3D Bio-Printing–A Review on Current Application and Future Prospects in Dentistry

Pati DolAva Teerdha[1], Mohammed Admali[1], Komal Smriti[2]*, Kalyana C Pentapati[3], R. Vineetha[2], Srikanth Gadicherla[4]

1. Manipal College of Dental Sciences, Manipal Academy of Higher Education, Manipal.
2. Department of Oral Medicine and Radiology, Manipal College of Dental Sciences, Manipal Academy of Higher Education, Manipal.
3. Department of Public Health Dentistry, Manipal College of Dental Sciences, Manipal Academy of Higher Education, Manipal.
4. Department of Oral and Maxillofacial surgery, Manipal College of Dental Sciences, Manipal Academy of Higher Education, Manipal.

Abstract

Three-dimensional (3D) printing refers to a process of deposition of materials to fabricate three dimensional objects by implication of materials such as plastic polymers, metal, ceramics or even living cells. The process involved in printing of living tissues is named as 3D bioprinting. Recent advances in the field of 3D Bio-printing has given rise to new possibilities in the manufacturing of customized patient-tailored (bioactive tissue) constructs which show a great degree of resemblance to the patient's native tissue for oral and craniofacial reconstruction.

Various application of additive manufacturing has gained much attention in the medical and dental domain. The improved quality and cost effectiveness has contributed to their increased use on patients. This review concisely summarized the different methods used in Bio printing, their various advantages and set-backs. It elaborates the concept behind bio-ink and focuses on different materials utilized to manufacture Bio-inks. Emphasis was given on various applications of bio printing in craniofacial and dental tissue regeneration, highlighting the recent advances in this upcoming field which would make bioprinting a routine procedure in our daily practices in future.

Review (J Int Dent Med Res 2019; 12(3): 1202-1210)

Keywords: Bioprinting, Bioink, Hydrogels, Tissue regeneration.

Received date: 10 September 2018 **Accept date:** 16 November 2018

Introduction

Three-dimensional (3D) printing also called as additive manufacturing is a process in which entities are fabricated by placing materials layer by layer to yield a three-dimensional assembly. This method can produce any 3D object with the help of computer aided design (CAD). Prof. Chuck Hull, the founder of stereo-lithography technology printed the world's first 3D model by solidifying a polymer material assisted with the help of a Laser. It has established its use in dentistry in many ways such as fabrication of customized prosthesis, personalized implants pertaining to an area and anatomical models to perform mock surgery prior to orthognathic surgery.it is done by using large and fast 3D printers by "rapid prototyping machines" to manufacture 3D models and molds. Another prospective extension of the 3D printing technology emerged in the early 21st century called as Bio-printing. It is an amalgamation of engineering and cell biology. This procedure enables artificial construction of living tissues and organs by a three dimensional, layer upon layer deposition of living cells along with a suitable protective and supportive matrix using various printing modalities.[1] This review explains the upcoming novel field of Bio printing, which shows promising solutions for treating comorbidities using tissue engineering and regenerative medicine. Although this method is in elementary stage but in future, it can revolutionize the treatments of a multitude of oral as well as oro-facial comorbidities by producing autografts and donor tissues such as jaw bones, periodontal ligament, cartilage, vascular network and blood vessels.

Corresponding author:
Komal Smriti
Department of Oral Medicine and Radiology,
Manipal College of Dental Sciences, Manipal Academy of Higher Education,
Manipal, Karnataka, India.
E-mail: komalmds1@gmail.com

Types of bio-printing methods

Many 3D printing technology have evolved over last two decades. Each one of them have their applications and limitations. The type of printing processes depends on the bio ink material chosen and its compatibility with the printer. A brief description of various bioprinting methods, their advantages and disadvantages are discussed below.

Inkjet bio-printing

The first Bio-printing technology developed, Inkjet Bio-printing[2] which produced droplets of well-regulated and controlled sizes, sourced from a pre-loaded cartridge containing the bio-ink material. It functions on the principle of thermal/piezoelectric deformation of printer head. The complete printing process is controlled electronically via a designated software which deposits cell containing droplets at frequencies of approximately 10,000/sec.[3–6] However, one of the major drawback is its non-compatibility with Bio-ink droplets having high viscosity (15mPa/s)/ Density (>1x10^6 cells/ml).[3,7,8]

Laser assisted bio-printing (LAB)

