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ABSTRACT

Introduction: This systematic review and proportional meta-analysis aims to evaluate the postoperative complication rate (CR%) of ceramic-based 3D-printed bone grafts based on the reported scientific articles conducted with human individuals.

Methods: MEDLINE and SCOPUS were used as information sources. The synthesis of the study was carried out from studies with human individuals and the use of 3D-printed bone graft-ceramic as inclusion criteria. Cohen's kappa (κ) was calculated for interrater reliability. Qualitative analysis was performed based on the characteristics and outcomes of the individual study, and quantitative analysis was performed using proportional meta-analysis for CR%.

Results: A total of 1352 records were identified through databases and resulted in 11 included studies (κ = 0.81–1.00) consisting of prospective clinical trials (64.63%), case series (16.67%), and case reports (18.18%). The overall postoperative complication rate was 14.3% (95% CI: 0.19–53.6). The postoperative complication rate for studies conducted on the cranial defect, the maxillofacial-zygomatic defect, and the tibial-femoral defect was 2.7%, 11.1%, and 15.6%, respectively. This review also highlights common 3D printing techniques, materials, and grafs' characteristics, as well as their clinical applications.

Conclusions: Ceramic-based 3D-printed bone grafts show potential as alternatives for bone tissue reconstruction.
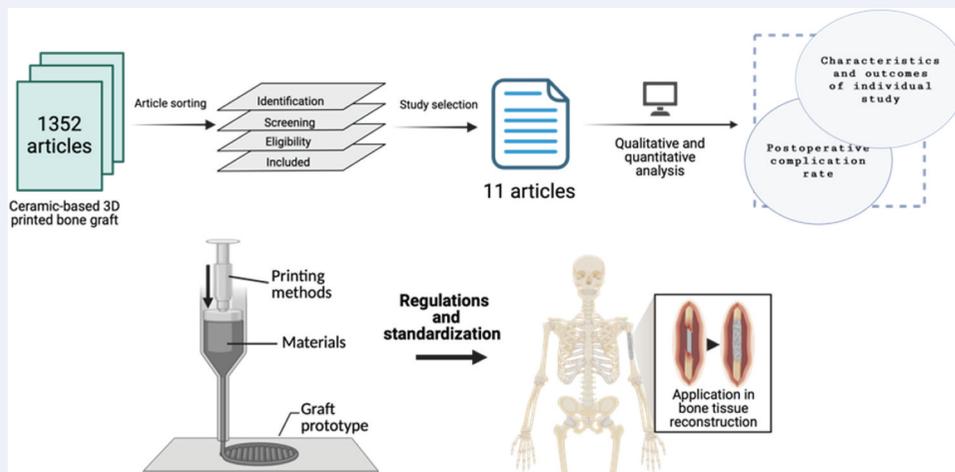
1. Introduction

Bone defects are serious tissue injuries that result from pathological circumstances, such as trauma, infections, and tumors [1]. Bones can heal themselves through self-healing mechanisms under suitable physiological and environmental conditions [1,2]. Secondary healing accounts for most fracture healing and is dependent on osteogenesis, osteoinduction, and osteoconduction factors. Osteoprogenitor cells that differentiate into osteoblasts

and osteoclasts are specifically provided by mesenchymal stem cells, which are found in the bone marrow, granulation tissue, periosteum, surrounding soft tissues, and blood vessels. Osteoinductive factors (pro-inflammatory cytokines, growth factors, angiogenic factors, etc.) are transported to the fracture site by the vasculature and stimulate this differentiation, while osteoconduction is provided by the hematoma and cartilage callus [3]. Despite this robust function of osteoinductive factors, conditions such as large bone defects have a poor healing process due to the large size of the defect (2.5 cm or greater) and limited vascularization in the injured tissue. As a result, bones cannot heal spontaneously during a patient's lifetime [3,4]. Since there is quite a large loss of bone tissue, a support that is strong enough mechanically is needed to support the lost tissue with expectations that natural healing of the tissue can occur.

Limitations in the natural healing of bone defects in critical-sized cases necessitate specialized treatment in which the bone implant used must be anatomically compatible with the patients. 3D printing has grown exponentially in recent decades in healthcare. This additive manufacturing paradigm unleashes significant design freedom, making the technique perfectly suited for fabricating patient-specific anatomic models [5]. The application of 3D-printed devices, such as bone grafts, has been proven to improve functional and aesthetic outcomes while shortening surgery time [6,7]. This technique also does not cause donor site morbidity as commonly found in autologous techniques and is favored by both patients and surgeons [8,9]. It is predicted that by 2028, the patient-specific 3D-printed medical devices market will vastly grow and reach $6.9 billion with an annual growth rate of 17.1% [10].

Ceramics are widely used as bone grafts due to their inertness and biocompatibility [11,12]. Ceramics, such as β-tricalcium phosphate (β-TCP) and hydroxyapatite (HA), have been shown to have positive impacts on bone regeneration whether used alone or as composites [13,14]. However, ceramics are recognized for their fragile characteristics [11,15]. As a result, using ceramic as a single material in bone reconstruction is ineffective, especially in load-bearing applications [11]. Combining biodegradable polymers offers an alternative to improved ceramic-based characteristics. Poly(lactic acid) (PLA) continues to be a favorable material for tissue engineering and bone fixation devices due to its biocompatibility, full biodegradability, and high stiffness [16]. A systematic review by Alonso-Fernández et al. [17] aimed to investigate the use of PLA/ceramics bone grafts in animal studies. The author detailed the biocompatibility and mechanical resistance of PLA/ceramics-based bone grafts have potential in clinical applications [17]. However, in recent years, the use of another biocompatible and biodegradable polymer called poly(ε-caprolactone) (PCL) is starting to dominate the bone regeneration field [16]. PLA and PCL differ in physical and mechanical properties, such as density, glass transition temperature, and melting temperatures [18,19]. This influences the end-point features of PLA/PCL-based bone grafts and how they perform in the *in vivo* environment which may influence the selection of the materials [17,20].

Unlike autologous bone grafts, 3D-printed bone grafts are still foreign to the host and may elicit an intense immune reaction. For example, HA is substantially more crystalline than bone mineral, which makes HA-based implants substantially less resorbable and was reported to induce inflammatory responses [11,21,22]. Other than HA, PLA is also an FDA-approved material and is used as a thermoplastic polymer in the 3D printing technique. However, PLA breaks down into lactic acid which may induce excessive inflammatory responses [18]. Because of this, further investigation is needed to evaluate the safety of ceramic-based 3D-printed bone grafts from the existing studies in humans.

To the best of our knowledge, no reviews have reported postoperative complications in the use of ceramic-based 3D-printed bone grafts in humans. Here, we systematically review the use of ceramic-based 3D-printed bone grafts in humans and statistically analyze the proportion of postoperative complication rate by using proportional meta-analysis. We used proportional meta-analysis because this type of analysis focuses on estimating the overall proportion, for example, the survival rate [23] or nonunion rate in the use of particular grafts [24]. We are also delving deeply into the commonly used 3D printing methods, the character of the most used materials, and how they influence the choice of materials for clinical applications. This review will pave the path for the commercialization and clinical applications of ceramic-based 3D-printed bone grafts, as well as for the potential replacement of autologous bone grafts as the 'gold standard' in clinical practices with 3D-printed bone grafts.

2. Methods

2.1. Protocol and registration

This review is to evaluate the postoperative complication rate (CR%) of ceramic-based 3D-printed bone grafts based on the reported scientific articles conducted with human individuals. We perform a systematic review before meta-analysis to comprehensively identify the evidence. The systematic review was conducted and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [25,26]. The review protocol was registered on the International Prospective Register of Systematic Reviews (PROSPERO) database with ID CRD42023478050. This study did not require ethical approval or informed consent.

2.2. Eligibility criteria

Eligibility criteria were considered concerning all human studies with population, intervention, comparator, and outcome (PICO) framework as presented in Table 1. The focused question of this review was 'Is ceramic-based 3D printed bone graft safe in clinical application?'

2.3. Information sources

The electronic databases used were MEDLINE (PubMed) and SCOPUS. Hand searches were also performed on the authors' electronic library and the references in the included articles. The final update for all electronic searches was completed on 18 October 2023.

Table 1. Search strategy and criteria for inclusion.

