

## **Tridimensional Bioprinting for Regenerative Dentistry: Systematic Review**

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### **ABSTRACT**

**Objective:** The aim of this work is headed in the systematic revision and comparison of different techniques for the use of bioprint within the area odontology used in the last 10 years.

**Methods:** Randomized or nonrandomized studies that apply bioprint in the face and neck region were included, the methods and techniques are summarized in the review. Electronic databases were reviewed as systemic search until June, 2016 a total of 212 articles matched with the criteria search and only 11 were focused on regenerative dentistry. The key words used were as follow: Bioprinting, 3D bioprinting, 3Dbioprinting dentistry.

**Results:** The question remains whether creating biomimetic tissue engineered constructs that recapitulate nature, even to a limited degree, will lead to significantly improved therapies, regardless if these constructs are immediately implanted or transplanted after culture.

**Conclusion:** A fundamental problem for designing bioprinting constructs is that we have only a very limited understanding of the underlying biology of regeneration. Even as a more complete understanding is gained, it will probably be impractical to attempt to replicate all of the hundreds to thousands of factors involved in tissue repair.

**Keywords:** Bioprinting, bioprinting dentistry, three-dimensional printing

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## **INTRODUCTION**

The ability to grow human tissues in three-dimensional (3D) cultures has proven useful both for regenerative medicine and for tissue development. Such “organoid” culture systems have been developed for several types of human tissues, including intestine, stomach, kidney, and brain(1).

Three-dimensional systems enable the formation of tissue-mimetic architectures and promote more realistic physiological responses than conventional 2D systems. It has been report, a previously unidentified layered 3D culture system to assay migration and maturation of human induced pluripotent stem cell (iPSC)- derived neural progenitor cells (NPCs) and reveal a genotype- specific effect of methyl-CpG-binding protein-2 (MeCP2) dysfunction on iPSC-derived neuronal migration and maturation in 3D layered hydrogels(2).

Polymeric collagen fell out of general use in the 1980s with the shift in focus to soluble collagen isotypes and gene sequences, rendering it virtually absent from the recent literature. Cell collagen materials for tissue engineering have largely taken the form of collagen gels based on acid-soluble collagen, or cross linked collagen(3,4).

Mesenchymal stem cells (MSCs) are an adult stem cell population found in multiple tissues throughout the body including bone marrow, adipose tissue, the synovial membrane, and trabecular bone. MSCs are of particular interest for therapeutic applications because the cells can be differentiated into many of lineages including chondrocytes, adipocytes, and osteoblasts. MSCs exist in very low numbers in the highly cellular and heterogeneous bone marrow, and lack unique identifying markers necessary for definitive isolation. Isolation and characterization of MSCs is currently based on a set of established properties set forth by the International Society for Cellular Therapy(5).

Mesenchymal stem/stromal cells (MSCs) are known for their proangiogenic qualities and are currently being developed to treat a wide variety of diseases in adults caused or

complicated by inadequate tissue perfusion and vascularization(6). Mesenchymal stem cells (MSC) are adult stem cells capable of self-renewal and differentiation into multiple lineages including cartilage, adipose, and bone. MSC are characterized by their ability to adhere to plastics under standard cell culture conditions. Along with their self-renewal property, MSC secrete factors, such as growth factors, both in an autocrine and paracrine fashion, which affect the surrounding microenvironment to promote angiogenesis, decrease inflammation, and enhance tissue repair. Moreover, MSC exert strong immunosuppressive properties, allowing them to be transplanted without any pre- or post-treatment. Additionally, they are easy to expand in culture and have multi-lineage differentiation potential and tropism toward neoangiogenic, tumor, and inflammatory sites(7).

Mesenchymal stem cells have the self-renewal potential and can differentiate into multiple cell types, such as osteoblasts, chondrocytes and adipocytes. Although it was, originally, supposed that injected MSCs might differentiate to replace injured cells, therapeutic effects were, frequently, observed without engraftment and differentiation of donor cells. Instead, paracrine secretion acted as an important mechanism for stem cell-based tissue repair. Cytokines produced by MSCs exhibited multiple beneficial functions, including promoting angiogenesis, inhibiting apoptosis, reducing inflammation and scavenging reactive oxygen species (8).

Human pluripotent stem cells (hPSC), which include both embryonic stem cells and induced pluripotent stem cells, play an important role in regenerative medicine, developmental biology and pathology, and drug screening, owing to their ability to give rise to any cells in the human body and indefinitely self-renew. Despite OF the benefits that iPSC offer, controlling their differentiation into targeted cell type(s) remains a challenge. Studies over the years have shown that stem cells respond to their microenvironment, composed of soluble and matrix based cues, to regulate their fate and commitment Synthetic 3D

bioprinting biomaterials have been used extensively to recapitulate tissue-specific physicochemical cues to direct self-renewal and differentiation of stem cells.(9) The bioprint technique includes different methods such as stereolithography, inkjet 3D printing and selective laser sintering among others. Table 1 summarize the different available techniques for 3D bioprinting.

