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Applications of 3D printing on craniofacial bone repair: A systematic review

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Abstract

Objectives

Three-dimensional (3D) bioprinting, a method derived from additive manufacturing technology, is a recent and ongoing trend for the construction of 3D volumetric structures. The purpose of this systematic review is to summarize evidence from existing human and animal studies assessing the application of 3D printing on bone repair and regeneration in the craniofacial region.

Data & sources

A rigorous search of all relevant clinical trials and case series was performed, based on specific inclusion and exclusion criteria. The search was conducted in all available electronic databases and sources, supplemented by a manual search, in December 2017.

Study selection

43 articles (6 human and 37 animal studies) fulfilled the criteria. The human studies included totally 81 patients with craniofacial bone defects. Titanium or hydroxylapatite scaffolds were most commonly implanted. The follow-up period ranged between 6 and 24 months. Bone repair was reported successful in nearly every case, with minimal complications. Also, animal intervention studies used biomaterials and cells in various combination, offering insights into the techniques, through histological, biochemical, histomorphometric and microcomputed tomographic findings. The results in both humans and animals, though promising, are yet to be verified for clinical impact.

Conclusions

Future research should be focused on well-designed clinical trials to confirm the short- and long- term efficacy of 3D printing strategies for craniofacial bone repair.

Clinical significance

Emerging 3D printing technology opens a new era for tissue engineering. Humans and animals on application of 3D printing for craniofacial bone repair showed promising results which will lead clinicians to investigate more thoroughly alternative therapeutic methods for craniofacial bone defects.

Keywords

Additive manufacturing, 3D bioprinting, Bone regeneration, Craniofacial, Systematic review

1. Introduction

1.1. Background

Three-dimensional (3D) bioprinting technology will play a pivotal role in medicine, offering a promising potential for bone reconstruction, rehabilitation and regeneration [1,2] and expanding treatment options in many field of operation [3]. The technique was first described in 1986 by Charles W. Hull under the name of stereolithography [4]. Since then, many diversified methods and manufacturing techniques have emerged, keeping to the same fundamental goal - to create intricate 3D structures that mimic the external and internal architecture of the hosted site [5] and provide essential framework for cell attachment and migration, thereby initiating tissue regeneration. Alternatively, such a custom-made framework behaves as filling material that rehabilitates the impaired site; 3D scaffolds, seeded with signaling biomolecules and stem cells, have recently been successfully transplanted into intended defects [6,7].

There is a variety of terminology for describing 3D printing, including: additive manufacturing (AM), solid freeform fabrication (SFF) and rapid prototyping (RP). 3D printing technologies involve building a well-defined 3D structure from a computer-aided design (CAD) model using layer by layer arrays [8]. The information for designing the model is collected by medical imaging technology, mainly computed tomography (CT) and magnetic resonance imaging (MRI). The acquired raw imaging data are processed and reconstructed as a volumetric model, which is then transmitted to a 3D bioprinter system. Computer-aided manufacturing (CAM) tools are used to produce 3D structures, based on the anatomical information of the tissue, to be regenerated or reconstructed. Finally, 3D printing scaffolds are fabricated, by addition of layered biological materials, with custom-made external shape and internal porosity, enriched with signaling biomolecules and seeding cells in several combinations [9,10] (Fig. 1).
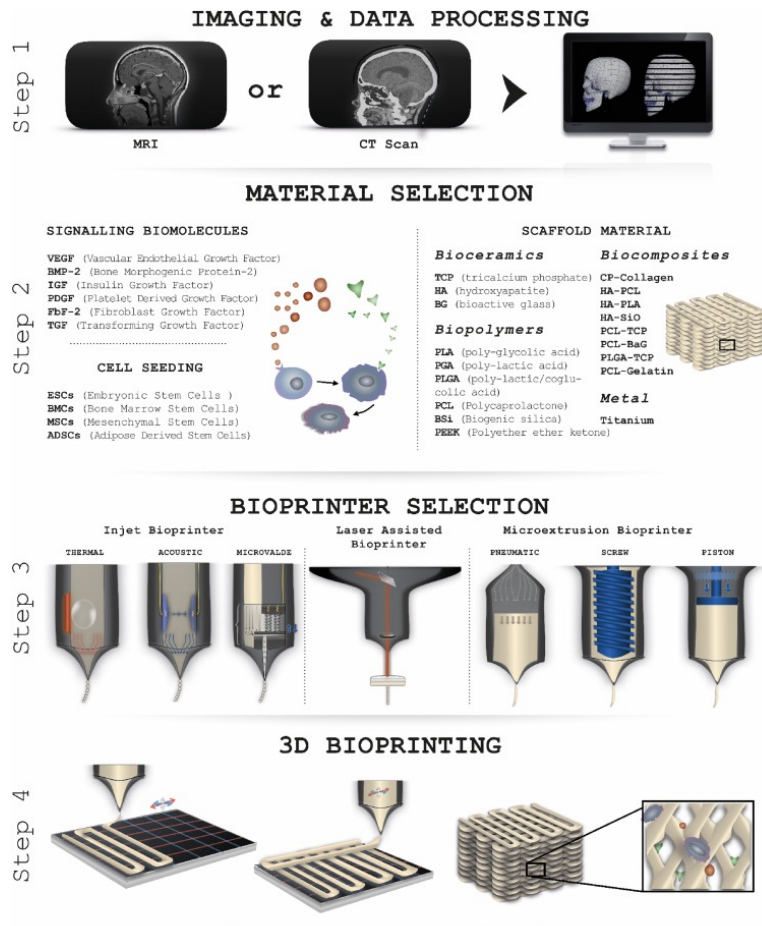
Fig. 1. Process of 3D bioprinting.

1.2. Categories of 3D printing systems

The technology of 3D tissue bioprinting comprises three main categories of printing systems: inkjet printers, laser-assisted printers and microextrusion printers. All these systems share the same coordinated spatial motion, differing in their bioink dispensing mechanisms. Factors such as surface resolution, biological material selection and cell viability, need to be taken into account to choose the appropriate printing system [11].

Inkjet printers are also known as drop-on-demand printers; controlled volumes of liquid are delivered to predefined sites of the substrate via diverse mechanisms [12]. Inkjet printers use thermal, microvalve or acoustic forces to create and eject droplets of biomaterial through an orifice and thereby to form the tissue substitute. Thermal inkjet printers use a heating element to separate the liquid into drops [13]. Microvalve inkjet printers

use consecutive opening/closing of a small valve, controlled by an electromagnetic field to expel the liquid [14]. Acoustic inkjet printers use the rapid change in shape of a piezoelectric crystal to generate an impulse in the liquid [15].

Laser-assisted printers are based on laser-induced forward transfer (LIFT) technology [16]. Typically, they consist of four components: a pulsed laser beam, a focusing system, a transparent glass slide (coated with laser-energy absorbing layer), and a layer of biological material/cells. The laser pulse is transferred to the absorbing layer and is then directed to the layer of biomaterial, generating a high-pressure bubble that propels the biomaterial drop-by-drop toward the receiving substrate. Variations of laser-based 3D printers include selective laser sintering (SLS), stereolithography (SLA) and selective laser melting (SLM) [17].