Search terms	Population	#1 – ((clinical study) OR (clinical trial) OR (RCT) OR (human) OR (subject) OR (participant))
	Intervention	#2 – ((3D print*) OR (3D bone) OR (fused deposition modelling) OR (selective laser sintering) OR (stereolithography) OR (digital light processing) OR (3D gel printing)) AND ((ceramic) OR (hydroxyapatite) OR (alumina) or (tricalcium phosphate) OR (zirconium oxide) OR (barium titanate) OR (silicon carbide) OR (silica carbide) OR (ferrite) OR (calcium silicate) OR (calcium carbonate))
	Comparison	#3 – ((transplantation) OR (autologous) OR (autografts) OR (tissue scaffold) OR (tissue harvesting) OR (organ harvesting))
	Outcome	#4 – ((bone regeneration) OR (bone formation) OR (graft rejection) OR (bone gain) OR (intraoperative complications) OR (postoperative complications) OR (complication) OR (side effect) OR (adverse effect) OR (treatment failure) OR (graft failure))
Search combination		#1 AND #2 AND #3 AND #4
Publication year		1 January 2000 to 26 September 2023
Database search	Electronic	PubMed, SCOPUS
	Journals	All peer-reviewed journals available in PubMed and SCOPUS. No filters were applied for the journals.
Selection criteria	Inclusion criteria	The study must be human studies (such as prospective clinical trials, case series, and case reports). The intervention must be a 3D-printed bone graft with ceramic as the main or substitute material that is used at any location related to the bone defect. The study must report if the intervention causes postoperative side effects or not.
	Exclusion criteria	*In vitro*, *in vivo*, *in silico* studies, literature review, and study protocol.

2.4. Search strategy

The initial search technique was designed and implemented by MAG and HDM. The search terms used are presented in Table 1. The search terms were combined with 'AND' and limited to articles published between 1 January 2000, and 26 September 2023. The search was then discussed by MAG, HDM, and IKA to confirm the number of discovered articles.

2.5. Study selection

Studies that met the inclusion criteria were included in this review (Figure 1, Table 2). Initial title and abstract screening were performed independently by MAG and HDM. The final list of studies for full-text analysis and data extraction was provided only after

the two investigators reached an agreement. Disagreements were resolved via a consensus discussion with IKA. Cohen's kappa (κ) was calculated for interrater reliability between investigators by using SPSS version 24.0 software (IBM Corporation, U.S.A.). During the study selection, studies that used other methods to fabricate personalized bone grafts were also included in separate tables in the data collection process to provide a border overview.

2.6. Data collection

MAG and HDM independently extracted the data from the included studies based on the required parameters and information that have been previously agreed between MAG, HDM, and IKA. This was related to the descriptive patient/population level information (e.g. sample size, mean patient age, gender
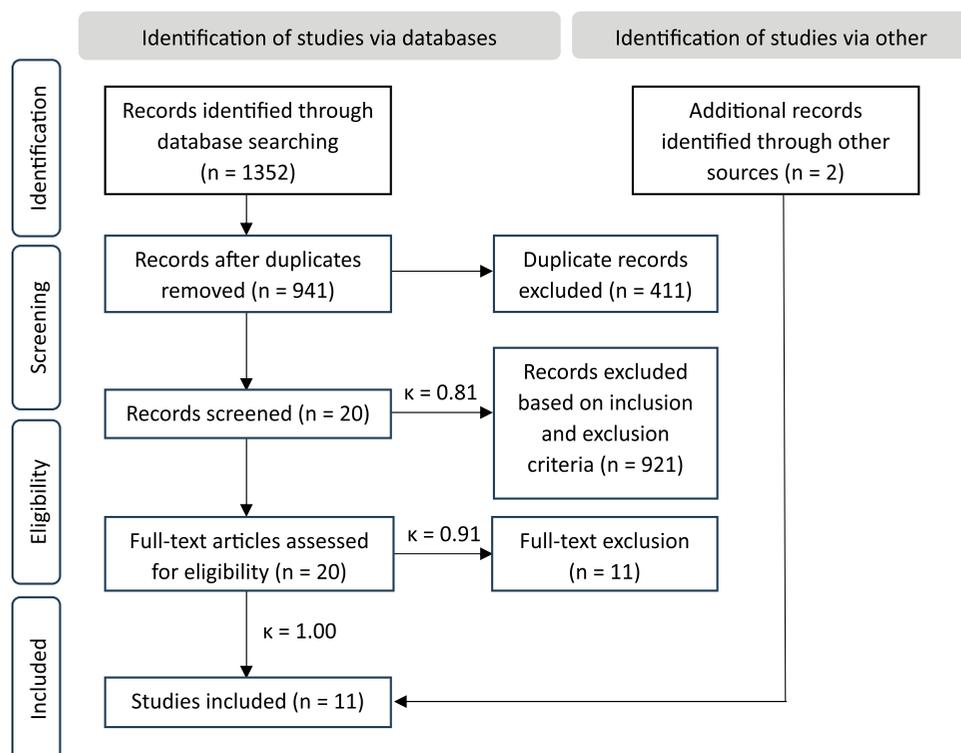

Figure 1. The flow-chart of study inclusion.

Table 2. The main reason for exclusion after full-text screening.

Main reason	Number (n)	References
Did not use 3D printing technology	9	[27–34]
Not human study	1	[35]
Did not report postoperative complications	1	[36]

distribution), treatment and fabrication technique, and study outcomes. Disagreements were resolved through discussion.

2.7. Missing data

Missing information regarding postoperative complications was requested through e-mail to the corresponding author of the study. In case of a non-response, the study was excluded.

2.8. Statistical analysis

A fixed effect was performed to determine the proportion of postoperative complication rate (CR%). Each study's data was extracted with 95% confidence intervals (95% CI) and analyzed using JAMOVI software version 2.3.28.0 (retrieved from https://www.jamovi.org). We also analyzed CR% of studies that examined 3D graft implantation for 1) cranial bone, 2) maxillofacial-zygomatic bone, and 3) tibial-femoral bone with 95% CI performed on the same software. I^2 statistics were used to assess heterogeneity, with the following interpretation guide: 0% to 25% indicate low heterogeneity; 25% to 75% indicate moderate heterogeneity; and 75% to 100% indicate significant heterogeneity. Funnel plots were created to test for publication bias.

2.9. Risk of bias of the included articles

The quality of each study was assessed independently by MAG and HDM. The study design of each included study was different. Thus, the quality assessment was conducted by using assessment instruments that matched the study design. Prospective studies with no control group were assessed by using NIH's Study Quality Assessment Tools for Before-After (Pre-Post)Studies With No Control Group, case series studies were assessed by using NIH's Study Quality Assessment for Case Series Studies, while case report studies were assessed by using critical assessment instruments from the Joanna Briggs Institute (JBI) for case reports. In case of disagreement, a third reviewer's opinion (IKA) was sought for further discussion to reach an agreement [37,38].

3. Results

3.1. Study selection

The selection process of this systematic review was based on the PRISMA guidelines (Figure 1). Initial search from databases delivered 752 and 600 studies from SCOPUS and PubMed, respectively, which made 1352 records in total. Title and abstract screened excluded 921 studies and 20 remaining studies were assessed for full-text screening. The reason for full-text exclusion is present in Table 2: Nine studies did not use 3D printing technology to fabricate the intervention used

and/or did not conduct 3D printing on the ceramic material but on other supporting devices used in the treatment, such as titanium mesh or reconstruction plates for bone [27–34,39]. One study is not a human study [35] and one study did not report postoperative complications after bone graft operation surgery. On the other hand, manual searches were also performed on the authors' library and references in the included articles, this resulted in two articles that were also included in the review [7,40]. Studies that used other methods to fabricate 3D bone grafts were also listed in Supplementary 3 and 4 to provide a border overview of personalized bone grafts.

3.2. Study characteristics

Of the 11 included studies, 7 studies (64.63%) are prospective clinical trials, 2 studies (16.67%) are case series, and 2 studies (18.18%) are case reports (Table 3). For studies that used other manufacturing methods, 5 studies (71.43%) are prospective clinical trials and 2 studies (28.57%) are case reports (Supplementary 3).

3.3. Qualitative analysis of the included studies

TCP was the most used ceramic in 3D-printed bone grafts in the included studies (81.81%), with 3 studies using α-TCP (27.27%), 2 studies using β-TCP (18.18%), and 4 other studies used TCP but did not report the form of TCP (36.36%). HA and BGS-7 were also used in one study, respectively (each counts 9.09%). Combination material was also used in the included studies with PCL count as the most used material (6 studies, 54.54%), and resin in one study in combination with HA (9.09%, Table 3). Moreover, 85.71% of studies that used other methods used HA ceramic, while one study used BCP (14.29%). The method used in these studies was CNC in 4 studies (57.14%), molding in 2 studies (28.57%), and cutting in one study (14.29%, Supplementary 3).