LAB functions on the Laser Induced Forward Transfer mechanism.[3] In this procedure the upper layer is a donor layer comprising of an energy absorbing top zone and bio-ink suspended at the bottom layer. A pulsed laser beam is focused on the energy absorbing zone. It absorbs the laser energy and creates increased gas pressure, causing the propulsion of the cell droplets towards the collector slide (Bio-paper/Culture plates/Scaffolds). Here individual tissue particles subsequently integrate to form a fully functional organ incorporated with desired spatial configuration of the 3D construct, obtained by guiding the path of the laser beam.[9] With LAB, a wider array of bio-ink material can be used [10] as this enables printing of highly viscous cell droplets (1-300 mPa/s). Laser assisted printing shows greater cell viability greater than (95%) the former (85%).[11]

Micro-entrusion bio-printing (MEB)

This method of bio-printing is based on fused deposition and solution deposition modeling technology. It uses a fluid dispenser along with an automated robotic system for extrusion printing. Fluid dispenser is based on either an air-driven or Piston pressure assisted system that deposits the Bio-Ink in the form of cylindrical filaments according to the required design.[12] It produces mechanically and structurally strong supportive polymeric constructs and 3D scaffolds which can be used in bio-printing of bone tissues.[11,13,14] Major concern with this method is cell deformation due to the sheer stress of highly viscous fluids which could potentially lead to cell death.[13,14] Detailed comparison of these three types are mentioned in table1.

Parameters	Inkjet	Laser assisted	Micro-extrusion
Resolution[15,16]	High (50µm)	High (100-600µm)	Moderate (5 µm–1mm)
Droplet size[15]	50-300µm wide droplets	<20µm	100 µm -1mm wide droplets
Accuracy[15]	Medium	High	Medium-Low
Print speed[3,15,17]	Fast (1-10,000 droplets/sec)	Medium (200-1600 mm/sec)	Slow (10-50 µm/sec)
Supported viscosities [14,15,18,19]	Low (3.5-12 mPa/sec)	1-300 mPa/sec	High (30 mPa/sec to above 6×10^7 mPa/sec)
Cell density[14,15]	Low (10^6 – 10^7 cells/mL)	Medium (cell spheroids)	High (10^6 – 10^7 cells/mL)
Cell viability[7,20]	>85 %	>85 %	40% - 80%
Structural and Mechanical integrity[15]	Low	High	Low/medium
Fabrication time[15]	Medium	Long	Short
Cost[16,21]	Low	High	Medium
Applications[22–24]	Blood vessel, bone, cartilage, neuron.	Blood vessel, bone, skin, adipose.	Blood vessel, bone, cartilage, neuron, muscle, tumor.
Materials for Bio inks[4,5,25–28]	Alginate, PEGDMA, Collagen	Collagen, Matrigel	Alginate, Gel MA, Collagen

Table 1. Comparison of Various Types of Bio-Printing Methods

Bio-ink

Three dimensional Bio-printers function on the suitable cell delivery media which support the growth, proliferation of intercellular communication and differentiation of living cells ultimately to achieve complete regeneration of the defect in concern.[29] The selection of the various biomaterials in designing the bio-ink is dependent on research-specific needs, type of organs, tissues and cells to be regenerated and the type of printer being used. An ideal bio-ink should have characteristic physical or chemical cell encapsulation with high cell viability during pre and post printing process.[30] The surface tension and viscosity must be under control. It should be bioactive to guide the cellular maturation and regeneration of the defects and be biodegradable in order to be replaced by the newly formed tissue.[31]

Bio-inks can be classified broadly as scaffold based and scaffold free materials.

Scaffold based constructs:

a. Hydrogels are the most widely used bio-ink material used in conjunction with Inkjet, laser assisted and extrusion based bio-printers.[1,30] It is a combination of an Extra-cellular matrix and living cells in the form of a pre-polymer solution that undergoes physical or chemical cross linking to form self-supported structures. The encapsulated cells in the Bio-ink are cultured in suitable media in the laboratory and are deposited at sub-human body temperatures.[32]

b. Decellularized matrix-based bio-ink is produced by the lysis and extraction of the cellular components of the native tissue with the conservation of the extracellular matrix. It is employed in the extrusion-based bio-printing and offers good bio-mimicry. In this process, tissue specific-customized constructs can be fabricated but it is expensive and lacks adequate mechanical strength required for fabrication of load bearing large constructs.[33]

c. Microcarriers are porous constructs of natural or synthetic materials used in Extrusion based bio-printing that facilitate cellular attachments, growth and maturation with improved mechanical properties. Clogging of the nozzle head, expense and subsequent decreased accuracy while printing are some of the issues reported previously.[34]