Microextrusion printers are robotically controlled dispensing systems, consisting of a material-handling print head, a dispensing system and a stage capable of three dimensional movement [18]. The dispensing system can be pneumatic or mechanical. Pneumatic extrusion is by use of compressed gas, whereas mechanical dispensing systems use metallic screws or pistons to push the material out, through a nozzle, on the stage [19]. The latter dispensing system provides more precise control over the material flow. The layers of the extruded biomaterial are deposited in continuous struts, rather than droplets, each layer serving as the foundation for the next [11]. Extrusion based variations of 3D printers include fused deposition modeling (FDM) and robocasting/direct ink writing (DIW) [20] (Fig. 1).

1.3. Objectives

The craniofacial complex comprises regions of diverse structural demands, each requiring appropriate design and materials for scaffolding, reinforced or not with biomolecules and cells for bone repair. A good understanding of the manufacturing concepts, biological mechanisms and applications of 3D printing is necessary for comparison with the traditional methods of bone reconstruction and consideration of such methods in treatment planning.

In light of the considerable differences among techniques, this systematic review sought to summarize evidence from existing human and animal studies assessing the application of 3D printing on bone repair and regeneration in the craniofacial region as well as to identify the success factors and potential complications of this intervention.

2. Data & sources

2.1. Protocol

The present systematic review was conducted according to the guidelines of the Cochrane Handbook for Systematic Reviews of Interventions version 5.1.0. [21] and followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [22].

2.2. Information sources and literature search strategy

The search strategy was conducted in the electronic databases of MEDLINE, EMBASE, COCHRANE LIBRARY, in October 2016 and was updated December 2017. Clinical Trials (www.clinicaltrials.gov) and National Research Register (www.controlled-trials.com) were also searched for unpublished studies. Attempts for personal communication with the authors were made in cases of incomplete data. Various combinations of the following keywords were inserted in according to the instructions of each search engine: 3D printing, 3D printed, bioprinted, bioprinting, 3D scaffold, bone, cranial, craniofacial, facial, craniomaxillofacial, maxilla, mandible, dental, dentistry. No language, publication status or year restriction was applied. Cross-checking of the included articles and relevant reviews, as well as a manual web search was conducted for unidentified article. The list of databases searched with the corresponding strategies is presented in Supplementary Table 1.

2.3. Inclusion/exclusion criteria

The eligible studies were chosen based on inclusion/exclusion criteria that were determined a priori according to the Participant-Intervention-Comparison-Outcome-Study (PICOS) schema (Table 1).

Table 1. Eligibility criteria used for the study selection.

Category	Inclusion Criteria	Exclusion Criteria
Participant characteristics	Studies on human participants of any gender with craniofacial bone defects (congenital or acquired) Animal interventional studies with craniofacial bone defects (congenital or acquired)	Clinical trials with fewer than five participants Defects at sites other than the craniofacial region
Intervention	Bone repair (reconstruction or/and regeneration) using 3D printed implanted biomaterials as scaffolds, solely or in combination with bone grafts, biomolecules or cell cultures	Bone repair using autologous bone, allogenic bone or xenograft as the only means of bone repair 3D printing used only for preoperative analysis or for simulation of a surgical case 3D printing used only for the fabrication of surgical splints, guides, temporary molds, dental implants or screws 3D printing used only for soft tissue repair
Comparison	Studies assessing bone repair after using 3D printed implanted biomaterials	Studies assessing bone repair by any other means of reconstruction
Outcome	Primary: Evaluation of immediate and long-term bone repair by histological or radiographic analysis Secondary: evaluation of serious complications intraoperatively and postoperatively (e.g. handling, exposure, infection of the biomaterial)	
Study design	Randomized controlled clinical trials Prospective controlled and uncontrolled clinical trials Retrospective controlled and uncontrolled clinical trials Case series with number of participants ≥5	Unsupported opinion of expert Books Case reports Case series with number of participants <5 Observational studies Narrative or systematic reviews

2.4. Study selection

The resulting studies after applying inclusion/exclusion criteria were first checked for duplicates, then the titles and abstracts were screened for relevance. The final stage involved retrieving and checking the full texts. The process was conducted independently by two of the authors (MM, GK) and any conflicts were resolved by consulting a third author (YR).

2.5. Data extraction

Data extraction was performed independently by two authors (MM, GK) and any discrepancies in data extraction between the two authors were likewise resolved by a third author (YR). The following data were recorded:

a. A Study's characteristics (author, year of publication, language, study design)
b. B Details of the type of intervention
c. C Details of outcome

In every study, the following intervention characteristics were recorded:

a. A Human or animal subjects
b. B Type of animal (for animal studies)
c. C Number of subjects
d. D Site of defect
e. E Origin of defect (congenital or acquired – for human studies)
f. F Type of additive manufacturing
g. G Type of scaffold (degradable or not)
h. H Material of scaffold
i. I Biomolecules or cell seeding
j. J Bone graft
k. K Follow-up period

2.6. Quality assessment of human studies

Initially, a tool was used to rate all the included studies according to their level of evidence, based on the Oxford Centre for Evidence-based Medicine Levels of Evidence [23].

Afterwards, the Cochrane Collaboration's Risk of Bias Tool was used to assess the risk of bias in randomized clinical trials [24]. Seven domains of bias (sequence generation, allocation concealment, blinding of participants and investigators, blinding of outcome assessors, missing outcome data, selective outcome reporting, other sources of bias) were estimated as "low", "unclear" or "high". A final overall classification was given to each study as follows:

- Low risk of bias (if all domains of the study were at low risk of bias)
- Unclear risk of bias (if one or more domains of the study were unclear)
- High risk of bias (if one or more domains of the study were at high risk of bias)

The ROBINS-I tool of Cochrane library was used to assess the risk of bias of non-randomized studies [25]. Seven domains of bias (confounding, selection of participants, classification of intervention, deviations from intended interventions, missing data, measurement of outcomes, selection of reported result) were estimated as "low", "moderate", "serious", critical" or "no information". Each study was finally assessed as follows:

- Low risk of bias (if all domains of the study were at low risk of bias)
- Moderate risk of bias (if all domains of the study were at low or moderate risk of bias)
- Serious risk of bias (if at least one domain were at serious risk of bias)
- Critical risk of bias (if at least one domain were at critical risk of bias)
- No information (if there is a lack of information in one or more domains of bias and there is no clear indication that the study is at serious or critical risk of bias)

Several confounders were considered for the assessment of risk of bias and these were the age of the patient, the oral hygiene, the initial periodontal health, the nature of the defect, the site of defects and force loading parameters.

It should be stated that the above tool was not applied to case studies without controls, because the risk of bias is inherently high and these studies were regarded to have low credibility.

2.7. Quality assessment of animal studies

The animal studies were qualitatively assessed according to the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) survey of experimental design and reporting, which is based on the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, as modified by Leim et al. [[26], [27], [28]]. A checklist of domains was applied in all included studies, which were further graded into 3 categories based on the percentage of the essential information they contained: 75% or more of positive answers (A), 50–74% (B), and less than 50% (C).