All eleven studies reported information regarding postoperative complications after bone graft treatment. Infection was the most common postoperative event with five cases. Events such as redness swelling and scaffolding failure also occurred (Table 4). For studies that used other methods to fabricate 3D bone grafts, postoperative complications vary, such as dehiscence of the bone grafts, scalp thinning, pain, hyperesthesia, and others (Supplementary 4). Outcomes related to bone regeneration vary between studies as presented in Tables 4 and Supplementary 4.

3.4. Proportional meta-analysis of the included studies

No study included a negative or positive control group. Thus, we presented the statistical analysis as a proportional meta-analysis. The overall postoperative complication rate was 14.3% (95% CI: 0.19–53.6, Figure 2). A low level of heterogeneity was found among the studies ($I^2 = 0\%$, $p = 0.763$ $n = 11$; Figure 2). A funnel plot analysis was performed to investigate potential publication bias (Supplementary 1). Furthermore, the postoperative complication rate for studies conducted on cranial defect was 2.7% (95% CI: 8.7–14.0, Figure 3), the maxillofacial-zygomatic defect was 11.1% (95% CI: 1.54–20.6,

Table 3. Characteristics of the included studies.

Study	Country	Study design	Type of defect	Treatment type	Software	Device	Sample size (n) Patient	Sample size (n) Region	Mean age (years)	Gender (n) Female	Gender (n) Male
Saijo 2009	Japan	Prospective clinical trial	Maxillofacial	α-TCP	NS	Z406 3D (Z-Corporation, U.S.A.)	10	10	35.0 (18–55)	9	1
Probst 2010	Germany	Case report	Cranial	PCL/TCP	Mimics (Materialise, Belgium)	FDM3000® (Stratasys Inc., U.S.A.)	1	1	11.0	NS	NS
Brie 2013	France	Prospective clinical trial	Cranial	Resin/HA	NS	NS	8	8	44.1 (27–63)	2	6
Kanno 2016	Japan	Prospective clinical trial	Maxillofacial	α-TCP-sodium chondroitin sulfate-disodium succinate (termed as CT-bone)	Mimics (Materialise, Belgium)	Z406 3D (Z-Corporation, U.S.A.)	20	20	31.7 (18–55)	14	6
Saijo 2016	Japan	Prospective clinical trial	Maxillofacial	α-TCP	NS	Z406 3D (Z-Corporation, U.S.A.)	20	23	31.7 (18–55)	14	6
Kobbe 2020	Germany	Case report	Femoral	PCL/TCP combined with autologous bone graft and BMP-2	NS (procced by Osteopore®)	NS (procced by Osteopore®, Singapore)	1	1	29.0	NS	NS
Lee 2020	South Korea	Prospective clinical trial	Zygomatic	CaOSiO2-P2O5-B2O3 glass-ceramic (BGS-7)	3 Matics (Materialise, Belgium)	NS (procced by Osteopore®)	10	10	36.0 (24–53)	9	1
Castrisos 2022	Australia	Case series	Maxillofacial	PCL/TCP	3 Matics (Materialise, Belgium)	NS (procced by Osteopore®)	1	1	12	1	-
			Tibial	PCL/TCP	3 Matics (Materialise, Belgium)	NS (procced by Osteopore®)	2	2	21.5 (16–27)	1	1
			Cranial	PCL/TCP	3 Matics (Materialise, Belgium)	NS (procced by Osteopore®)	1	1	25	-	1
Jeong 2022	South Korea	Prospective clinical trial	Zygomatic, maxillary	PCL/β-TCP	3 Matics (Materialise, Belgium)	NS	8	8	36.4 (19–51)	4	4
Laubach 2022	Germany-Australia	Case series	Femoral, tibial	PCL/TCP combined with autologous bone graft	Geomagic (3D Systems, U.S.A.) or Autodesk Meshmixer (Autodesk Inc., U.S.A.)	NS (procced by Osteopore®)	4	4	30.5 (23–42)	NS	NS
Park 2022	South Korea	Prospective clinical trial	Cranial	PCL/β-TCP	Mimics (Materialise, Belgium)	NS (procced by TnR T&R Biofab, South Korea)	7	7	34.3 (20–62)	NS	NS

α-TCP, alpha-tricalcium phosphate; β-TCP, beta-tricalcium phosphate; PCL, polycaprolactone; NS, not specified.

Figure 4), the tibial-femoral defect was 15.6% (95% CI: 16.2–47.4, Figure 5).

3.5. Risk of bias of the included articles

The results of the quality assessment of the included studies are shown in Supplementary 2. The included studies are considered to have good/fair quality with a low risk of bias.

4. Discussion

This review demonstrates that a variety of techniques are employed to create 3D ceramic-based bone grafts in the clinical trials that have been documented thus far. This proportional meta-analysis found that the total postoperative complication rate for 3D ceramic-based bone grafts was 14.3%, while the postoperative complication rate for studies conducted on the cranial defect, the maxillofacial-zygomatic defect, and the tibial-femoral defect was 2.7%, 11.1%, and 15.6%, respectively. The benefit of utilizing a 3D-printed bone graft is that there is no

donor site morbidity, which is known to play a significant part in postoperative problems with autologous bone grafts [8,9]. Here, we discussed in detail the fabrication methods that have been used to produce 3D ceramic-based bone grafts, their characteristics, as well as their performance in bone healing.

4.1. Fabrication technique for custom-made 3D bone graft

The fabrication technique used to produce 3D bone grafts varies between clinical investigations included in this review. In this section, here we discussed in detail the general fabrication method of 3D bone grafts, which includes conventional cutting or molding methods, layer-by-layer 3D printing without biological material, and layer-by-layer 3D printing with biological materials.

4.1.1. Cutting or molding methods

Non-layer-by-layer fabrication is one technique to produce customized 3D bone grafts. By using the computer numerical

Table 4. Outcomes of individual study.

Study	Months of follow-up, mean (range)	Outcomes		
		Assessment method	Parameter	Results
Saijo 2009	6, 12	CT scan, clinical symptom	Intraoperative complications	NM
			Postoperative complicatuons	0/10 patients
			Compatibility	10/10 patients
			Bone union after 12 months	10/10 patients
			Satisfaction	10/10 patients
Probst 2010	6, 9, 12	CT scan, clinical symptom	Intraoperative complications	NM
			Postoperative complications	Not present
			Overall result	The scaffold was well integrated and beginning bony consolidation was detected.
Brie 2013	1, 6, 12	CT scan, clinical symptom	Intraoperative complications	NM
			Postoperative complications	0/8 patients (in terms of infection or fracture of the implant)
			Satisfaction	8/8 patients
Kanno 2016	36.555 (12–87)	CT scan, clinical symptom	Intraoperative complications	NM
			Postoperative complications after 1 year	0/20 patients
			Postoperative complications after 1–5 years	4/20 patients Infections were present in 4 sites of 4 patients which made the CT bone removed
			Compatibility	23/23 sites
			Chronological change	0/23 sites
			Bone union	18/21 sites
			Satisfaction	18/20 patients
Saijo 2016	55.526 (13–115)	CT scan, clinical symptom	Intraoperative complications	NM
			Postoperative complications	5/20 patients An artificial bone of 1 patient was broken, 1 patient was an MRSA carrier and infection occurred early after surgery, patients experienced redness and swelling on the implantation regions
Kobbe 2020	12	CT scan, X-ray, clinical symptom	Intraoperative complications	NM
			Postoperative complications	Not present
			Bony fusion	Almost complete
Lee 2020	6	CT scan, clinical symptom	Intraoperative complications	NM
			Postoperative complications	0/10 patients
			Bone fusion	10/10 patients, average fusion rate of 76.97% (58.33–88.24%)
			Immobilization	Average displacement 0.415 mm (0.1155–0.889 mm)
			Satisfaction	9/9 patients, 1 patient lost to follow-up
Castrisos 2022	15.3 (4–35)	CT scan, X-ray, clinical symptom	Intraoperative complications	0/4 patients
			Postoperative complicatuons	1/4 patients 1 patient had extensive blistering of the native skin distal to the CPCF skin paddle on postoperative day two (tibial defect)
Jeong 2022	6	CT scan, clinical symptom	Intraoperative complications	NM
			Postoperative complications	1/8 patients 1 patient had wound dehiscence due to delayed wound healing
			Volume conformity	Mean of 79.71% (70.89–86.31)
			Bone volume fraction	Mean of 23.34% (7.81–66.21)
			Tissue density	Mean of 188.84 hU (151.48–291.74)
Laubach 2022	15.250 (8–23)	CT scan, X-ray, clinical symptom	Intraoperative complications	0/4 patients
			Postoperative complications	0/4 patients
			Compatibility	4/4 patients
			Bony ingrowth	3/4 patients
			Comprehensive bone regeneration and full weight-bearing	1/4 patients Present in the case with a 10 cm tibia shaft
Park 2022	8.386 (6–17)	CT scan, clinical symptom	Intraoperative complications	NM
			Postoperative complications	1/7 patients 1 patient had a seroma at 3 months after operation
			Increased soft tissue volume at 2 weeks	15.800 cm^3 (6.3–53.3)
			Increased soft tissue volume at 6 months	14.871 cm^3 (6.3–48.7)
			Symmetry after surgery	6/7 patients 1 patient had partial symmetry
			Smoothness on the implant edges	6/7 patients 1 patient had a slightly irregular edge

NM, not mentioned.

control (CNC) method, it is possible to automate the control of machine tools using software embedded in a computer to cut or mold a particular material [41]. In the screening and full-text assessment of our systematic review, we found 7 studies that used the non-layer-by-layer fabrication method. References number [28,41–43] used the CNC milling technique, references number [44,45] used the molding technique, and references number [46] used the cutting technique (Supplementary 3).