A laser-assisted bioprinting technique, one of the bottom up approaches, is applied to “living cells” to arrange keratinocytes and fibroblasts in three-dimensional patterns as multicellular constructs mimicking native skin architecture. This most recent technology of three-dimensional bioprinting could translate into the TE of oral mucosa. Since the ECM communicates with cells and modulates phenotypes and function of those cells, there is a need for a new generation of biomaterials because TE strategies depend on a three dimensional scaffold design. Although, for a decade, single-phase scaffolds have been employed for dermal substitutes, biphasic and multi-phasic or gradient scaffolds may better recapitulate the complex internal structure of the underlying connective tissue, including basement membrane which may provide a niche for repopulated cell (15).

Numerous bone-grafting options exist for head and face reconstruction to fulfill various needs depending on the specifics of the defect and the patient’s clinical condition. Bone-grafting is widely adopted because of the superior osteogenic, osteoinductive, and osteoconductive properties of native bone grafts. The membranous bone grafts such as those harvested from cranium are superior to endochondral bone grafts in terms of the volume maintenance, while highly vascularized grafts such as vascularized bone flap from fibula or iliac bone have advantage of rapid incorporation into the host bone and vascular flow. Finally, osteochondral grafts can repair composite defects of bone and fibrocartilage. Autologous grafts are considered the gold standard for head and face reconstruction due to their bioactivity, mechanical competence, and immediate cellular function. However, the

restricted volume of the bone available for harvest, donor site morbidity, the lack of precision in carving delicate shapes of craniofacial analysis and interpretation (16).

This article aims to review the literature the term biofabrication and different definitions, and applications as recorded in literature since 2006 to present days. Even more important, we believe that there is a need to clarify the position in the bioprinting dental field with a practical approach due to its implementation and evolution. In this context, it is proposed to investigate keywords such as bioprinting, 3D printing and bioprinting in dentistry.

## **SUBJECTS AND METHODS**

This review follows the criteria and standards for the systemic review. It aims to give a broader focus on bioprinting in the dental area and materials that have been used in course of time to the period of 2016. Randomized and quasi-randomized, controlled or nonrandomized studies published from 2006 to 2016. Nonrandomized studies to increase the scope of the review and has been shown that generally does not generate any bias were included. Inclusion criteria were based on articles that research was carried out at the level of face and neck mostly focused on the dentistry field.

### **Search strategy**

The identification of the studies was based on a search strategy for each electronic database (the Cochrane Central Register of Controlled Trials, MEDLINE via PubMed and Embase); the search was conducted on March, 2016. Screening procedures were restricted to SUMMARY search Title/Keyword (Figure 1). The search was restricted to English language. Neither the authors nor the journals were blinded to researchers. Two researchers identified and selected the studies by title and summary.

*Data extraction and evaluation*

Data from eligible studies were extracted independently by 2 reviewers using electronic spreadsheet Excel. Data were recorded in accordance with the selection criteria. To extract the data the follow variables were taking: used materials, technique and diagnosed application in the face and neck region.

*Perspective of 3D bioprinting in dentistry*

The different bioprinting technologies reported exhibit promise in the field of regenerative craniofacial and dentistry. However, each tissue currently requires a specified technology and the bioprinting of multi cellular tissue constructs is difficult. Table 1 summarized the different technology with bioprintinf for regenerative dentistry. Moreover, the mechanical stability of current materials such as hydrogels and bioinks are not improving for craniofacial reconstruction with the need for long-term. Pre and clinical studies are necessary by using different materials and ultimately good manufacturing production (GMP) of bioprinted constructs. A promising future approach for the treatment of external craniofacial tissues could be a handheld bioprinting device that will enable the delivery of cells into tissues such as skin or cartilage. From here, future studies should be focused on the optimization of bioprinting technologies to enhance the self-repair capabilities of tissues in the craniofacial and dentistry area.

**RESULTS**

Advances in image-guided fabrication of biocompatible, osteoinductive scaffolds such as 3D bioprinting, which in theory enables simultaneous printing of biofactors and antibiotics within the patient-specific scaffold for sustained release can potentially lead to single stage reconstruction procedures, accelerated recovery time, and improved the clinical outcome.