The potential bias of the animal studies was assessed using a simplified version of the Cochrane Collaboration's risk of bias tool for systematic reviews of interventions [21].

2.8. Outcomes evaluation

The outcome assessment was conducted with regard to the inclusion-exclusion criteria. The primary outcome was the immediate and long-term bone repair, for the time of observation, assessed by histological or radiographic evaluation. The secondary outcome was the presence of serious complications, either peri- or post-operatively (i.e. handling, exposure, infection of biomaterial) that may affect the success of the intervention. Human trials were regarded as successful if the aesthetic and functional result was satisfactory in accordance to primary and secondary outcomes.

3. Results

The search yielded 838 articles; 803 from the databases and 35 from other sources. One hundred thirty-eight full texts were retrieved out of these initial results, after eliminating duplicates and checking titles and abstracts. Only 43 articles fulfilled the predetermined criteria, including 6 human and 37 animal studies. The flow diagram of the systematic review is presented in Fig. 2.
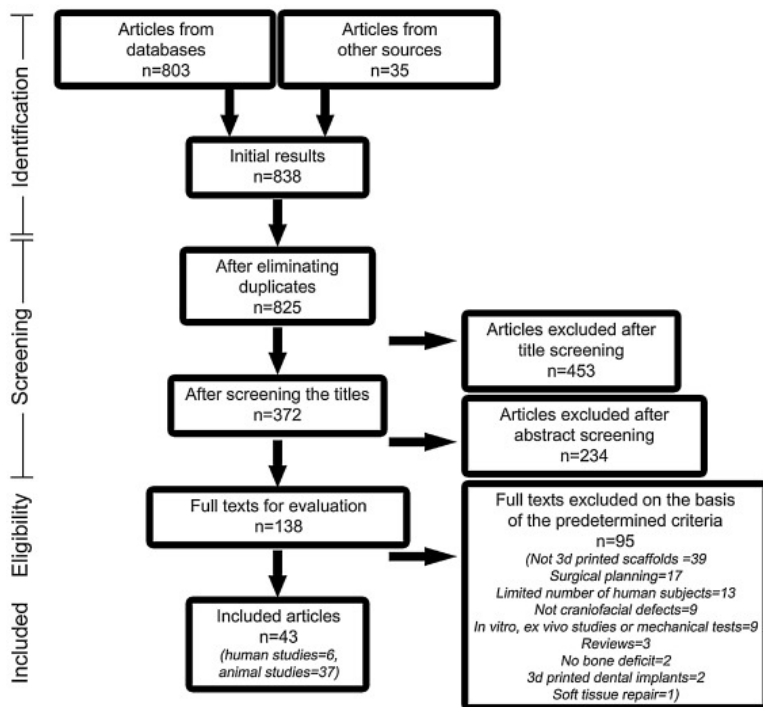
Identification

Articles from databases
n=803

Articles from other sources
n=35

Initial results
n=838

Screening

After eliminating duplicates
n=825

Articles excluded after title screening
n=453

After screening the titles
n=372

Articles excluded after abstract screening
n=234

Eligibility

Full texts for evaluation
n=138

Full texts excluded on the basis of the predetermined criteria
n=95
*(Not 3d printed scaffolds =39
Surgical planning=17
Limited number of human subjects=13
Not craniofacial defects=9
In vitro, ex vivo studies or mechanical tests=9
Reviews=3
No bone deficit=2
3d printed dental implants=2
Soft tissue repair=1)*

Included

Included articles
n=43
*(human studies=6,
animal studies=37)*

Fig. 2. Flow diagram of the systematic review.

4. Human studies

4.1. Study selection

All 6 human studies were published in scientific journals during the period 2009–2017, 5 in English and 1 in Chinese [29]. They consisted of two prospective clinical trials (one randomized and one non-randomized) [30,31] and four retrospective case series [29,[32], [33], [34]]. The two clinical trials compared 3D printed scaffolds of different type, biomaterials and with other techniques [30,31].

4.2. Study characteristics

Overall, the studies included 81 patients; 19 patients from the clinical trials and 62 patients from the case series. The clinical trial included six to 13 patients each [30,31] the case series including eight to 23 patients each [29,[32], [33], [34]].

Three-dimensional printed biomaterials were most often implanted in mandibular bone defects, followed by calvarial, maxillary and nasal defects. The vast majority of the defects were acquired after tumor resection or trauma. Laser printing was most commonly used [31,32,34], followed by inkjet printing [29,33] and microextrusion [30]. Non-absorbable biomaterials were usually applied. Hydroxyapatite ceramic scaffolds were most commonly implanted [29,33,34], followed by titanium metal [31,32] and lastly by PCL polymer [30]. Two studies used a scaffold enhanced with a bone graft [31,33]. The follow-up period ranged between 3 and 12 months. The data extraction of the included human studies is presented in Table 2.

Table 2. Data extraction of human studies.

Study	Level of evidence	Study design	Sample size	Defect site	Application	AM	Scaffold material	Material type	Bone graft	Follow-up	Success	Serious complications
Goh et al. 2014	1	RCT	6 (scaffold) 7 (no scaffold)	Maxilla, mandible	Post-extraction ridge preservation	MEP	PCL polymer	Absorbable	No	6 months	6/6 (100%)	Exposure 2/6 (33%) No bone in-growth 1/6 (17%)
Sumida et al. 2015	3	Clinical trial	13 (custom-made scaffold) 13 (commercial scaffold)	Mandible	Guided bone regeneration for ridge augmentation	LP	Ti metal	Non absorbable	Yes	n/a	13/13 (100%)	Experimental group: exposure 1/13 (8%), infection 1/13 (8%) Control group: exposure 3/13 (23%), infection 3/13 (23%)
Park et al. 2016	4	Case series	21	Calvaria	Large bone defect reconstruction	LP	Ti metal	Non absorbable	No	6-24 months	20/21 (95%)	Infection 1/21 (5%)
Shen et al. 2014	4	Case series	23	Mandible	Bone defect reconstruction	IJP	HA ceramic	Absorbable	No	3-10 months	23/23 (100%)	None
Brie et al. 2013	4	Case series	8 (divided in 3 groups by scaffold design)	Calvaria and nasal bones	Large bone defect reconstruction	LP	HA ceramic	Non absorbable	No	12 months	8/8 (100%)	None
Saijo et al. 2009	4	Case series	10	Maxilla, mandible	Bone defect reconstruction / augmentation	IJP	HA/a-TCP composite	Non absorbable	No	12 months	10/10 (100%)	None

Abbreviations: additive manufacturing (AM), inkjet printing (IJP), laser printing (LP), micro-extrusion printing (MEP), polycaprolactone (PCL), titanium (Ti), hydroxyapatite (HA).