Hydrogel	Advantages	Disadvantages	Cell type used
Agarose[40]	Good mechanical strength characteristics, economical	Exhibits poor cell adhesion and attachment	Human neural stem cells
Chitosan[41]	Highly biocompatible, Antibacterial and antifungal properties	Restricted to small constructs, delayed gelation, poor mechanical properties	Human neural stem cells and human Adipocyte derived mesenchymal stem cells (ADSC)
Alginate[42]	Good strength characteristics can be used in fabrication of complex 3-D structures, fast gelation, economical, and adequate stability	Poor cell adhesion, clogging issues	Human neural stem cells
CollagenI[41,43]	Facilitates cell adhesion and attachment and good printability	Gelation process is slow, poor mechanical properties, Absence of uniform distribution of cells	Human Bone and amniotic fluid derived stem cells
Fibrin[44]	Promotes angiogenesis, Favorable gelation properties	Immunologic concerns, risk of microbial contamination, poor mechanical stability	Human amniotic fluid stem cells, human Bone Marrow derived Mesenchymal stem cells (BMSC)
Gelatin[4542]	Promotes cell adhesion	Unstable/fragile/weak mechanical properties, poor abilities without combining with other materials	Human cardiac progenitor cells
Hyaluronic Acid (HA)[42,43]	Vital for ECM development, promotes angiogenesis and cell proliferation, fast gelation	Rapid biological degradation, poor mechanical stability and characteristics	Human cardiac progenitor cells, Human BMSCs
Matrigel[46]	Produces mechanically strong constructs with high cell vitality, promotes cell differentiation and integration with host tissue	Clogging issues made from tumor cells	Human epithelial cells

Table 2. Overview of Various Available Hydrogels

Scaffold free Bio-inks: Scaffold free Bio-inks are used for printing highly dense cellular constructs with the absence of any supporting hydrogel or matrix. It applies extrusion-based bio-printing method. It consists of cell suspensions in suitable growth media that facilitate cellular

interactions and deposition of extra cellular matrix. The tissue spheroids produced exhibits enhanced tissue bio-mimicry and cellular interactions.[35,36] Tissue spheroids, are spherical aggregates of cells with favorable structural integrity used for tissue engineering and drug testing. However, the whole process is very labor intensive with difficulty in extraction of prematurely fused cellular aggregates. Cell pellets and tissue strands are viewed as alternatives in scaffold free Bio-inks.[37,38]

Cell pellets are concentrated cellular aggregates wherein the growth of these cells is dictated by a supporting polymer mold. Tissue strands are manually-guided to synthesise constructs in dissolvable alginate tubes that have properties equivalent to cartilaginous tissue regeneration.[39]

Natural hydrogels: Various available natural hydrogels and their compatible cell types were presented in table 2.

Synthetic hydrogels: Various available synthetic hydrogels and their compatible cell types were presented in table 3.

Applications of Bio-printing in the field of craniofacial and dental tissue regeneration:

Bone:

Engineering of craniofacial bone tissue has been done by scaffold-based approaches and various biomaterials have been used in craniofacial bone reconstructions. They function as cores for tissue synthesis which enable cell division, growth and also facilitate cell differentiation. It provides conducive mechanical properties and biomechanical features such as bio-resorbability, biocompatibility, bioactivity and porosity of the construct. They can be broadly classified into natural polymers, synthetic polymers and bio-ceramics.[49] Biomaterials are commonly required for the reconstruction of the defects which arise due to trauma, congenital deformities and post tumour surgery.[50]

Bio-printing of Bone tissue has mainly been done using the Extrusion based or laser-based bio-printing modalities using polymeric hydrogels as a component of the bio-ink. In rapid prototyping, these scaffolds are often seeded with osteoprogenitor cells such as adult adipocyte derived mesenchymal stem cells. They are infused with growth factors such as bone morphogenic proteins, fibroblasts and endothelial growth factors that support the maturation of the stem cells and to lay down the extracellular matrix[51] The maturation process of the infused scaffold happens in a Bioreactor.[52] A Bioreactor is a system which mimics the native environment by providing mechanical and biochemical stimuli that induce the cells to organize uniformly, differentiate and lay down the extracellular matrix. This enables the formation of new tissue in the construct with improved mechanical properties.[53]