Study	Event	P	LL	UP
Saijo 2009	0/10	0.000	0.00000	0.326
Probst 2010	0/1	0.000	0.00000	0.829
Brie 2013	0/8	0.000	0.00000	0.378
Kanno 2016	4/20	0.200	0.07676	0.423
Saijo 2016	5/20	0.250	0.10982	0.474
Kobbe 2020	0/1	0.000	0.00000	0.829
Lee 2020	0/10	0.000	0.00000	0.326
Castrisos 2022	1/4	0.250	0.03953	0.710
Jeong 2022	1/8	0.125	0.00500	0.495
Laubach 2022	0/4	0.000	0.00000	0.550
Park 2022	1/7	0.143	0.00954	0.536
Overall 95% Cl.	12/93			

Heterogeneity: $I^2 = 0\%$

Figure 2. Forest plot showing postoperative complication rate % for all included studies (Cl, confidence interval).

Study	Event	P	LL	UP
Probst 2010	0/1	0.000	0.00000	0.829
Brie 2013	0/8	0.000	0.00000	0.378
Lee 2020	0/10	0.000	0.00000	0.326
Castrisos 2022	0/1	0.000	0.00000	0.829
Park 2022	1/7	0.143	0.00000	0.536
Overall 95% Cl.	1/27	0.027	-0.0870	0.140

Heterogeneity: $I^2 = 0\%$
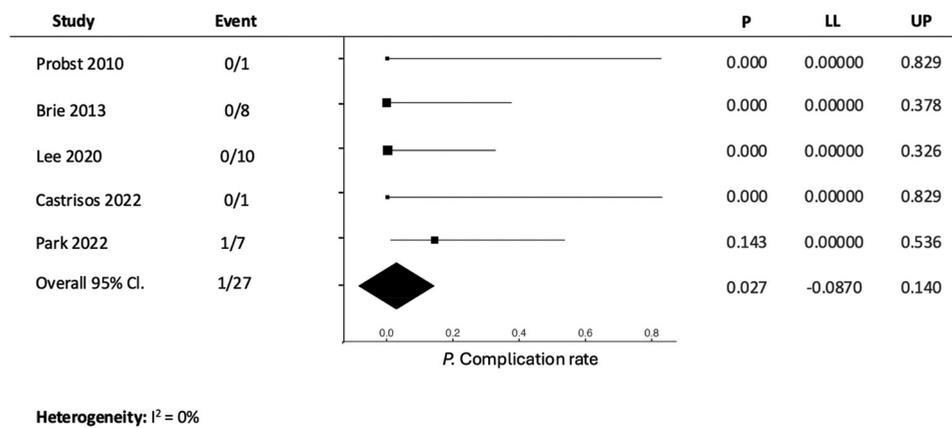
Figure 3. Forest plot showing postoperative complication rate % for cranial defect (Cl, confidence interval).

Study	Event	P	LL	UP
Saijo 2009	0/10	0.000	0.00000	0.326
Kanno 2016	4/20	0.200	0.07676	0.423
Saijo 2016	5/20	0.250	0.10982	0.474
Lee 2020	0/10	0.000	0.00000	0.326
Castrisos 2022	0/1	0.000	0.00000	0.829
Jeong 2022	1/8	0.125	0.00500	0.495
Overall 95% Cl.	10/69	0.111	0.0154	0.206

Heterogeneity: $I^2 = 21.186\%$

Figure 4. Forest plot showing postoperative complication rate % for maxillofacial-zygomatic defect (Cl, confidence interval).

Study	Event		P	LL	UP
Kobbe 2020	0/1		0.000	0.0000	0.829
Castrisos 2022	1/2		0.500	0.0999	0.900
Laubach 2022	0/4		0.000	0.0000	0.550
Overall 95% Cl.	1/7		0.156	-0.162	0.474

P. Complication rate

Heterogeneity: I² = 54.07%

Figure 5. Forest plot showing postoperative complication rate % for tibial-femoral defect (CI, confidence interval).

The outcomes of each study are presented in Supplementary 4.

CNC milling technique uses porous ceramic blocks, such as HA [28,41] or BCP [42] to fabricate 3D bone grafts. This technique is great for shaping materials by generating small chip fragments [47]. However, since no layer-by-layer processing is applied, the porosity of the material is difficult to customize during the fabrication process. For instance [48], used gel casting foam to produce customized bone grafts by using a porous hydroxyapatite foam. The porosity of the graft was 83.3% and had an average compressive strength of 2 MPa. Fabris *et al.* [49] also fabricated zirconia grafts with periodic open cellular structures using CNC machining from zirconia blocks. Different drill sizes used in the study (1 to 2 mm diameter driller) produced bone grafts in distributed pore sizes with porosity starting from 39 to 57% with compressive strength starting from 667 MPa to 300 MPa, respectively [49]. This proved that controlled pore size and/or porosity of bone grafts fabricated by using this CNC machining can only be reached by using suitable drill sizes. Other than driller sizes, machining parameters such as spindle speed, feed rate, depth of cut, and machining direction were reported to have impacts on cutting force and surface roughness or ceramic matrix [50].

The bone graft performance made from the cutting/molding techniques varies between studies (Supplementary 3). The study by Mangano *et al.* [41] resulted in 1/10 postoperative complications. The authors reported that a patient experienced dehiscence of the graft two months after the reconstructive surgery [41]. Hardy *et al.* [46] also reported postoperative complications after HA graft implantation. However, the was no additional investigation if the complications were related to the bone graft or not. Only a study by Staffa *et al.* [45] reported that *Neisseria meningitidis* infection experienced by a patient was accelerated because of the grafts' porosity. This showed that bone graft characteristics closely determine the graft's performance in bone healing.

4.1.2. Layer-by-layer conventional 3D printing methods

During the past decades, there has been a shift in the use of conventional methods to layer-by-layer 3D printing methods in fabricating 3D ceramic-based bone grafts. For example, clinical investigation series from Mangano *et al.* [28,41,42] used the CNC milling technique to fabricate ceramic-based 3D bone grafts, however, years later, the researchers started using layer-by-layer 3D printing method to produce 3D BCP

bone grafts [36]. There have been several developed methods for layer-by-layer 3D printing. Here, we discussed in detail each method that has been used for bone tissue reconstruction.

4.1.2.1. Stereolithography (SLA) digital light processing (DLP). SLA uses photosensitive resin material to fabricate 3D objects by controlling the laser through a computer. Surface scanning of the liquid photo resin occurs based on the specific information of the layer. The thin resin layer in the scanned area is cured by photopolymerization to form a thin layer of the part. After one layer is completed, the platform will move down and a new layer will be produced until the 3D part is obtained [51]. One advantage of SLA is its faster printing rate and excellent resolution [52]. The improvement of the SLA method is called digital light processing (DLP), which uses a single projector light source. A beam of light is used to pass across the layer of resin mixture all at once, which simplifies the printing process [52]. However, material choices are limited and expensive for this method [52].

Studies included in this review did not specify which kind of method was used to fabricate layer-by-layer 3D-printed bone grafts. However, other studies have proved that SLA can produce grafts with controlled porosity and pore sizes that result in desired mechanical strength. Sodeyama *et al.* [53] reported that a 3D polymer-infiltrated ceramic network made from SLA exhibited a nano-sized dual-network structure with a similar Vickers hardness to enamel, and a similar elastic modulus to dentin [53]. In fabricating bone grafts, SLA can also be combined with other methods. For example, Hann *et al.* [54] fabricated a biomimetic nano-bone tissue construct with a perfusable by combining SLA and FDM methods. Experiments in physiological conditions revealed that during up to 20 days of observation, the graft improved vascular network formation and osteogenic maturation of the structures [54]. This proved that SLA can produce grafts with specific characteristics that contribute to their beneficial action in physiological conditions.