4.3. Risk of bias within studies

The risk of bias of the included randomized and non-randomized clinical trials can be seen in Table 3, Table 4 respectively. According to the Cochrane risk of bias tool, the one identified randomized control trial [30] was evaluated as having high risk of bias. Although allocation concealment (sealed envelopes) was applied, no information was given regarding random sequence generation. It is unclear if the participants and personnel were blinded. As far as the assessors are concerned, no blinding is mentioned for all means of outcome evaluation, except for radiographic grading. Multiple outcome measurements raise some concerns for other sources of bias. According to the ROBINS-I tool, the one identified non-randomized trial [31] was evaluated as having serious risk of bias. Confounding parameters were not adjusted, the classification of intervention was not well defined and the assessors were aware of the intervention received by the study participants.

Table 3. Risk of bias assessment of identified randomized control trials (RCTs).

AUTHOR	OUTCOMES	Sequence generation	Allocation concealment	Performance bias	Detection bias	Attrition bias	Selective reporting	Other sources of bias	Overall Bias
Goh et al. 2014	Alveolar ridge height/width (evaluation of bone resorption)	Unclear (not possible to conclude if randomization was successful)	Low risk (sealed envelopes)	Unclear (no information provided; blinding of participants/ personnel is not easily possible)	High risk (no blinding mentioned for all aspects of analyses; outcome is objective and blinding is feasible)	Low risk (one patient drop-out reported; unlikely to result in imbalance)	Low risk (all reported results correspond to intended outcome)	Unclear (residual bias cannot be excluded)	High risk of bias (the study is judged to be at high risk of bias in at least one domain for this outcome)

Table 4. Risk of bias assessment of identified non-randomized trials.

AUTHOR	OUTCOMES	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of intervention	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of reported result	Overall Bias
Sumida et al. 2015	1.postoperative infection 2.operative time 3.mucosal rupture 4.number of fixation screws	Serious risk (reliability or validity of measurements of an important domain was low enough that we expect serious residual confounding)	Low risk (all participants eligible for target trial were included in the study, start of follow-up and start of intervention coincided	Serious risk (intervention status is not well defined)	Low risk (any deviations from usual practice were unlikely to impact on the outcome)	Low risk (data were reasonably complete)	Serious risk (The outcome measure was objective, but the assessors were aware of the intervention received by study participants)	Low risk (all reported results correspond to all intended outcomes)	Serious risk of bias (the study is judged to be at serious risk of bias in at least one domain)

Because of the heterogeneity of the research methods and the intervention characteristics as well as the high risk of bias of studies, only qualitative analysis of the data of the included studies was performed. Meta-analysis was not feasible.

4.4. Outcomes evaluation

The immediate and long-term bone repair was successful for the time of observation and only one study reported failure of one case [32]. Regarding serious complications, three studies reported infection and/or exposure of the biomaterial, and fibrous invasion of the scaffold instead of bone infiltration [[30], [31], [32]]. Nevertheless, all the authors stated that these complications were successfully managed and in no case was the sustainability of scaffold affected.

5. Animal studies

5.1. Study selection

The 36 included animal studies were published in scientific journals in English, during the period 2007–2017 [[35], [36], [37], [38], [39], [40], [41], [42], [43], [44], [45], [46], [47], [48], [49], [50], [51], [52], [53], [54], [55], [56], [57], [58], [59], [60], [61], [62], [63], [64], [65], [66], [67], [68], [69], [70], [71]]. One study was published as poster presentation [72].

5.2. Study characteristics

Overall, the studies included 614 animal subjects. Each study included one to 68 animals; with only one study [53] not reporting the number of the animals used. Rabbits were most commonly used [40,41,49,50,[54], [55], [56],59,60,62,[68], [69], [70],72], followed by rats [52,53,57,61,62,[64], [65], [66]], mice [39,44,45,48,58,67], pigs [36,46,47,51], sheep [35,37,42,63] and dogs [38,71].

The vast majority of the defects were calvarial [35,37,[39], [40], [41], [42],[44], [45], [46], [47], [48], [49], [50],[52], [53], [54], [55], [56], [57], [58], [59], [60], [61], [62],[64], [65], [66], [67],69,73], followed by mandibular [38,51,57,63,68,70,71] and maxillary [36] ones. Microextrusion was most commonly used [[36], [37], [38],42,44,46,47,49,[53], [54], [55],61,64,66,67,[69], [70], [71], [72]], followed by inkjet printing [39,45,50,56,[58], [59], [60],62,65] and laser printing [35,40,41,48,52,57,68]. Most studies used degradable biomaterials, with only two exceptions [35,68]. Hydroxyapatite and tricalcium phosphate scaffolds and/or composites were most commonly implanted [[36], [37], [38],42,44,47,48,51,[53], [54], [55], [56], [57],59,60,62,64,65,67,[69], [70], [71], [72]]. In approximately half of the studies, the scaffold was enhanced with biomolecules, such as BMP-2 and/or stem cells [35,36,39,40,42,[44], [45], [46], [47],[51], [52], [53], [54],57,58,[65], [66], [67], [68]]. Three studies implanted non-3D printed dermal matrices, enhanced with 3D printed biomolecules [39,45,58]. The total time of observation, of the included animal studies ranges from 4 to 26 weeks. The data extraction of included animal studies is presented in Table 5.

Table 5. Data extraction of animal studies.

Author	Animal	n	Defect origin	AM	Scaffold material	Biomolecules & cell seeding	Time of observation	Serious complications
Cooper et al. 2010	Mouse	68	Calvaria	IJP	dermal matrix (non 3D printed)	BMP-2 (3D printed injection)	4 and 8 wks	None
Herberg et al. 2014	Mouse	19	Calvaria	IJP	dermal matrix (non 3D printed)	BMP-2, SDF-1β, TGF-β1 (3D printed injection)	4 wks	None
Ishack et al. 2015	Mouse	15	Calvaria	MEP	HA/β-TCP composite	Dipyridamole, BMP-2	2, 4 and 8 wks	None
Keriquel et al. 2010	Mouse	30	Calvaria	LP	n-HA ceramic	–	1 and 2 wks, 1 and 3 months	None
Li/Xu et al. 2016	Mouse	42	Calvaria	MEP	PLGA/nHA composite	LV-pdgfb cells	2, 3, 4, 5, 6, 7 and 8 wks	None
Smith et al. 2012	Mouse	8	Calvaria	IJP	dermal matrix (non 3D printed)	BMP-2 (3D printed injection)	4 wks	None
Jensen et al. 2013	Pig	16	Calvaria	MEP	PCL polymer	Mononuclear cells, BMP-2	8 and 12 wks	2 deaths (unrelated)
Jensen et al. 2016	Pig	14	Calvaria	MEP	PCL polymer, HA/β-TCP composite	BM stromal cells, DP stromal cells	5 wks	None
Dadsetan et al. 2015	Rabbit	10	Calvaria	LP	PPF polymer with CP coating	BMP-2	6 wks	None
Ge et al. 2009	Rabbit	18	Calvaria	LP	PLGA polymer	–	4, 12 and 24 wks	1 death intraoperatively
Goetz et al. 2013	Rabbit	8	Calvaria	MEP	HA/β-TCP composite	–	8 and 16 wks	None
Kim et al. 2016	Rabbit	40	Calvaria	MEP	MgP ceramic	–	4 and 8 wks	None
Komlev et al. 2015	Rabbit	5	Calvaria	IJP	OCP ceramic	–	6.5 months	None
Shao/Ke et al. 2017	Rabbit	24	Calvaria	MEP	CSi–Mg6 ceramic	–	4, 8 and 12 wks	None
Shim et al. 2014	Rabbit	36	Calvaria	MEP	PCL/PLGA/β-TCP composite	BMP-2	4 and 8 wks	None
Simon et al. 2007	Rabbit	16	Calvaria	MEP	HA ceramic	–	8 and 16 wks	None
Simon et al. 2008	Rabbit	16	Calvaria	IJP	HA ceramic	–	8 and 16 wks	None
Tamimi et al. 2009	Rabbit	8	Calvaria	IJP	TCP ceramic	–	8 wks	None
Torres et al. 2011	Rabbit	8	Calvaria	IJP	TCP ceramic	–	8 wks	None
Hwang et al. 2017	Rat	32	Calvaria	MEP	PCL/PLGA/β-TCP composite	–	2 and 8 wks	None
Kwon et al. 2017	Rat	30	Calvaria	IJP	PLLA/β-TCP composite	MG-63 human osteoblastoma cells	2, 4, 6, 8, 10 and 12 wks	None
Lee et al. 2012	Rat	12	Calvaria	LP	PPF/PLGA composite	BMP-2, AD stem cells	11 wks	None
Li/Chen et al. 2017	Rat	24	Calvaria	MEP	PCL polymer	Platelet-rich plasma	4, 8 and 12 wks	None