Scaffolds used in rapid prototyping and additive manufacturing:

Nano-hydroxyapatite scaffolds employing laser-based bio-printing (LBB) have shown adequate osteoinductive features, Acellular dermal matrix with BMP-2, TGF-β2, SDF-β1(DBB) and bioactive glass based scaffolds have exhibited controllable osteogenesis. However, the mechanical properties of these scaffolds seem inferior and demonstrate printability concerns.[19,48,54] This problem seems to be rectified by extrusion based bioprinting employed silk fibroin and gelatin scaffolds, Type 1 collagen and agarose constructs and calcium deficient hydroxyapatite hybrids.[55–57] However, these scaffolds in turn exhibit individual disadvantages of different origins. Scaffold free constructs demonstrate favorable mechanical strength characteristics right since their fabrication and do not rely on time-dependent maturation and development of mechanical properties. However, the current technology limits the production of these constructs to small defect sizes.[58,59]

The most commonly observed limitation is the release of growth factors within the scaffolds cannot be regulated.[60] Another drawback of engineering bone tissue is attributed to lack of neo-vascularization which affects osteogenesis and host integration.[61,62]

The porosity of the scaffold material has been showed to affect osteogenesis and vascularization. It has been suggested that a minimum of 100 μm pore size is essential for diffusion of oxygen and nutrients for maintaining the cell vitality of the scaffold. Pore size of 200-300 μm is considered optimal for bone growth.[63] However, increased porosity of the material leads to decreased mechanical properties. Modulus of elasticity (MOE) of the mandibular trabecular bone varies from 3.5 – 240 MPa depending on the presence or absence of the cortical plates[64] with reports of MOE upto 910 Mpa in the midline-ramus regions. The hydrogels however have

MOE values ranging from 300-350 Kpa, thus necessitating improvements in the mechanical properties of the bio-printed constructs.[65]

To tackle these challenges, the upcoming research has emphasized on use of Bio-printing technology employing an image analysis system (CAD) based on computed tomography (CT) or magnetic resonance imaging (MRI) of the craniofacial defect.[66–68] this allows the designing of the architecture of the entire graft including the Nano topography of the scaffold and also the spatial deposition or printing of different cells in these scaffolds to generate complex tissues as dictated by the requirements of the defect. This process has provided bio-fabrication via precision printing. Hybrid scaffolds with multi-material Bio-inks are being fabricated in attempt to combine the crypto-biocompatibility of hydrogels with the superior mechanical properties of synthetic/ polymeric based scaffolds.[67,69]

Kang *et al*. developed an integrated tissue organ printer that engaged Human amniotic fluid derived stem cell laden multimaterial bio-ink in a hybrid mixture of Polycaprolactone (PCL), Pluronic 127 and tricalcium phosphate to reconstruct human mandible and rat calvarial defects. Formation of neovascularised healthy bone in the entire scaffold was reported in vivo.[53]

Recent studies on hybrid scaffold of decellularised bone and PCL scaffolds with fibrinogen and thrombin growth factors exhibited the regeneration of mineral and collagenous components on the reconstructed defects and yielded superior results in comparison to synthetic scaffolds in rat calvarial defects.[70] They also used these scaffolds to successfully design customized temporomandibular joint condyles and concluded that this hybrid scaffold is superior to pure synthetic scaffolds.

Kuss *et al*. reported that short term (7-21 days) hypoxic conditioning of PCL/Hydroxypatite and Stromal vascular fraction cells (SVFC) laden hydrogel based hybrid bio-ink construct promoted vascularisation and enhanced integration with host vasculature. The osteogenic differentiation potential of the stem cells was not affected in both in vitro and in vivo testing.[71]

Temporomandibular joint (TMJ)

Both autogenous and alloplastic materials have been used in TMJ reconstruction. However, autogenous grafting is associated with donor site harvesting causing morbidity and alloplastic materials do not exhibit responses towards chemical and bio-mechanical stimuli in congruence with native tissues.[72] Schek *et al*. in 2014 used MRI/CT to design the scaffold of the joint to be reconstructed using biphasic composite scaffolding of HA and polylactic acid. They were seeded with chondrocytes in the polymeric phase and transduced fibroblasts ion in ceramic phase of the composite scaffold to produce cartilage and bone simultaneously.[73] They concluded that these bi-composite scaffolds generated osteochondral tissue with vascularized and organized subchondral bone-cartilage interphase and is effective in regenerating osteogenic tissue in situ.