(Table 1) The question remains whether creating biomimetic tissue engineered constructs that recapitulate nature, even to a limited degree, will lead to significantly improved therapies, regardless if these constructs are immediately implanted or transplanted after culture? To be successful, significant challenges will have to be overcome. A fundamental problem for designing bioprinted constructs is that we have only a very limited understanding of the underlying biology of regeneration. Even as a more complete understanding is gained, it will probably be impractical to attempt to replicate all of the hundreds to thousands of factors involved in tissue repair. However, as tissue engineers gain new knowledge, this will provide them with the insight and intuition to help them select the minimum number of variables needed to create the simplest tissue engineered constructs capable of achieving desired clinical outcomes (17).

## **DISCUSSION**

Craniofacial regeneration strategies seek to mimic or promote oral developmental processes by using biomaterials and growth factors to induce tissue formation via stimulation of specific cellular function, both *in vitro* and *in vivo*, table 1 summarizes the studies performed. Craniofacial tissues, including bones, teeth, cartilage, muscles, and ligaments, as well as their fundamental building blocks, such as blood vessels and nerves, form complex systems responsible for a number of critical functions in the body. For instance, these structures work synergistically to ensure physiologic respiration, speech, digestion, and craniofacial support, among other specific roles. In nature, these tissues are organized with complex heterotypic 3-dimensional architectures, specific cell-cell interactions, anisotropic mechanical properties, and heterogeneous distribution of growth factors. Because of the complex anatomy of craniofacial structures, full recovery of craniofacial tissues from trauma, respective surgeries,

or congenital malformations is extremely challenging. Despite important recent advances in the field, conventional regenerative strategies still largely fail to mimic the 3D complexity and the multicellular interactions occurring in native craniofacial tissues.

## **CONCLUSION**

There are a large number of methods for three-dimensional printing with great success in its application, but they remain a great challenge in terms of durability and biocompatibility. It can be predicted that while bioprinture of monolayer and hollow complexes of less complexity can be achieved in the foreseeable future, the manufacture of functional solid organs will only become a clinical reality for future generations.



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Table 1: A summary of the most commonly used 3D printing techniques in medical application

<b>3D printing techniques</b>	<b>Pros</b>	<b>Cons</b>
Stereolithography;	Current gold standard High resolution Increased efficiency with increase in print size Detailed fabrication of internal structures	>1 day of printing time required Require extensive post-production manual handling High cost related to the materials, the printer, and the maintenance (10)
Multijet modeling	High resolution Minimal post-production manual handling Multiple materials	High cost related to the material and printer Poorer surface finishing than SLA (11)
Selective laser sintering;	Not require support structures Smooth surface finishing Print delicate structures Print in metal	Require post-production manual handling High cost related to the materials, the printer, and the maintenance Require expert handling of the printer (12)
Binder jet technique;	Not require support structures Multiple colors Multiple materials	Brittle Require extensive post-production manual handling Poor surface finish (16)
Fused deposition modeling.	Low cost maintenance of printers High availability	Require post-production manual removal of support structures Poor surface finish Mono-color and mono-material with the current technology (11,14)

Table 2: Bioprinting materials used for regenerative dentistry

Reference	Model	Procedure	Cell Type	Graft Material	Technique	Methods
<b>Ciocca et al, 2011 (22)</b>	Human	Atrophic maxillary arch reconstruction	—	Titanium	DMLS	CT
<b>Ciocca et al, 2013 (23)</b>	Sheep	Condyle reconstruction	BMCs	HA	NS	Histology and histomorphometry
<b>Mangano et al, 2013 (26)</b>	Human	Oral rehabilitation with dental implants	—	Ti-6Al-4V alloy powder	DMLS	Radiography
<b>S_andor et al, 2014 (18)</b>	Human	Craniomaxillofacial hard tissue defect reconstruction	ASCs	b-TCP, bioglass BMP-2	SLS	CT
<b>Wang et al, 2012 (28)</b>	Human	Ramus defect and condylar fracture reconstruction	—	Titanium	NS	CT
<b>Wolff et al, 2013 (16)</b>	Human	Mandibular ameloblastoma resection defect reconstruction	ASCs b-	TCP + BMP-2	SLS	Radiography and CT
<b>Mina D. et al, 2015 (21)</b>	Human	Mineralized dental tissues	ASCs	NF-MS	NS	P
<b>Bertassoni a et. al 2014 (22)</b>	bovine	Bone reconstruction	GelMA Hg	cell-laden photolabile ECM-derived	Novogen MMX Bioprinte	CT histomorphometry
<b>Rasperini. et al. 2015 (23)</b>	Human	Periodontal Repair	ASCs,	rhPDGF-BB	DMLS	CT
<b>Obregon et al 2014 (24)</b>	Human	Craniofacial	NS	Hg	NS	CT, P
<b>Hossein E et. al 2015 (25)</b>	Human	Dentoalveolar defects	HG	HA	FDM	CT
<b>Caton et al 2010 (26)</b>	Human	Periodontal defects	NS	HA	NS	CT