Pati et al. 2014	Rat	N/A	Calvaria	MEP	PCL/PLGA/β-TCP composite	TM stem cells	8 wks	None
Zhao et al. 2015	Rat	18	Calvaria	MEP	Sr-MBG polymer	–	8 wks	None
Tamimi et al. 2014	Rat, Rabbit	6, 16	Calvaria	IJP	TCP ceramic	–	8 wks	None
Adamzyk et al. 2016	Sheep	1	Calvaria	LP	PEKK	Mesenchymal stem cells	12 wks	None
Carrel/Moussa et al. 2016	Sheep	12	Calvaria	MEP	HA/α-TCP composite	–	8 and 16 wks	None
Haberstroh et al. 2010	Sheep	9	Calvaria	MEP	PLGA polymer, TCP/Col composite	Chitosan	14 wks	None
Smeets et al. 2016	Rat	10	Calvaria, Mandible	LP	PDLLA/β-TCP composite	Mesenchymal stem cells	10 and 30 days	None
Carrel/Wiskott et al. 2016	Dog	1	Mandible	MEP	HA/α-TCP composite	–	8 wks	None
Shim et al. 2017	Dog	3	Mandible	MEP	PCL/β-TCP composite	–	8 wks	None
Konopnicki et al. 2015	Pig	2	Mandible	IJP	PCL/β-TCP composite	BM progenitor cells	8 wks	None
Rockies et al. 2017	Rabbit	12	Mandible	LP	PEKK	AD stem cells	10 and 20 wks	1 case (exposure & infection)
Shao/Sun et al. 2017	Rabbit	16	Mandible	MEP	TCP, CSi, CSi-Mg10, bredigite (Bred) ceramic	–	8 and 16 wks	None
Ciocca et al. 2017	Sheep	1	Mandible	n/a	PCL-HA composite	–	3 months	None
Abarrategi et al. 2012	Pig	8	Maxilla	MEP	HA/β-TCP composite	Chitosan/BMP-2	3 months	None

Abbreviations: additive manufacturing (AM), inkjet printing (IJP), laser printing (LP), micro-extrusion printing (MEP), polycaprolactone (PCL), hydroxyapatite (HA), tricalcium phosphate (TCP), polylactide-co-glycolide acid (PLGA), magnesium phosphate (MgP), octacalcium phosphate (OCP), calcium silicate (CSi), poly-L-lactide (PLLA), propylene fumarate (PPF), poly-D,L-lactide (PDLLA), polyetherketoneketone (PEEK), bone morphogenetic protein (BMP), stromal cell-derived factor (SDF), transforming growth factor (TGF), dental pulp (DP), adipose-derived (AD), turbinate tissue-derived mesenchymal (TM), bone marrow (BM).

Notes: All studies reported bone formation. All materials are degradable except for PEKK. Goetz et al.2013 is a poster presentation. Table entries sorted by defect origin, and then by animal.

5.3. Quality assessment and risk of bias within studies

Regarding the quality assessment of the included animal studies, most of them were of moderate quality (B), but five were graded as high (A) and nine as low quality (C). The reasons why most of the studies were graded as moderate were because outcome assessments and scores were not blinded, sample sizes were not justified, methods of randomization were not stated, blocking experiments were not performed or no raw data were available (Supplementary Table 2).

As for the risk of bias, the vast majority of the animal studies were evaluated as having high risk of bias regarding the sequence generation, the allocation concealment and the blinding parameters. On the contrary, the great majority of the animal studies were evaluated as having low risk of bias regarding the outcome data and the selective reporting parameters (Table 6).

Table 6. Risk of bias assessment for animal studies.

Author	Adequate sequence generation	Risk of bias	Allocation concealment	Risk of bias	Blinding	Risk of bias	Incomplete outcome data addressed	Risk of bias	Free from selective reporting	Risk of bias
Abarrategi et al. 2012	−	H	−	H	−	H	+	H	+	L
Adamzyk et al. 2016	−	H	−	H	+	L	−	L	+	L
Carrel et al. 2016a	+	L	−	H	−	H	+	H	+	L
Carrel et al. 2016b	−	H	−	H	−	H	−	L	+	L
Ciocca et al. 2017	−	H	−	H	−	H	−	L	+	L
Cooper et al. 2010	−	H	−	H	−	H	−	L	+	L
Dadsetan et al. 2015	−	H	−	H	−	H	−	L	+	L
Ge et al. 2009	−	H	−	H	−	H	+	H	−	H
Goetz et al. 2013	−	H	−	H	−	H	+	H	−	H
Haberstroh et al. 2010	+	L	−	H	−	H	−	L	+	L
Herberg et al. 2014	+	L	−	H	−	H	−	L	+	L
Hwang et al. 2017	−	H	−	H	−	H	+	H	+	L
Ishack et al. 2015	−	H	−	H	−	H	−	L	+	L
Jensen et al. 2014	+	L	−	H	−	H	−	L	+	L
Jensen et al. 2016	+	L	−	H	+	L	−	L	+	L
Keriquel et al. 2010	−	H	−	H	−	H	+	H	+	L
Kim et al. 2016	−	H	−	H	−	H	−	L	+	L
Komlev et al. 2015	−	H	−	H	−	H	−	L	+	L
Konopnichi et al. 2015	−	H	−	H	−	H	−	L	+	L
Kwon et al. 2017	−	H	−	H	−	H	−	L	+	L
Lee et al. 2013	−	H	−	H	−	H	−	L	+	L
Li et al. 2016	+	L	−	H	−	H	+	H	+	L
Li et al. 2017	−	H	−	H	−	H	−	L	+	L