4.1.2.2. Selective laser sintering (SLS) and selective laser melting (SLM). SLS is a technology that heats powdery material such as wax or plastic powder to fabricate 3D objects. In this method, the powder is heated below its melting point and then flattened using a leveling stick [51]. Under computer control, the laser beam is selectively

sintered based on the delamination-specific information. After one layer is finished, the next layer is sintered, and any remaining powder is removed [51]. To obtain optimal sintering conditions for powders, parameters including laser power and scan speed should be optimized [55]. SLS can print a wide variety of materials and has fast processing speed as well as a high precision of the 3D graft. The advantage of SLS is also supports structures that can be easily removed [52]. Selective laser melting (SLM) is similar to SLS and was developed as an improvement. The primary distinction between them is the heating temperature range. SLS heats the powder surface slightly below the melting temperatures, while SLM heats the powder bed to the material's melting point [52].

SLS/SLM is widely used to print metals for reconstruction plates [56–58]. However, studies also started reporting the use of this method in printing 3D ceramic bone grafts. In combination with polymer, ceramic-based material can be printed with SLS/SLM. A study by Park et al. [59] compared SLS and extrusion-based methods in fabricating a 3D bone graft made from PCL and HA. The authors reported that printing modality had inherent characteristics that impact printing outcomes and eventually implant performance [59]. Another study also showed that HA and PCL printed by SLS showed favorable activities for bone cells [55]. However, SLS/SLM may destroy biological material and thus can not support tissue engineering applications [51].

4.1.2.3. Fused deposition modeling (FDM).
Fused Deposition Modeling, also known as Fused Lamination Modeling, is a part of the extrusion method that employs a heater plug to melt and extrude filaments through the nozzle [51,52]. Similar to another 3D printing method, FDM uses computer controls to selectively coat the material on the workbench based on cross-sectional profile information [51]. After quick cooling, a cross-sectional layer develops. When one layer is completed, the machine table falls a height that is the thickness of the layer and then creates the next layer until the full solid frame is produced [51]. Because of this, the main material that should be used for this method is heat-shrinkable polymers, such as polylactic acid and polycaprolactone [11,21]. FDM is a simple and low-cost method for the fabrication of 3D bone scaffolds. However, it is not ideal for the accuracy of the printed prototype. The involvement of high temperatures in this method can destroy heat-sensitive material, making it non-applicable for the printing of growth factors, proteins, and cells that are mostly involved in tissue engineering [51].

Despite the fact that it can only print a limited number of materials, since it has low cost of manufacturing, FDM has been utilized to create a wide variety of 3D ceramic grafts, such as for HA [60], BCP [61], and TCP [62,63]. This method helps incorporate ceramics into existing bone grafts with various incorporation methods, for example by making filaments/blends containing various materials including ceramic, and using the filaments/blends for 3D printing, which was used by Kim et al [64] with PCL and HA. On the other hand, other researchers used thermoplastic materials as a 'frame' before incorporating the ceramic into the 3D-printed frame with chemical reactions such as hydrolysis [65,66].

The success of this method in fabricating 3D-printed grafts has been widely reported. Wang et al. reported that FDM can produce PLA/nano β-TCP graft with desired internal pores and external structures [67]. The successful fabrication of 3D ceramic-based material was also reported by other researchers [60,63]. In addition, FDM was also reported to produce inversely printed 3D bone grafts. In this technique, lead structures from PLA were printed with FDM for directional bone growth, filled with β-TCP slurry, and then burnt [62]. The compressive strength of the grafts was reported as 3.4 ± 0.2 MPa for 500 μm spacing and reduced when incubated in simulated body fluids [62]. In vitro assays also showed that bone cells were able to adhere and proliferate in the grafts [62]. Given the benefits and the current success of FDM, it is predicted that the application of this method in generating 3D ceramic bone grafts will continue in the coming years.

4.1.3. Layer-by-layer 3D bioprinting method
Increasing demand for tissue/organ transplantation making the additive manufacturing technology for biological materials improved in recent years [52]. 3D bioprinting is one method that prints 3D parts containing biological material to mimic human tissue/organs [68]. The material that is being printed is called 'bioink' which is usually a combination of living cells, biomaterials, or active biomolecules [68]. Based on the method used, 3D bioprinting can be classified as either inkjet, extrusion, or laser-based bioprinting [52,68].

Inkjet, also called droplet bioprinting, was the first printing method that progressed from 2D to 3D and bioprinting. The printer in this method prints ink in droplets on the surface and forms a layer over time [52,69]. In designing a method for inkjet bioprinting, printing conditions should be taken carefully since this may alter culture conditions, especially the pH and temperature. A study by Firaldo et al. [70] used microwave-inkjet bioprinting with mesenchymal stem cells (MSCs) and collagen-based bio-ink to develop a cellularized human meniscus. The authors reported that only about 50% of the cells survived after 5 days of printing. However, after 28 days, the cells were able to grow and colonize in the graft [70]. Cell encapsulation in collagen gels, where the fiber network traps cells, is a common tissue engineering technique. Collagen gel pore size may be modified based on concentration for different uses and cell types [70]. However, bioprinting also can be performed without cell encapsulation. Gao et al. [71] used inkjet bioprinting to fabricate a 3D graft using poly(ethylene glycol) dimethacrylate, gelatin methacrylate, and human MSCs (hMSCs), in order to mimic bone and cartilage tissue. The fabrication was successful with more than 80% of the cells surviving during the printing process without extra steps for cell encapsulation. Graft also showed excellent osteogenic and chondrogenic differentiation capacity [71].

Another method that has also been used in 3D bioprinting technology is the extrusion bioprinting. Extrusion is the process of pushing extrusion-based material through a nozzle using an external source such as air pressure, a piston, or a screw [52]. The printing cost for this method is usually categorized as medium and has a low precision of printed parts [72]. Kang et al. [73] used extrusion-based technology to

fabricate a microchannel networks-enriched 3D hybrid scaffold composed of decellularized extracellular matrix, gelatin, chitosan, and nano-hydroxyapatite (nHA). The scaffold was extruded with pre-gel filaments at 20°C–25°C, crosslinked, frozen at −80°C, lyophilized, and combined with human adipose-derived stem cells (ADSCs) exosomes [73]. Authors reported that the addition of nHA improved the antibacterial properties of the scaffold, while the addition of exosomes promoted cell attachment and proliferation, as well as osteogenesis and vascularity regeneration in vitro and in vivo [73]. Other than gelatin and chitosan, collagen is one of the popular materials used in bioprinting technology due to its high affinity for cells adherent [74]. However, a lack of printability and low mechanical strength hampered collagen applicability through 3D bioprinting. This can be solved by altering the properties of the collagen bioink, for example by the addition of ceramic. It was proved that the addition of HA increased the strength of the scaffold, with BMSCs on the scaffold kept living and proliferating on the scaffold [75]. To archive a specific graft property, bioink also can be varied and combined. For instance, a study by Shen et al. [74] used extrusion-based technology to develop a bone tissue-engineering scaffold. The authors used two types of bio-ink; one is a photo-crosslinked extracellular matrix hydrogel supplemented with MSCs for osteogenesis, and the other is a templating bioink which is a thermosensitive hydrogel supplemented with endothelial cells (ECs) for angiogenesis [74]. This technique aims to enable ECs to form in situ vascular networks within a bone tissue-engineering scaffold [74]. The results showed that a coupling effect between angiogenesis and osteogenesis was archived in vitro, and also excellent performance in bone formation in vivo [74].

Other than inkjet and extrusion-based bioprinting, one printing technology that has been used to print biological material is laser-assisted bioprinting. Similar to SLA, laser-assisted bioprinting uses photopolymerization to create 3D structures with high printing resolution. Like the two existing bioprinting methods, collagen-based bioink is the most commonly used in laser-assisted bioprinting. However, other types of bioink also starting to be developed. Touya et al. [76] developed tricalcium silicate-based ink for laser-assisted bioprinting. The developed bioink confirmed all aspects of the formulation including rheological impact. Besides, the bioink also had great cytocompatibility, influencing cell motility and osteogenic differentiation response in vitro, promoting bone formation in vivo, and can deliver active compounds [76]. Laser-assisted bioprinting method is costly but offers rapid and high-resolution prototype technologies that allow for the exact organization of biomaterials in a predetermined configuration, which is suitable for in situ bioprinting [72,76–78]. Another study used laser-assisted bioprinting to organize endothelial cells in a mouse calvaria bone defect filled with collagen-containing MSCs and vascular endothelial growth factor. The in situ bioprinting was successful; defined local cell density and printing parameters allowed the generation of microvascular networks, which resulted in vascularization and bone regeneration into critical bone defects after two months in vivo [77]. A similar result was also reported by Kerequel et al. [78]. The authors utilized the laser-assisted method for in situ bioprinting by using bioink that contained MSCs, collagen, and nHA for bone regeneration. Results showed that MSCs used in the study remained viable and proliferated in vitro and in vivo [78]. This proved that laser-assisted bioprinting method is a great approach for in situ bioprinting and tissue engineering.