Grayson *et al*. used image guided fabrication of anatomical decellularised bone scaffold which was cultured with human adipose derived stem cells (hASCs) and subjected to a customized Bioreactor which enabled controlled growth and development of the TMJ construct.[49] The final results showed that the mechanical properties and geometry of the final graft was exactly similar to the native bone with high cell densities.

Periodontium:

The successful regeneration of the periodontium involves a coordinated multi-response from the periodontal fibers, gingiva, alveolar bone and cementum. Guided tissue regeneration is the conventional procedure involving the placement of a barrier membrane in the periodontal defect site to promote selective repopulation of the periodontal cells. However, the clinical outcomes of this method have been unpredictable.[74]

Recently, use of multiphasic scaffolds which consists of a complex construct with varying microarchitecture such as porosity, pore organization as well as the chemical composition has shown promising clinical outcomes, as these scaffolds closely mimic the native periodontal architecture.[75] Carlo Reis *et al*. developed a Polylactide-co-glycolide acid and Calcium phosphate based bi-layered biomaterial scaffold which was tested in class II furcation defects in dogs and reported to enhance cementum, bone and periodontal fibers insertion.[76] Park et al designed a compartmentalized hybrid scaffold containing PCL and Polyglycolic acid with a biomimetic architecture using a computational CAD/CAM. It was later seeded with Human

periodontal cells and Ad BMP-7-hAF (Adenovirus mediated Bone morphogenetic protein and human amniotic fluid).[77] Lee *et al*. developed a tri-phasic PCL-HA scaffolds consisting of phase A for the cementum dentin interphase, phase B for periodontal fiber attachment and Phase C for alveolar bone regeneration. Phase A had 100µm micro-channel supported by Human amelogenin. Phase B constituted of connective tissue growth factor and an architecture consisting of 600um micro-channel. Bone morphogenetic protein containing phase C construct had 300µm micro-channel. Seeding was done with progenitor cells and on incubation it was found that the same progenitor cell differentiated and produced cementum, periodontal and alveolar bone complex as guided by the scaffold design and architectural construct.[78]

Tooth regeneration:

The Adult human dentition exhibits very limited potential for repair and regeneration. The current treatment modalities of missing teeth are based on prosthetic rehabilitation with dentures or dental implants. The enigma of replacing missing teeth with complete natural teeth has led to research on bio-printing technology to congregate regeneration of edentulous regions. Nakao *et al*. developed bioengineered tooth germ by injecting epithelium and mesenchymal derived stem cells at high cell densities (5×10^8 cells/ml) with a collagen bio-ink. This process exhibited formation of a tooth germ with cell compartmentalization between epithelial and mesenchymal derived cells that were capable of generating the whole tooth. Later, they conducted in-vivo experimentation by transplanting early primordia of an incisor prior to bell stage partially developed in a sub renal capsule over a 10 day period, into a cavity created by extraction of mandibular incisor in mice which exhibited successful penetration of nerve fibers and blood vessels.[79] In a similar study conducted by Ikeda *et al*., one bio-engineered molar tooth germ in early bell stage was successfully implanted into the upper first molar region in the alveolus of an 8 week old adult murine transplant model. The bio-engineered tooth underwent complete development similar to that of a natural tooth and reached the occlusion with identical periodontal ligament space and constitution.[80]

Dental pulp:

Although conventional endodontic therapy has been successful in treating non-vital teeth with infected root canals, restitution of complete healthy pulp tissue is not achieved yet. Bio-printing has given hopes for pulp regeneration. Constraints while bio-printing of dental pulp is the limited viability of the cell laden hydrogel material based construct due to the absence of highly vascularized network.[75]

Athirasala and Bertessoni *et al*. conducted an in vitro study on extracted adult molar and premolar teeth to simulate vascularized dental pulp like tissue constructs in root canals. They used 10 and 15% Gelma –hydrogel matrix, seeded with OD 21(odontoblast 21) cells and endothelial cells which were later incorporated into an endodontically treated tooth. The OD 21 cells exhibited proliferation towards dentinal walls which gave rise to monolayers after 7 days. Thus, a concept was proposed, promising future for further studies and to achieve a goal of regeneration of vital pulp tissue.[81]

Skin:

