can also serve as mineralization nucleation sites, inducing extracellular matrix mineralization and thereby accelerating bone tissue formation [16]. The schematic diagram below illustrates the key mechanism by which the CaSR-mediated signaling pathway regulates the process of osteogenic differentiation [17].

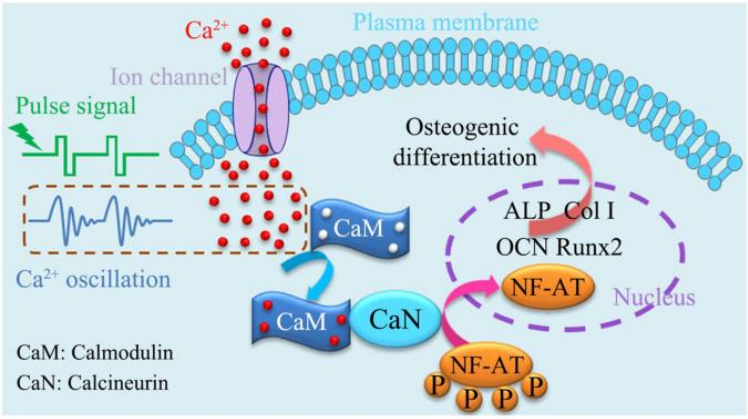

Figure 2. The binding of calcium ions to CaM activates the mechanism of calcium ion signaling pathway, which further mediates osteogenic differentiation by upregulating the expression of osteogenic related genes [17]

3.3. Regulatory Role of Biological Signals

In the bone regeneration microenvironment formed by β-TCP scaffolds, multiple biological signals synergistically regulate the osteogenic differentiation process of DPSCs through a multidimensional network. The core mechanism of this process involves bone morphogenetic protein-2 (BMP-2), a key soluble growth factor: after sustained release from the scaffold, BMP-2 activates the BMPR-Smad1/5/8 signaling pathway in DPSCs; subsequently, phosphorylated Smad proteins bind to Smad4 as a complex and are translocated into the nucleus to directly promote the Runx2 transcription factor expression directly, thereby upregulating the levels of osteogenic marker genes such as alkaline phosphatase (ALP) and osteocalcin (OCN) [18]. Meanwhile, surface-modified integrin ligands (e.g., RGD peptides) collectively promote mineralized nodule formation by activating the Integrin α3/β-catenin signaling axis, which converts mechanical signals during cell adhesion into biochemical signals that promote differentiation [19]. In addition, natural nanocarriers represented by exosomes can be loaded with functional molecules such as miR-129-5p, which can target the inhibition of DKK2 (a Wnt pathway repressor) and attenuate its inhibitory effect on β-catenin, and then positively regulate the process of bone formation at the post-transcriptional level by activating the Wnt/β-catenin signaling pathway [20]. Efficient induction of BMP-2, integrin-mediated adhesion signaling, and exosome-involved paracrine fine-tuning-these three types of bio signals constitute a synergistic network in β-TCP scaffolds, which together enhance the osteogenic efficacy of DPSCs. The figure 3 clearly shows the BMP-2-Smad/MAPK-Runx2 core axis [21]. The BMP/Smad signaling pathway has the function of regulating osteogenic differentiation of bone progenitor cells. In the activated MAPK pathway, the expression and activation of Runx2 are regulated through p38 and ERK1/2 signaling. Although TNF - α and IL-1 β activate p38, ERK1/2, and JNK1/2 signaling pathways, their effects are opposite, counteracting the effects of BMP-2 on Runx2 expression and osteoblast differentiation. In addition, without the positive influence of BMP/Smad signaling, both JNK1/2 and ERK1/2 signaling were activated while BMP-2-induced Runx2 activity was inhibited, leading to inhibition of osteoblast differentiation.

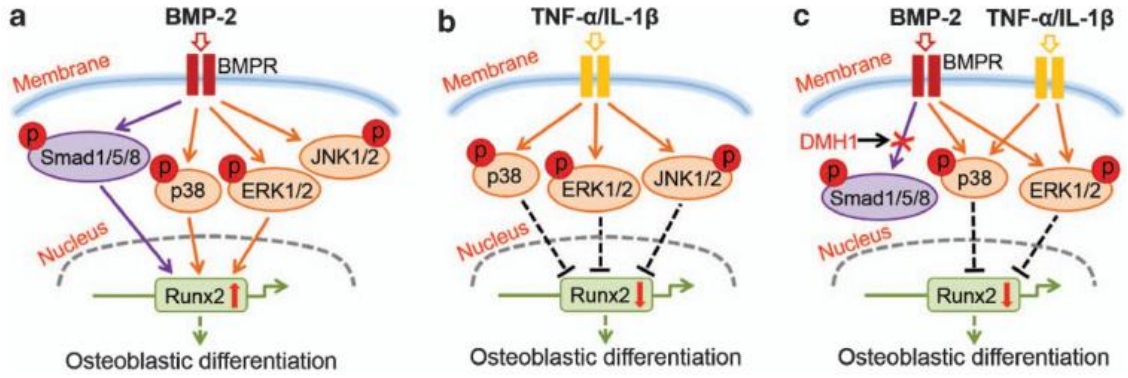

Figure 3. The MAPK signaling pathway (activated by TNF-a/IL-1b - and BMP-2) acts on Runx2, further regulating the BMP-2-induced osteogenic differentiation pathway [21]