Pati et al. 2014	+	L	–	H	–	H	–	L	+	L
Roskies et al. 2017	–	H	–	H	–	H	+	H	+	L
Shao et al. 2017a	+	L	–	H	–	H	+	H	+	L
Shao et al. 2017b	–	H	–	H	–	H	–	L	+	L
Shim et al. 2014	+	L	–	H	–	H	–	L	+	L
Shim et al. 2017	+	L	–	H	–	H	–	L	+	L
Simon et al. 2017a	–	H	–	H	–	H	+	H	+	L
Simon et al. 2017b	–	H	–	H	–	H	–	L	+	L
Smeets et al. 2016	–	H	–	H	–	H	–	L	+	L
Smith et al. 2012	–	H	–	H	–	H	+	H	+	L
Tamimi et al. 2009	–	H	–	H	–	H	–	L	+	L
Tamimi et al. 2014	–	H	–	H	–	H	–	L	+	L
Torres et al. 2011	+	L	–	H	–	H	–	L	+	L
Zhao et al. 2015	+	L	–	H	–	H	–	L	+	L

Abbreviations: YES (+), NO (-), high (H), low (L).

5.4. Outcomes evaluation

The immediate and long-term bone repair was successful for the time of observation among animal studies demonstrated by histological, biochemical, histomorphometric or microcomputed tomographic findings. However, some studies reported only bone formation along the scaffold structure and not inside. Only two studies exhibited complications, related to scaffolds; one incidence of surgical complications [41], one incidence of minor exposure and infection [68].

6. Discussion

6.1. Overview of existing studies

The present systematic review included only in-vivo studies in order to evaluate the application of the 3D printed scaffolds in live subjects. For better understanding of these strategies both human and animal studies were included. The systematic search, based on the inclusion criteria, yielded a limited number of human studies and a greater number of animal studies. It was decided to include animal studies in the review, since clinical application of 3D-printing in humans is still scarce in the literature. The human studies reported high success with limited serious complications, but they were all evaluated as having high risk of bias; the quality assessment revealed that none fulfilled the requirements of a high-quality study design. Nevertheless, these studies provide valuable findings for a cutting-edge technology, applied on human beings. On the other hand, animal studies provide a bridge between in-vitro and human studies, by illustrating significant parameters of the histological and cellular background of scaffold integration and bone regeneration, even though the quality of these studies is regarded as moderate. Nevertheless, within limitations and concerns of assessing animal studies and relating such findings to applications in human beings, it needs to be acknowledged that animal studies constitute a first in-vivo level of evidence.

6.2. Scaffold materials & combinations

Many types of scaffold materials, alone or combined, have been proposed in an attempted to integrate many desirable properties, such as osteoinductivity, osteoconductivity, printability, biocompatibility and durability [6,9].

Bioceramics are the materials most commonly selected. Calcium phosphate compounds (mainly β-TCP) exhibit favorable biodegradability, chemical bonding with hard tissues and wear resistance, all necessary for load-bearing craniofacial defect sites [74]. The challenge is to maintain a low temperature of sintering in an attempt to avoid transformation of β-TCP to α-TCP, which is more chemically unstable [75]. In addition, HA has been deemed a scaffold material for bone repair with outstanding biocompatibility, because of the stoichiometric similarity to the mineral phase of natural bone [76]. However, it is frequently combined with other bioceramics or biopolymers due to its inherent weak interaction with the binder liquid during 3D printing process [77]. Bioglasses are also used, because they show great osteoconductivity and bonding to hard tissue, nevertheless, they slowly degrade and provoke cytotoxicity on the surrounding tissue [78]. Their major advantage is that they upregulate osteogenesis and nicely interact with cells [30,31], but they are too brittle for implantation in load-bearing craniofacial sites [2]. A human study of our review confirms this disadvantage. Saijo et al. [33] used HA/a-TCP composite scaffolds for maxillomandibular defects and emphasized the difficulties of composition and fabrication of an ideal scaffold to fulfill strength and dimensional requirements.

Biopolymers have also been widely used. Alginate is usually chosen for cartilage repair, because it induces chondrocyte proliferation and is compatible with cartilaginous tissue [79,80]. However, it does not interact with cells and is not suitable for load-bearing applications due to its very low stiffness [11,81]. Biogenic polyphosphates (bio-polyP), such as PCL and PLGA, have remarkable printing resolutions and porosity, biocompatibility and osteoconductivity [82,83]. Biogenic silica is another biopolymer which is highly

osteoproductive in unfavorable environmental conditions and ensures effective nutrient diffusion in hard craniofacial tissues with moderate vascularity. It is suitable for cell and proteins adhesion [84,85]. In general, the superiority of biopolymers over other materials is their prime printability and their ability to efficiently promote osteogenesis in the scaffold complex.

Metal applications are predominately by titanium. It has incredible biocompatibility and mechanical properties, such as elastic modulus, fatigue strength and toughness; features which are comparable with natural bone [86,87]. It is preferable for craniofacial vault reconstruction, where the size of the defect is extensive. It nicely redefines the shape and aesthetic condition of the affected site. Although non-absorbable, it has the potential for bone ingrowth, when 3D printed Ti plates incorporate porosity in the periphery, or if 3D printed Ti meshes have been added to preserve bone grafts [88]. Up to now, the AM technology focused on using ceramics or polymers for bone tissue engineering, the 3D printing of titanium in microscale has posed considerable challenges [86].

Composites are the combination of bioceramics with biopolymers to achieve the desirable properties; no single biomaterial is able to satisfy all the prerequisites for a bone graft material. Composite materials incorporate the characteristics of their components (e.g. the high wear resistance of ceramics and high toughness of polymers) [2]. Calcium phosphate/collagen, hydroxyapatite/polyamide, TCP/HA/polyP and others have been proposed for better chemical and mechanical properties [89,90] as well as controlled porosity and cell interaction [91]. These materials have been introduced for craniofacial application [92].

6.3. Growth factors & cells

Two strategies have been followed to incorporate biomolecules and cells in scaffolding structures. One method is to print acellular scaffolds and then seed them with cells through chemical binding. The suitable binder (e.g. phosphoric acid) should prevent pH-related damage of seeded cells. Alternatively, in bioprinting, the scaffold material and cells are printed simultaneously. This latter method is superior in precise cell distribution into the scaffold, however it suffers from low mechanical strength (5 kPa) and temperature- and pressure-related damage to the cells during printing. These are the main reasons that bioprinting has not yet been used for human craniofacial tissue regeneration; instead, the method of acellular printing and subsequent cell seeding seems to be the preferable choice [1]. Cell proliferation should follow a balanced momentum; the viability of the scaffold can be influenced by insufficient proliferation, whereas too much proliferation can evoke hyperplasia and apoptosis. Long-term survival and controlled proliferation of cells are essential to achieve tissue homeostasis of the newly formed bone [6].