In this review, no included articles used the 3D bioprinting method. This is due to the challenges of the graft characteristics when fabricated by the bioprinting method. The mechanical properties of grafts produced with bioprinting are commonly lower compared to grafts produced by other methods. For instance, the compressive strength of HA bone grafts made by the FDM method was reported to reach ~ 40 MPa due to the combination with a thermoplastic material [67], while the compressive strength of nHA bone graft by 3D bioprinting was about 7 to 16 MPa [73]. This issue makes the 3D bioprinted bone graft have difficulty adapting to the unique mechanical environment of load-bearing bones, which limits their potential applications [79]. In this study, we also detailed the utilization of these 3D printing methods, the materials used, and their characteristics in Table 5.

4.2. Materials used for 3D printed bone graft

Before being used in medical applications, the 3D bone grafts must first be designed to meet existing demands. Mechanical strength, elasticity, interconnected pores, and topography of the bone graft are important considerations for supporting bone healing in defective tissue [52,105]. These properties can only be achieved by selecting suitable materials, besides the 3D printing methods. We discussed in detail the characteristics of the ceramics and polymeric materials commonly used to fabricate 3D bone grafts, and also their impact on the properties of 3D printed bone grafts.

4.2.1. Ceramic material

Ceramics are commonly used in bone graft fabrication due to their inertness and biocompatibility. Tricalcium phosphate (TCP) and hydroxyapatite (HA) are the most used ceramics that are utilized as dental and orthopedic implants. TCP is a calcium salt of phosphoric acid, also known as tribasic calcium phosphate and bone phosphate of lime, with the chemical formula $Ca_3(PO_4)_2$. TCP has three polymorphs which are β-TCP, α-, and α'-TCP. β-TCP is the polymorph that is stable at room temperature and at 1125°C transforms to α-TCP and can be preserved after cooling to room temperature [12]. α-TCP is known to degrade more rapidly than β-TCP; α-TCP block began to degrade in the fourth week, while β-TCP in the eighth week when applied as augmenting highly resorbed alveolar ridges in rabbits [106]. This rapid degradation rate of α-TCP is due to the high solubility of α-TCP compared to β-TCP [107]. β-TCP is widely used as a mono- or biphasic bioceramic and bone graft composite [11,12], which is also used in the included studies of this review [14,108–113]. This is because the solubility of β-TCP is close to that of bone minerals, and as a result, can be resorbed by osteoclasts [11]. On the other hand, α-TCP is used as hydraulic bone cement due to its high solubility, hydration reaction, and bioresorbability [12,107].

Table 5. Outlined of ceramics material, 3D fabrication method, and characteristics of produced graft.

Type of Ceramics	Methods	Ceramics precentage	Additional Materials	Characteristics						Ref
				Material's Strength	Elasticity	Biodegradability	Porosity	Pore Size	Contact Angle	
nHA	FDM	30 and 50 wt%	PLLA (fiber preloaded with ceramics)	29.68 MPa (30 nHA wt%), 14.22 MPa (50 nHA wt%)	NA	11% (30 nHA wt%) and 15% (50 nHA wt%) mass degraded after 12 days	60%	NA	87.2° (30 nHA wt%), 77.4° (50 nHA wt%)	[80]
nHA	FDM	10, 20, 30, 40, and 50 wt%	PLA	~15–30 MPa (positively correlated with ceramic content)	NA	Fully degraded at day 17	~50–60%	NA	NA	[81]
nHA	FDM	10–20 wt%	PCL	4.5–8.67 MPa	3.83–8.51 MPa	NA	85.1% (10 wt%), 70.1% (20 wt%)	6.92 (10 wt%), 15.6 (20 wt%)	NA	[82]
HA	FDM	5, 10, 25, 20, 25 wt%	PCL	9 MPa (5 wt%) and 11 MPa (10 wt%)	24 to 30 Gpa	NA	60.0–65.4%	~550 μm	67° (25 wt%) to 87.8° (5 wt%)	[83]
nHA and mHA (each)	FDM	20 wt%	PCL	23.29 MPa fot nHA and 20.25 for mHA MPa (tensile strength)	NA	NA	65.27% (nHA), 63.28% (mHA)	Length 239 μm and width 204 μm (nHA), length 217 μm and width 190 μm (mHA)	NA	[84]
nHA (biogenic)	FDM	15 wt%	PCL	NA	316 MPa (cuttlebone HA), 219 MPa (mussel HA), 203 MPa (eggshell HA)	NA	NA	328 μm (cuttlebone HA), 422 μm (mussel HA), 461 μm (eggshell HA)	NA	[85]
HA	FFF with ultrasonic vibration assistance	10 and 20 wt%	PLA	143.98–173.03 MPa (90 W ultrasonic vibration power)	NA	NA	NA	NA	NA	[86]
HA	FFF	15 wt%	PLA, polyhydroxyalkanoates, poly(3-hydroxybutyrate), magnesium	4.09–39.75 MPa (tensile strength)	31.86–119.31 MPa (Young's modulus)	NA	6.25–6.80%	0.8 mm	27.10–56.94°	[87]
HA (bovine)	SLA	5, 10, 20 wt%	Ceramic resin (slurries), silica	45 MPa for 5 wt% and 68 MPa for 10 wt % (compressive strength), 36 GPa for 5 wt% and 52 GPa for 10 wt%	20 MPa for 5 wt% and 40 Mpa for 10 wt% (flexural strength)	NA	NA	NA	NA	[88]
HA and TCP	SLA	HA:TCP (6:4)	Acrylic monomers, photo initiator	2.80 MPa	NA	NA	NA	NA	NA	[89]
HA	SLA	NA	Photocurable binder matrix	~1.60 MPa	~513 Mpa (elastic modulus)	NA	81.8%	≤1 μm and increased with CS content increase	NA	[90]
β-TCP	FDM	15 vol%	PLA	54.344–69.711 MPa (tensile strength)	1.685–2.161 GPa (Young's modulus)	NA	NA	NA	NA	[91]
β-TCP	FDM	10 wt%	PCL, PLGA	10.3–15.7 N (maximum tensile load)	594.7–803.0 (elastic modulus)	NA	~40%	200 μm	NA	[92]

(Continued)

Table 5. (Continued).

Type of Ceramics	Methods	Ceramics precentage	Additional Materials	Characteristics						Ref
				Material's Strength	Elasticity	Biodegradability	Porosity	Pore Size	Contact Angle	
β-TCP and calcium sulfate	FDM	10, 15, 20 wt%	PCL	NA	NA	NA	50.92 (20% each), 43.96 (15% each), 54.12 (each 10%)	400–500 μm	120.3° (20% each of β-TCP and calcium sulfate)	[93]
β-TCP	SLA	50 wt%	Poly(D, L)-lactide	13 MPa–23 MPa (0.5 W laser power)	NA	NA	NA	NA	NA	[94]
Calcium silicate (CS) and β-TCP	SLA	0, 20, 40, 60, 80, 100 wt% (CS wt% to β-TCP)	NA	Decreased when CS content increase	NA	CS 100 wt% lost the least weight in 30 days compared to CS 0 wt%	Increased with CS content increase	≤1 μm and increased with CS content increase	NA	[95]
β-TCP	Liquid crystal display	10, 20, 30, 35 wt%	PLA	50 MPa (10 wt%), 48 MPa (20 wt%), 25 MPa (30 wt%), 10 MPa (35 wt%)	NA	10.5% (10 wt%), 11.5% (20 wt%), 12.5% (30 wt%), 12.97% (35 wt%)	48.3% (10 wt%), 45.9% (20 wt%), 43.6% (30 wt%), 40.4% (35 wt%)	NA	NA	[96]
β-TCP	SLS	NA	PCL or alendronate (coated)	5.74 MPa (uncoated PCL), 5.58 MPa (PCL coated)	NA	NA	NA	311 μm	NA	[97]
Calcium phosphate powders (HA, β-TCP, BCP)	Inkjet	NA	Polyvinyl butyral, polyvinyl alcohol, polyethylene glycol	5.5 Mpa (60% porosity), 3.0 Mpa (70% porosity), 1.0 Mpa (80% porosity)	NA	NA	50% (pore size 200 μm), 75% (pore size 700 μm)	200 μm, 400 μm, 700 μm	NA	[98]
Bioglass BGS-7	FDM	20, 40, 60 wt%	PCL	2.5–2.9 Nmm-1 (60 wt%) (increased as the bioglass content increase)	4.3–6.3 MPa (bending stress max, decreased as the bioglass content increase)	NA	42.3–43.1	~200 μm	Decreased with the increase of BGS content	[99]
Bioactive glass	FDM	5, 10, 20 wt%	PCL	36.75–43.52 MPa (compressive strength, increased as the bioglass content increase), 4.63–5.82 MPa (20%) (tensile strength, decreased as the bioglass content increase)	NA	NA	45.70–48.10%	372.3 μm	87.99–116.04° (decreased as the bioglass content increase)	[100]
Bioactive glass 63S	FDM	5, 10, 20 wt%	PCL	NA	4.79977 N/mm2 (highest elasticmodulus)	22.5–45% (increased as the bioglass content increase)	~50%	NA	82.85–86.75°	[101]
Bioactive glass 45SS	FDM	10, 15, 20 wt%	PCL	~270–340 MPa (compressive modulus), ~8.5–11 MPa (0.2% offset yield strength)	NA	10.61–20.68 (increased as the bioglass content increase)	~52%	264–330 μm (internal region)	~50–69°	[102]
Bioglass S53P4	FFF	5%, 10%, 20 wt%	PLA	100–180 Mpa	NA	NA	NA	180 μm–2 mm	NA	[103]
Bioactive glass 13–93	SLS	50, 60 wt%	Polymeric binder	5.9–20.4 Mpa	NA	NA	58.8%	300–800 μm	NA	[104]