4. Parameters of Extrusion-based 3D Printing β-TCP-Containing Bioscaffolds

Extrusion 3D printing is a type of additive manufacturing technology that continuously extrudes thermoplastic materials or bio-inks through a print head and molds them layer by layer into a stack. This technology is widely used in various fields, such as prototyping, medical implants, and customized parts, due to its lower cost and ease of operation. Using extrusion 3D printing technology, β-TCP scaffolds with precisely controllable pore sizes in the range of 100-400 μm could be successfully prepared. A study by Qiuju Miao showed that scaffolds with large pore sizes (e.g., 400 μm) significantly promoted macrophage polarization toward a reparative M2 phenotype and induced the release of angiogenesis-promoting factors such as PDGF-bb. Through this regulatory mechanism, endothelial cells enhance their tubule-forming capacity in vitro and accelerate the processes of

angiogenesis and new bone formation in bone defect regions within animal models [1]. This technology has been introduced into clinical practice for personalized repair of small bone defects, achieving favorable osseointegration outcomes [1]. When preparing β-TCP-containing bio-scaffolds via extrusion-based 3D printing technology, the mass ratio of β-TCP to polymer materials (such as PCL) is typically set between 30% and 50% to balance the material's bioactivity and printability [22]. This ratio maintains the required level of biological activity while ensuring smooth extrusion of the material. When configuring printing parameters, the nozzle diameter (0.2–0.5 mm) and layer thickness (0.1–0.3 mm) should be matched and adjusted according to the pore size conditions required for dental pulp stem cell (DPSC) growth [10]. The degradation rate of scaffolds can be modulated through subsequent treatments such as crosslinking or sintering, thereby influencing cellular behavior and tissue regeneration processes.

5. Limitations and Future Outlooks

β-TCP bio scaffolds constructed based on extrusion 3D printing technology currently have several limitations in the application process of promoting osteogenic differentiation of DPSCs. First, ceramic scaffolds tend to exhibit low mechanical strength due to their intrinsic brittleness; they are more susceptible to brittle fracture in wet environments, thus making it difficult to meet the mechanical load-bearing needs of bone tissue. In addition, the bioactivity of pure β-TCP scaffolds is still relatively limited while maintaining good osteoconductive properties, and the ability to induce directed osteogenic differentiation of DPSCs is not yet sufficient; moreover, the degradation rate of the scaffolds has not yet achieved a good match with the rate of new bone generation, which still needs to be optimized further. Currently, achieving precise modulation of micro- and nanoscale structural features and spatiotemporal distribution properties of bioactive factors during the printing process is still a significant challenge, a limitation that constrains the accuracy of scaffolds for targeted modulation of cellular behavior.

In the future, research in this field is expected to further deepen the development of multifunctional integration, precise regulation, and individual adaptation. On the one hand, composite bioinks consisting of β-TCP with polymers (e.g., gelatin, chitosan) or functional bioceramics (e.g., strontium/zinc-doped calcium phosphate) can be developed to synergistically optimize the mechanical properties and bioactivity of the scaffolds. On the other hand, by employing surface functionalization strategies, such as encapsulating exosomes derived from DPSCs or growth factors such as BMP-2/VEGF, biologically active biomimetic microenvironments can be constructed, thus facilitating the precise spatiotemporal and spatial regulation of the osteogenic differentiation process of DPSCs. At the technical level, the integration of multi-scale fabrication strategies and artificial intelligence-assisted parameter optimization methods will facilitate the development of personalized scaffolds with gradient pore characteristics and spatial programming capabilities of active factors, thereby enhancing the efficacy and precision of bone tissue defect repair.

6. Conclusion

In summary, this paper systematically explores the application and mechanism of extruded 3D bioprinted β-TCP-based scaffolds in promoting osteogenic differentiation of DPSCs. Present research confirms that these scaffolds, with their highly permeable porous structure and modifiable components, effectively promote the adhesion and proliferation of DPSCs. Simultaneously, they significantly enhance the expression activity of early osteogenic markers such as alkaline phosphatase (ALP). On one hand, the microenvironment formed by calcium and phosphorus ions released through β-TCP demineralization induces enhanced bone formation. On the other hand, it enhances the transcriptional stability of key genes such as Runtx2 through epigenetic mechanisms like m6A methylation. Although these scaffolds still exhibit shortcomings in mechanical properties, degradation rate matching, and the precision of bioactivity regulation, they demonstrate significant

application value and research potential in the field of bone regeneration through functional gradient structural design, multi-material composites and the incorporation of bioactive factors. Through a systematic review of existing research, this overview establishes a theoretical foundation for in-depth exploration in this field and identifies feasible technical pathways. It provides significant guidance for advancing the clinical application of personalized, functionalized bone tissue engineering scaffolds.

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