Abbreviations: 3D, 3-dimensional; AM, additive manufacturing; ASCs, adipose stem cells; BMCs, bone marrow stem cells; BMP-2, bone morphogenetic protein-2; CBCT, cone-beam computed tomography; CT, computed tomography; DMLS, direct laser metal sintering; FDM, fused deposition modeling; HA, hydroxyapatite; nHA/PA, nanoscale hydroxyapatite and polyamide; NA, not available; NS, not specified; PGA/PLA, polyglycolic acid and polylactic acid scaffolds; PCL,  $\epsilon$ -caprolactone; RP, prototyping; SLS, selective laser Q13 sintering; TCP, tricalcium phosphate. Hg, Hydrogels, nanofibrous microspheres NF-MS, rhPDGF- BB, human platelet.

## Bioprinting for Regenerative Dentistry

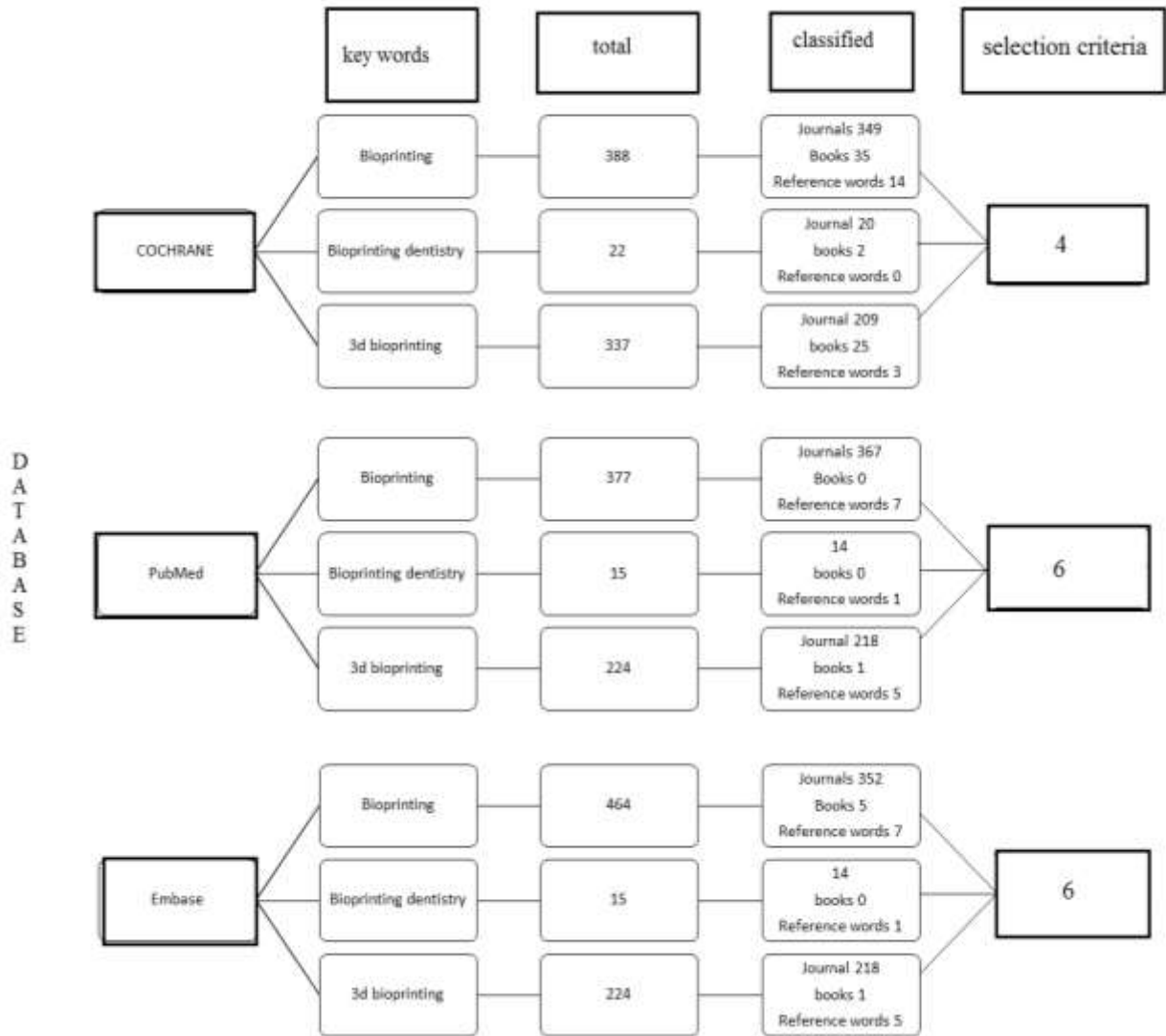


Figure: PRISMA flowchart. Systematic review.