NA, not available.

One article in the included studies used hydroxyapatite (HA) as ceramic material in the 3D printed graft [7]. HA is the most stable of all calcium orthophosphates with the chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ [12]. HA is a widely used biomaterial in the orthopedic and dental fields. As a biomaterial, HA is known to have biocompatible, biodegradable, osteoconductive, and osteoinductive properties due to their inertness in chemical and physical features with bone inorganic components [13,114,115].

Although both are calcium phosphate derivatives and extensively utilized as biomaterials, β-TCP and HA have different properties that may influence the selection of these ceramics for bone graft fabrication. HA is substantially more crystalline than bone mineral, making HA-based implants chemically more stable and consequently non-degradable upon implantation [11,21]. This makes HA degrade more slowly and less resorbable compared to TCP [21]. Because of this property, HA is usually used as a scaffold for bone ingrowth, giving a stable framework for calcification to occur in place [116]. Due to its osteoinductivity, HA is also been used as a coating material in metal and ceramic-based bone grafts, including TCP [117]. HA coating in β-TCP porous graft greatly increased alkaline phosphatase and bone sialoprotein levels in preosteoblasts [117].

Several factors in the synthesis process can alter the solubility, reactivity, bioresorbability, and behavior in the biological environments of the ceramics. Metal ions including Sr^{2+}, Zn^{2+}, and Mg^{2+} change the stability of α- and β- phases by replacing Ca^{2+} ions in the TCP lattice, which stabilizes the β-TCP phase [107]. In HA, cation doping was reported to alter particle size and adsorption capacity [118]. This doping is frequently an uncontrolled side consequence of elemental contaminants in the synthesis method. However, it can also be employed in a controlled setting to alter biological features [11]. Continuously released Zn^{2+} and Ca^{2+} from doped-PLGA/β-TCP graft made by 3D exhibited higher osteogenic and anti-inflammatory properties compared to PLGA/β-TCP graft alone [119], which is also in line with other reports [120,121]. However, the commercial success of ion-doped ceramic for medical applications is still undetectable due to the regulatory burden in commercialization [11].

TCP and HA are widely used as 3D-printed bone grafts together with polymeric material. It has been proved that they can induce new bone growth and have biodegradable properties [7,122]. However, the use of HA and β-TCP and its combination with polymeric material as a 3D-printed bone graft must be carefully considered by the surgeon and depends on the needs of the patient. Ceramic 3D-printed bone graft is not suitable for conditions that require permanent replacement of bone tissue considering that most of the material in the graft is biodegradable.

4.2.2. Synthetic polymeric material

Ceramics such as TCPs and HA, however, have brittle character [11,15]. Because of this, the use of ceramic as a single material in bone regeneration is not effective, especially for load-bearing applications [11]. Most ceramics are formulated with other materials for application as bone scaffolds. For 3D printing fabrication, synthetic polymers have been widely used to improve bone grafts' mechanical properties. In 3D printing, ceramic can be combined with polymers to up to 30% to meet the mechanical requirement, osteogenesis, and prevent needle clogging [123]. These are polycaprolactone (PCL), polylactic acid (PLA), polyglycolic acid (PGA), polylactic-co-glycolide (PLGA), poly(methyl methacrylate) (PMMA), polyethylene (PE), and polyetheretherketone (PEEK) [18]. Among them, PCL and PLA are the most used synthetic polymers for 3D printing applications [17,18].

It should be emphasized that the compressive strength of trabecular bone varies substantially with anatomical location and individual parameters such as bone density [124]. As a result, the use of bioceramic scaffolds must be tailored to the strength of the bones they will support. The addition of ceramic to PLA/PCL-based bone grafts has been reported to significantly alter the mechanical strength of the grafts. Nyberg et al. [60] reported that the addition of TCP did not change the compressive modulus of the PCL graft. However, the addition of HA significantly increased the compressive modulus in the solid and porous form of the grafts [60]. HA also was reported to increase the stiffness of the PCL-based graft [125]. These findings explained that HA has strong interactions with the PLA/PCL matrix, which transfers the load effectively between the ceramic and polymer in both compression and tensile tests. On another hand, Wang et al. [67] showed that the addition of nanosized β-TCP increased the roughness and surface hydrophilicity, but lowered the mechanical strength of the PLA-based grafts. Another study also reported similar results with the PCL-based bone grafts [63]. The study showed the addition of TCP to 60% decreased the yield strength of the PCL-based filaments, this was also proportional and inversely proportional to the grafs' surface roughness and contact angle [63]. Nevertheless, the slight decrease in strength did not affect the performance of the grafts which was proven by the superior osteogenic ability and bone repair capacity of the bone grafts [63,67]. However, additional considerations are required, notably adjustments to the architecture and character of the injured bone to ensure the stability of bone graft fixation, especially in situations with critical-sized bone defects.

PLA is a biodegradable thermoplastic, semi-crystalline polymer with a slow rate of crystallization that comes from renewable resources such as corn starch and sugarcane [18]. A previous systematic review explored the use of PLA/ceramics bone grafts in animal studies [17]. The author outlined the biocompatible and mechanically resistant character of PLA/ceramics-based bone grafts have promising applications in clinical cases [17]. However, our review showed that there are no human studies that used PLA/ceramics material, yet, PCL/ceramics 3D composite [14,110–113,126]. This shift in thermoplastic polymer selection in animal and human studies is suggested due to the character preference of the polymer. PLA and PCL differ in physical and mechanical properties. In comparison, the density of PLA and PCL are 1.21–1.25 g/cm³ and 1.11–1.14 g/cm³, the glass transition temperature of PLA and PCL are 45–60°C and (−60)–(−65) °C, and the melting

temperatures of PLA and PCL are 150–162°C and 58–65°C, respectively [18,19]. PLA is more brittle compared to PCL [18]. Important notes that PLA may cause an inflammatory response in the host [18]. This happens if the surrounding tissue can not eliminate the lactic acid produced from its breakdown results [127]. Moreover, PLA also presents hydrophobicity and low cell affinity which negatively affects the cells' adhesion to the PLA-based graft [17]. This biological effect might represent the reasons why PLA is not employed as a thermoplastic polymer in 3D printing applications for humans. Modifying the surface characteristic of PLA can provide beneficial surface properties while simultaneously minimizing the innate immune response by regulating cytokine production [17,20]. Nevertheless, further studies on this need to be conducted strictly, including in clinical settings.