The human skin is complex arrangement of different cell types positioned relative to each other at a high degree of specificity which allows the various cellular interactions to take place between each layer giving rise to functionality. Although at present none of bio-printed skin can fully mimic native skin in terms of its protective, regulatory and sensory functions,[82] resemblance of bio-printed skin post maturation shows a promising future wherein it could serve as custom tailored autologous grafts for various defects such as burns, traumatic injuries as well as surgical deficits. While dealing with large wounds, the harvesting of skin grafts from donor sites not only leads to donor site morbidity, also a large site requirement which unfortunately might not always be available.[83] Koch *et al*, using Laser assisted bio-printing, arranged the vital skin cells (keratinocytes and fibroblasts) embedded in collagen on a sheet of Matriderm® scaffold to obtain a skin tissue construct which greatly resembled native skin.[84] Michael *et al*., demonstrated the integration of the bio-printed skin tissue constructs onto a group of 12 nude mice which were given an intentional punch biopsy wound, following which the printed skin was fixed onto the specimens. The results were very promising with successful integration of the

printed skin graft with the surrounding connective tissue which greatly resembled the native skin tissue.[85]

Bio-inks used till date have their own drawbacks, future research should focus on fabrication of an ideal bio-ink by combining all the desirable properties of various existing bio-materials emerging as hybrid Bio-inks. Multi-material hydrogels and hybrid bio-inks are now being used to combine the superior printability and cell interaction features of natural polymers with the mechanical strength of synthetic polymers.[86] PCL-polyethylene glycol and collagen/ chitosan and have been used in bone regeneration. Hydrogels incorporating interpenetrating complex networks of nanoparticles of gelatin, fibrinogen, hydroxyapatite and glycerol have the potential for developing bio-ink with enhanced printability properties along with high cell vitalities, structural integrity, and mechanical strength after the printing process.[87] To understand the behavior of the bio-ink and their construct post printing, 3D printing is now evolving into 4D Bio-printing. In the later, growth and shape transformation of the printed tissue constructs can be achieved in response to various stimuli such as temperature, light, humidity and magnetic fields. Thus by the recent advances, shape transformation in the bio- materials can be added as the fourth dimension to three dimensional Bio-printing.[30]

Conclussion

Bio-printing technology has seen immense development in the past two decades and its application in the regeneration of defects in oral and craniofacial region has shown promising results in animal trials. Prior to further research and its application to clinical and chairside treatments, specific Standardization protocols by regulatory and legal bodies regarding the Bioprinters, stem cell engineering are yet to be laid down. Bioprinting and bioink designing could be established as a field of scientific specialty in the future oriented towards craniofacial applications. This gives us hope of a future where autologous grafts and alloplastic materials will be replaced by Bioprinted products.