Understandably, animal studies have tested a greater variety of materials, alone or in combination with biomolecules and seeding cells (stem or progenitor cells), scaffolding innovations which have not been adequately studied in humans. The animal studies show that there is a positive interaction between the scaffold material and the biomolecules/cells [40,45,47,51]. Noteworthy, the biomolecules/cells can be incorporated in material rods during layering and not only after microporous fabrication [48]. There is a synergetic stimulation of bone formation in the scaffolding area through the osteoconductive properties of some scaffold materials and the osteoinductive properties of the seeding cells [41,42,44,53,67]. Factors, such as TGFβ, BMP-2, MSCs, BMCs, Chitosan and stromal cells, promote osteogenesis by inducing cell transform towards the bone cell lineage. Leaving sufficient time for cell cultivation (1–2 weeks) is essential to create a favorable microenvironment before scaffold implantation [51].

6.4. Capabilities of 3D printing techniques

The in-depth knowledge of 3D printers' capabilities and their compatibility with scaffolding materials lays the groundwork for successful application of 3D printing in human and animal subjects. Inkjet printers have high

print speed, low cost, high resolution and compatibility with many biological materials and cells [2]. Also, printing can be with noncontact and overprinting can be achieved [39]. Nevertheless, some disadvantages, such as nozzle clogging, alteration of cell viability, uncontrolled droplet size and directionality, should be taken into account. This type of printer is suitable for low viscosity materials in liquid form and low cell concentrations [93]. Laser printers lack a nozzle, and therefore, the problem of material/cell clogging is avoided [11]. They are compatible with materials with high range of viscosity, do not have a detrimental effect on cell viability and achieve high resolution, although at a high cost and a low flow rate [16,94]. The micro-extrusion method is suitable for bioprinting of a broad array of materials and has the ability to deposit very high cell densities [6]. However, cell viability is lower than the other two types of bioprinting and printing resolution, as well as printing speed, is often problematic [95].

6.5. Scaffold kinetics & biocompatibility

A controllable degradation rate of the scaffold is required. Ideally, this rate should match the ability of the cells to replace the biomaterials with their own extracellular matrix (ECM) through a mechanism in which embedded cells secrete proteases and subsequently produce ECM proteins that define the new tissue [6]. It is also challenging to have control over degradation byproducts, the whole process should be nontoxic and the degradation products should rapidly metabolize without producing a detrimental environment to cell viability and function. The swelling and shrinkage of the biomaterial can unfavorably affect the success of the scaffold, evoking contamination and inflammation at defect boundaries as well as immune system reaction. Besides this, biocompatibility should be considered not only as the means to prevent local/systemic effects, but also the scaffold should have the ability to actively contribute to all biological and functional aspects [96].

The animal studies that use absorbable materials confirm and augment the above findings. Attempts to fabricate scaffolds that biodegrade in rates comparable to autologous block grafts [38,97] have shown that it is difficult to duplicate the regenerative ability of native bone (it differs between bones; i.e. ilium has more blood supply and osteoprogenitors than others) and match degradation of the biomaterial with new bone apposition [59]. Mostly the higher porosity facilitates more rapid biodegradation [49]. This issue becomes even more difficult, since the fact that degradation kinetics and mechanical properties are irreversibly proportional (faster degradation, lower mechanical strength) [44,57]. The high rate ensures fast bone turnover, avoids any inflammatory process and protects from fibro gingival dehiscence and invasion [57]. On the other hand, scaffold coverage should be performed without soft tissue tension (the block edges should be rounded) to avoid exposure and unnecessary degradation by macrophages and osteoclasts [38]. Also, the degradation rate should not be too fast, but in a physiological rate, to encourage more tissue penetration as well as nutrient exchange without affecting the scaffold stability [41,42,52].

6.6. Structural scaffold design & mechanical properties

Several parameters of scaffold design are important to fulfill bone in-growth. Regarding the scaffold macro-geometry, the scaffold should precisely fit in the bone defect without having a complicated outline, otherwise it may not yield to 3D printing [6]. The micro-architecture of the scaffold should be well-structured and with sufficient porosity and interconnectivity for bone in-growth, cell transportation and nutrient diffusion [11]. The scaffold should be bioactive by incorporation of mineral phases for osteoinductivity (chemical binders to create a mineralized structure that can house cells) [1]. Moreover, mechanical properties should be analogous of native bone. The selection of the biomaterial is driven by the size and load-bearing demands of the affected site. Human bone exhibits a wide range of physical properties; for example, the human trabecular bone within the condyle has an elastic modulus ranging between 120–450 MPa and within the mandibular body ranging between 112–910 MPa [10]. Unfortunately, many 3D printed scaffolds range much lower than these requirements (10–100 MPa) and we believe that this is the main reason for limited reports of applications in load-bearing sites.

Perhaps, this is the reason that most of the studies used predominately 3D printed applications in calvaria. In humans, the case series by Brie et al. [34] compared three hydroxyapatite (HA) ceramic scaffolds, the first scaffold type was not perfectly adapted to the defect, the second type was perfectly adapted to the defect and the third had additional peripheral macro-porous areas. They concluded that the hydroxyapatite implants are well suited to reconstruct large (greater than 25 cm^2) or complex calvarial or front-orbital defects, as these scaffolds eliminate the necessity of bone grafting and facilitate bone reconstruction, giving external shape and internal porosity. Nevertheless, mimicking the 3D complexity of natural tissues and functionally integrating the scaffold in bone defects represent significant challenges to which the authors answered with a scaffold with dense core for mechanical strength and peripheral porosity for bone integration. The case series by Park et al. [32] used Ti metal scaffolds for calvarial defects with a honeycomb structure to raise the strength-to-weight ratio. These scaffolds were considered the perfect choice for rehabilitation of large size defects, where the large amount of required autologous bone affects donor morbidity.

The animal studies emphasized that 3D printing controls the external and the internal architecture of the material and the scaffold acts as a template for cell colonization and extracellular matrix formation [36,47,50,52]. External surface topography should be rough enough to increase surface ground for cell attachment and proliferation as well as to firmly consolidate with the adjustment native bone [35,36,38,63]. Moreover, external surface should act as a barrier for fibrous tissue invasion, since that is a major factor of scaffold failure [41,46,48]; another way to avoid that is to cover the scaffold with a membrane [38,53,54,64]. Open channels in the periphery of the scaffold increase the migration of osteogenic factors [56]. The internal surface should be porous enough to facilitate penetration of bone agents and vessels into the scaffold, but to an extent that the mechanical resistance is not affected [40,71]. It is the fundamentals of 3D printing that constitute scaffolds as linear porous structures which are controllable in size and patency over the entire length of the blocks [41,65,66]. These structures are superior to particulate materials, since 3D scaffold provides a stable environment for multi-level bone augmentation and an organized arrangement of channels/pores for the progression of a "mineralization front" with its accompanying vascular system [37,51,54,55]. Dead-ends of tubes and channels increase the concentration of osteogenic factors [56].