4.3. Standardization and future perspective

Despite being a customized product, 3D bone grafts must be standardized before use for clinical applications. Standardization of 3D-printed materials must refer to the regulations of each country. However, the use of references such as ISO (International Organization for Standardization) can be considered. 3D-printed ceramic bone graft is a 'medical device' that is included in the implant group and can be used alone or in combination for human beings with exposure which is generally more than 30 days. Based on ISO 10993, physical and chemical information on materials that are in direct contact with patients must be obtained which makes materials characterization required. Chemical characterization by using ICP and XRD is required for ceramics-based medical devices. Biological evaluations must be carried out including cytotoxicity, haemocompatibility, implantation effects, and degradation test of the graft. Moreover, toxicokinetic studies that evaluate absorption, distribution, metabolism, and excretion should be considered if the medical devices are designed to be absorbable or to be in long-term contact with bone tissue [128]. Dental implants that contain ceramics should be tested in a low pH solution and in vivo pH solution to check the possible degradation product of the ceramics. On the other hand, degradation products from polymeric grafts can be examined by using the accelerated test, and if necessary real-time degradation test can be used [128]. Biocompatibility of the material can be defined first by using cytotoxicity assay using cell lines and can be done with extract, direct contact, or indirect contact with the graft. Testing for biocompatibility may include, but is not limited to sensitization, genotoxicity, implantation, chronic toxicity, and carcinogenicity [128].

Functional testing is required to make sure the manufactured graft can perform as intended in addition to tests to guarantee the graft's safety. As a bone support, for instance, the graft needs to be at least as strong as the cortical bone tissue it supports, especially in load-bearing districts. In comparison to maxillofacial-application grafts, higher mechanical strength is anticipated when the graft presents a weight-bearing intended use in the load-bearing districts, such as the femur, tibia, or spine. Ninarello et al. reviewed marked grafts for load-bearing applications and found that the orthopedic/spine group was almost 3.3 times higher than the mean

value for the oral/cranio-maxillofacial application group (22.4 MPa vs 6.8 MPa). Mechanical testing should also be based on a particular standard, for example referring to the materials used. ISO 13175–3:2012 can be used to characterize calcium phosphate grafts including HA, while grafts made from rigid plastic can be characterized following the procedure in ASTM D695–23 (2023) [129].

Furthermore, standardization of manufacturing techniques and the characteristics of the manufactured bone graft should be implemented (Figure 6). In the U.S.A., 3D-printed medical equipment is regulated by the FDA's Center for Medical Devices and Radiological Health (CDRH). However, in terms of the addition of biological components, it is required the additional involvement of the Center for Biologics Evaluation and Research (CBER) [130]. The FDA categorizes the additive manufacturing process of a medical device into five phases: Designing, software workflow, building, post-processing, and final testing consideration. In the designing process, patient CT or MRI data is transformed into a DICOM-compatible format. During the software workflow stage, an image segmentation system separates the anatomical region of interest from the rest of the scan. The file can be optimized further before being converted to a 3D printer-compatible format, such as standard triangulation language (STL). Then the build stage is the material selection stage. The selection stages must be adjusted to the patient's needs, especially regarding the condition of the defective tissue and its characteristics along with accompanying medical conditions in the existing patient. The operator must choose a suitable printing technique based on the materials and desired properties of the bone graft. In this review, we also outlined the 3D-printed bone graft fabrication methods, the materials used, and the characteristics of the fabricated grafts (Table 5). This may help manufacturers and/or surgeons in general in selecting materials and methods or products based on the needs of each patient.

Aside from that, post-processing is a stage that includes sterilization of the bone graft. To be FDA-cleared for intraoperative usage, all 3DMD must be validated at a certain sterility assurance level (SAL) [131]. The SAL is defined as the predicted likelihood of a live microbe after sterilization. The FDA specifies a $SAL < 10^{-3}$ for devices that contact the skin and $SAL < 10^{-6}$ for implanted devices [5,130,131]. Sterilization of bone grafts must still maintain their existing characteristics. For example, bone grafts with thermoplastic polymers should not be sterilized with high temperature, but the sterilization method may be different if the main material used is titanium [5,130,131]. Further, the device can then be characterized at the final testing consideration stage. This stage is to determine whether the bone graft's characteristics meet the desired requirements depending on the properties of the damaged tissue that will receive the bone graft [130]. In the end, the development process of the 3D-printed bone graft requires the collaboration of surgeons, manufacturers, biomedical engineers, and regulatory bodies.

This review has several shortcomings. First, we include all types of human studies since there are only limited clinical reports discussing 3D-printed ceramic bone grafts. The types of study can indirectly influence the outcomes reported in the
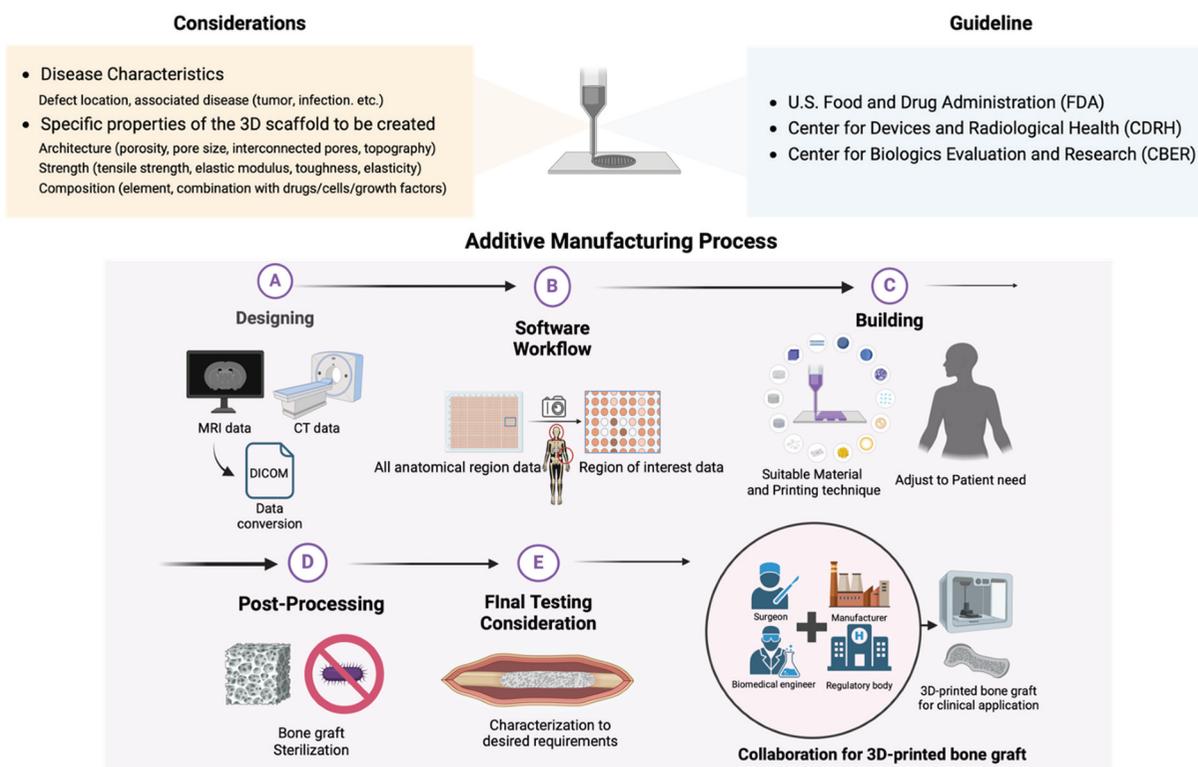
Figure 6. Work-flow and quality control of 3D-printed medical bone graft.

included articles and, thus, may influence the postoperative complication rate (CR%) reported in this review. Second, most of the reports only focus on existing outcomes, whereas how the bone graft is fabricated and its characteristics are difficult to track. Third, the identification of postoperative complications was also based on the decision of each surgeon in the included studies, and most of the studies did not report additional investigation if the complications were related to the bone graft or not. Fourth, in this study, we did not analyze the CR% based on the longevity use of the graft due to limited articles in the included studies and the limited information about this in each study. However, we believe that information about postoperative complications in the clinical uses of 3D-printed ceramics grafts, as well as comprehensive details about materials and fabrication methods, can provide valuable information for researchers in this field and help industry and clinicians to choose materials and fabrication processes for 3D-printed ceramic bone grafts.

5. Conclusion

Here we review in detail the outcomes of the use of ceramic-based 3D-printed bone grafts reported in humans. The proportional meta-analysis revealed that the total postoperative complication rate for 3D ceramic-based bone grafts was 14.3%. In this review, we also detailed the use of ceramic and synthetic polymers in customized bone grafts, as well as the methods used. We discovered that the presence of synthetic polymers, such as PLA/PCL, increases the mechanical strength of grafts and causes controlled graft breakdown. On the other hand, the effect of ceramic on the characteristics of the composite depends on the nature of each ceramic. The addition of other materials, such as growth factors or cells, improves the bioactive and osteoinductive features of bone grafts and allows for tissue engineering applications. Similarly, standardization in bone graft production is critical, which includes material selection, production techniques, and the expected characteristics of the finished bone graft. This is inextricably linked to the patient's condition and the damaged tissue that will receive the bone graft.

Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

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Author contributions

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