References

1. Munaz A, Vadivelu RK, St. John J, Barton M, Kamble H, Nguyen N-T. Three-dimensional printing of biological matters. J Sci Adv Mater Devices 2016;1(1):1-17. doi: 10.1016/J.JSAM D.2016.04.001.
2. Tuan RS, Boland G, Tuli R. Adult mesenchymal stem cells and cell-based tissue engineering. Arthritis Res Ther 2003;5(1):32. doi: 10.1186/ar614.
3. Guillotin B, Souquet A, Catros S, *et al*. Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. Biomater 2010;31(28):7250-7256. doi: 10.1016/j.biomaterials.2010.05.055.
4. Cui X, Breitenkamp K, Finn MG, Lotz M, D'Lima DD. Direct human cartilage repair using three-dimensional bioprinting technology. Tissue Eng Part A 2012;18(11-12):1304-1312. doi: 10.1089/ten.TEA.2011.0543.
5. Cui X, Breitenkamp K, Lotz M, D'Lima D. Synergistic action of fibroblast growth factor-2 and transforming growth factor-beta1 enhances bioprinted human neocartilage formation. Biotechnol Bioeng 2012;109(9):2357-2368. doi: 10.1002/bit.24488.
6. Cui X, Gao G, Qiu Y. Accelerated myotube formation using bioprinting technology for biosensor applications. Biotechnol Lett 2013;35(3):315-321. doi: 10.1007/s10529-012-1087-0.
7. Xu T, Jin J, Gregory C, Hickman JJ, Boland T. Inkjet printing of viable mammalian cells. Biomater 2005;26(1):93-99. doi: 10.1016/j.biomaterials.2004.04.011.
8. Pepper ME, Seshadri V, Burg TC, Burg KJL, Groff RE. Characterizing the effects of cell settling on bioprinter output. Biofabrication 2012;4(1):011001. doi: 10.1088/1758-5082/4/1/ 011001.
9. Vijayavenkataraman S, Fuh JYH, Lu WF. 3D Printing and 3D Bioprinting in Pediatrics. Bioeng (Basel, Switzerland) 2017;4(3). doi: 10.3390/bioengineering4030063.
10. Koch L, Kuhn S, Sorg H, *et al*. Laser printing of skin cells and human stem cells. Tissue Eng Part C Met 2010;16(5):847-854. doi: 10.1089/ten.TEC.2009.0397.
11. Mandrycky C, Wang Z, Kim K, Kim D-H. 3D bioprinting for engineering complex tissues. Biotechnol Adv 2016;34(4):422-434. doi: 10.1016/j.biotechadv.2015.12.011.
12. Zein I, Hutmacher DW, Tan KC, Teoh SH. Fused deposition modeling of novel scaffold architectures for tissue engineering applications. Biomater 2002;23(4):1169-1185.
13. Khalil S, Sun W. Biopolymer deposition for freeform fabrication of hydrogel tissue constructs. Mater Sci Eng C 2007;27(3):469-478. doi: 10.1016/J.MSEC.2006.05.023.
14. Murphy S V, Atala A. 3D bioprinting of tissues and organs. Nat Biotechnol 2014;32(8):773-785. doi: 10.1038/nbt.2958.
15. Dai G, Lee V. Three-dimensional bioprinting and tissue fabrication: prospects for drug discovery and regenerative medicine. Adv Heal Care Technol 2015;1:23. doi: 10.2147/AHCT.S69191.
16. Ozbolat IT, Yin Yu Y. Bioprinting Toward Organ Fabrication: Challenges and Future Trends. IEEE Trans Biomed Eng 2013;60(3):691-699. doi: 10.1109/TBME.2013.2243912.
17. Xu T, Baicu C, Aho M, Zile M, Boland T. Fabrication and characterization of bio-engineered cardiac pseudo tissues. Biofabrication 2009;1(3):035001. doi: 10.1088/1758-5082/1/3 /035001.
18. Chang CC, Boland ED, Williams SK, Hoying JB. Direct-write bioprinting three-dimensional biohybrid systems for future regenerative therapies. J Biomed Mater Res B Appl Biomater 2011;98(1):160-170. doi: 10.1002/jbm.b.31831.
19. Keriquel V, Guillemot F, Arnault I, *et al*. In vivo bioprinting for computer- and robotic-assisted medical intervention: preliminary study in mice. Biofabrication 2010;2(1):014101. doi: 10.1088/1758-5082/2/1/014101.
20. Catros S, Guillotin B, Bačáková M, Fricain J-C, Guillemot F. Effect of laser energy, substrate film thickness and bioink viscosity on viability of endothelial cells printed by Laser-Assisted Bioprinting. Appl Surf Sci 2011;257(12):5142-5147. doi: 10.1016/J.APSUSC.2010.11.049.
21. Billiet T, Vandenhaute M, Schelfhout J, Van Vlierberghe S, Dubruel P. A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering. Biomater 2012;33(26):6020-6041. doi: 10.1016/j.biomaterials.2012.04.050.