Almost all researchers of animal studies pointed out two critical features of scaffold material, important for scaffold survival. The first one is their porosity (pore size, pore morphology, pore interconnectivity and distribution) [41,46,49,56,63,72,98]. Most studies conclude that the pores size of scaffolds should be 100–500 μm to steer the migration/proliferation/differentiation of mesenchymal stem cells, supply with oxygen/nutrients and to induce the diffusion of factors that trigger inner bone formation [35,37,42,46]. Pore size below 100 μm promotes chondrogenesis and subsequently ossification, whereas pore size above 100 μm stimulates direct ossification, but to a size (∼500 μm) that the durability of scaffold is not affected [98]. Very small size of pores hinders cellular ingrowth and evokes a foreign body response [46]. The second feature related to scaffold survival is the ability to withstand physical forces. This is an important property when considering replacement of load-bearing bone and critical-sized defects [61,68,70]. Mechanical strength depends on the chemical composition, size, shape, surface modification and porosity of the scaffold [40,47,52,68,69]. Mechanical strength of bone can vary even for the same bone, depending on gender, age and health condition of the body [98].

6.7. 3D printing applications & topography of bone regeneration
3D printing technology in the craniofacial region has three main application areas: a) rehabilitation of a defect site with a custom-made prosthesis to restore normal facial appearance, in cases of large bone or soft tissue defects after trauma and tumor ablation. b) for reconstruction purposes, 3D models, fixation devices, cutting guides and implanted medical devices can be printed to facilitate and optimize surgical intervention by creating the essential framework and primary stability for bone grafts. This application is beyond the scope of this

review. c)regeneration aiming to preserve existing bone and stimulate osteogenesis for ultimate bone repair and normal anatomic and functional restoration [1,11]; this application is still at an initial stage of research. The researchers seem to emphasize the regeneration potential of the scaffolds, without present long-term follow-up of scaffold degradation/bone regeneration or in-depth investigation of their clinical sustainability.

The only randomized clinical trial by Goh et al. [30], used polycaprolactone (PCL) polymer scaffold, developed by a micro-extrusion 3D printer, to preserve maxillary and mandibular alveolar ridge height after tooth extraction in preparation for final implant restoration, and compared two groups, with or without scaffold. However, the sample size was too small and underpowered. Bone resorption was noted as evident in both groups, but the insertion of the 3D bioresorbable scaffold in fresh extraction sockets allowed normal bone healing and better ridge height maintenance mainly at the mesio-buccal aspect after 6 months as compared to the control group without the insertion of a scaffold. Nevertheless, the material had not actually resorbed during these months and that may be the reason for maintenance of alveolar ridge. Interestingly, they suggested the combination of PCL- tricalcium phosphate (TCP) for favorable degradation and resorption kinetics than PCL alone, without blocking new bone ingrowth. The positive results of bone formation in animals should be evaluated with caution, since there are studies which report predominately superficial or irregular bone formation and no or less bone distribution in the inner structure [38,41,60,62]. The most bone formation was noted at the interproximal surface of scaffold to adjacent bone [40] and progressively less bone towards the center of the structure [49,50,55]. However, more bone formation in depth may be expected over time [56,66]. In addition, the defect geometry plays an important role to successful bone ingrowth; it is preferable to have extensive surface connection with native bone and thereby proximity with osteoprogenitors [58,59]. The quality of formed bone seems to be very good with trabecular- and marrow-like structures [49,55,72]. Interestingly, some studies report that the critical degree of bone filling in the defect area for placement of a dental implant is less than optimal volume (100%) and the studies report 40–60% of bone filling as adequate for support of a dental implant [59,62,99,100].

6.8. Operative manipulation & complications
3D printed technology enables the meticulous study, design, fabrication and surgical position of the scaffold/implant. Virtual planning and fewer surgical steps can be achieved, minimizing operative and postsurgical complications [101]. The combined findings in human studies illustrate that 3D scanning technology provides the specific shape of the craniofacial surface and 3D printing accurately replicates the defect. Surgical maneuvers are easier and operative time is less. The implants can obtain detailed surface design that enhances the strength-to-weight ratio, tissue integration and bone ingrowth, resulting in high durability, aesthetics and low inflammatory rate. Dentoalveolar defects require a rapidly resorbing matrix to avoid wound dehiscence, exposure, and subsequent microbial contamination.

The non-randomized trial by Sumida et al. [31] compared custom-made to prefabricated Ti scaffolds for guided bone regeneration of mandibular sites for dental implants. Their findings agree with other studies [35,102], in that the 3D-printed custom-made scaffolds have more ideal shape, better surgical handling, and considerably less intraoperative time, eliminating postoperative infections and mucosal rupture. The accuracy of the fabrication procedure leads to improved functional and aesthetic results [103]. The case series by Park et al. [32] used Ti metal scaffolds for calvarial defects and highlighted the advantages of perfect anatomic alignment and aesthetic result, shorter operating time and lower risk of infection due to reduced manipulation. Shen et al. [29] used absorbable HA ceramic scaffolds in mandibular defects after mandibular angle osteotomy and pointed out the same benefits; the accuracy of the 3D structure ensures successful replacement of the defect, saving operative time and stimulating postoperative recovery.

6.9. Strength & limitation of the present review

The strengths of the current systematic review include the comprehensive literature search including grey literature, the robust use of methods for qualitative synthesis and the open provision of the review's dataset as an attempt to increase transparency and reproducibility. No language restrictions were applied to avoid language bias. No publication status or year restriction was applied, thereby maximizing data yield. By including both human and animal studies, the pool of human studies was enhanced with animal studies, as an effort to collect information for a recent clinical approach and ongoing technology. Though no unanimously accepted method for quality assessment of animal studies is available, broad but clinically relevant eligibility criteria were carefully set in this review to include studies that examined various combinations of biomaterials and types of 3D printing.

Several limitations of the study should be noted. Due to scarcity of randomized and prospective non-randomized studies, several retrospective case studies were included. Uncontrolled studies with methodological limitations were part of the review. On the other hand, case series with fewer than five participants which were excluded to avoid low quality evidence may have interesting findings. Another limitation is that the type and number of animals as well as the time of observation vary among studies, giving great heterogeneity in many domains.

6.10. 3D printing: Current facts & prospects

The application of 3D printing technology for tissue engineering is not yet universally accepted. 3D printing strategies on bone repair, especially in the regeneration field, seems to have many obstacles to overcome. In our mind, ongoing research will overcome surgical difficulties, material manipulation and potential criticisms of the use of certain regenerative biomolecules in humans. The fabrication of 3D printed scaffolds should be considered as a promising alternative for bone tissue repair in craniofacial deficiency on the supposition that several parameters should be taken into account to ensure the success and wide-spread application of 3D printing bone scaffolding. Firstly, an important factor is the collaboration between medical and engineering experts, and familiarization with 3D bioengineering abilities. Secondly, the printing devices should scale up to be faster with high resolution, compatible with biomaterials/living cells and affordable price; nevertheless, the necessary equipment is expected to have lower cost over the years. Thirdly, the science of biomaterials should produce compounds in optimal combinations to achieve the desired functional, mechanical and supportive properties. Direct control over cell proliferation and differentiation as well as well-characterized and reproducible source of cells are required. Scaffolds should be developed to actively induce vascularization, innervations, replacement by bone and bone maturation; not just be inert structures. Lastly, human research should focus on high quality clinical trials, which will provide evidence to assess 3D printing scaffolds over conventional grafting strategies.

Acknowledgements

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