22. Chang R, Emami K, Wu H, Sun W. Biofabrication of a three-dimensional liver micro-organ as an in vitro drug metabolism model. Biofabrication 2010;2(4):045004. doi: 10.1088/1758-5082/2/4/045004.
23. Huang TQ, Qu X, Liu J, Chen S. 3D printing of biomimetic microstructures for cancer cell migration. Biomed Microdevices 2014;16(1):127-132. doi: 10.1007/s10544-013-9812-6.
24. Ker EDF, Nain AS, Weiss LE, *et al*. Bioprinting of growth factors onto aligned sub-micron fibrous scaffolds for simultaneous control of cell differentiation and alignment. Biomaterials 2011;32(32):8097-8107. doi: 10.1016/j.biomaterials.2011.07.025.
25. Nahmias Y, Schwartz RE, Verfaillie CM, Odde DJ. Laser-guided direct writing for three-dimensional tissue engineering. Biotechnol Bioeng 2005;92(2):129-136. doi: 10.1002/bit.20585.
26. Ozbolat IT, Chen H, Yu Y. Development of 'Multi-arm Bioprinter' for hybrid biofabrication of tissue engineering constructs. Robot Comput Integr Manuf 2014;30(3):295-304. doi: 10.1016/j.rcim.2013.10.005.
27. Wang Z, Abdulla R, Parker B, Samanipour R, Ghosh S, Kim K. A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks. Biofabrication 2015;7(4):045009. doi: 10.1088/1758-5090/7/4/045009.
28. Xu C, Chai W, Huang Y, Markwald RR. Scaffold-free inkjet printing of three-dimensional zigzag cellular tubes. Biotechnol Bioeng 2012;109(12):3152-3160. doi: 10.1002/bit.24591.
29. Hospodiuk M, Dey M, Sosnoski D, Ozbolat IT. The bioink: A comprehensive review on bioprintable materials. Biotechnol Adv. 2017;35(2):217-239. doi: 10.1016/j.biotechadv.2016.12.006.
30. Ji S, Guvendiren M. Recent Advances in Bioink Design for 3D Bioprinting of Tissues and Organs. Front Bioeng Biotechnol 2017;5:23. doi: 10.3389/fbioe.2017.00023.
31. Derakhshanfar S, Mbeleck R, Xu K, Zhang X, Zhong W, Xing M. 3D bioprinting for biomedical devices and tissue engineering: A review of recent trends and advances. Bioact Mater 2018;3(2):144-156. doi: 10.1016/J.BIOACTMAT.201 7.11.008.
32. Ozbolat IT. Scaffold-Based or Scaffold-Free Bioprinting: Competing or Complementing Approaches? J Nanotechnol Eng Med 2015;6(2):024701. doi: 10.1115/1.4030414.
33. Gilpin A, Yang Y. Decellularization Strategies for Regenerative Medicine: From Processing Techniques to Applications. Biomed Res Int 2017;2017:1-13. doi: 10.1155/2017/9831534.
34. Li J, Chen M, Fan X, Zhou H. Recent advances in bioprinting techniques: approaches, applications and future prospects. J Transl Med 2016;14:271. doi: 10.1186/s12967-016-1028-0.
35. Leberfinger AN, Ravnic DJ, Dhawan A, Ozbolat IT. Concise Review: Bioprinting of Stem Cells for Transplantable Tissue Fabrication. Stem Cells Transl Med 2017;6(10):1940-1948. doi: 10.1002/sctm.17-0148.
36. Polo-Corrales L, Latorre-Esteves M, Ramirez-Vick JE. Scaffold design for bone regeneration. J Nanosci Nanotechnol 2014;14(1):15-56.
37. Mironov V, Prestwich G, Forgacs G. Bioprinting living structures. J Mater Chem 2007;17(20):2054. doi: 10.1039/b617903g.
38. Nguyen DG, Funk J, Robbins JB, *et al*. Bioprinted 3D Primary Liver Tissues Allow Assessment of Organ-Level Response to Clinical Drug Induced Toxicity In Vitro. PLoS One. 2016;11(7):e0158674. doi: 10.1371/journal.pone.0158674.
39. Choudhury D, Tun HW, Wang T, Naing MW. Organ-Derived Decellularized Extracellular Matrix: A Game Changer for Bioink Manufacturing? Trends Biotechnol 2018;36(8):787-805. doi: 10.1016/j.tibtech.2018.03.003.
40. Duarte Campos DF, Blaeser A, Weber M, *et al*. Three-dimensional printing of stem cell-laden hydrogels submerged in a hydrophobic high-density fluid. Biofabrication 2012;5(1):015003. doi: 10.1088/1758-5082/5/1/015003.
41. Duarte Campos DF, Blaeser A, Korsten A, *et al*. The stiffness and structure of three-dimensional printed hydrogels direct the differentiation of mesenchymal stromal cells toward adipogenic and osteogenic lineages. Tissue Eng Part A 2015;21(3-4):740-756. doi: 10.1089/ten.TEA.2014.0231.
42. Wüst S, Godla ME, Müller R, Hofmann S. Tunable hydrogel composite with two-step processing in combination with innovative hardware upgrade for cell-based three-dimensional bioprinting. Acta Biomater 2014;10(2):630-640. doi: 10.1016/j.actbio.2013.10.016.
43. Park JY, Choi J-C, Shim J-H, *et al*. A comparative study on collagen type I and hyaluronic acid dependent cell behavior for osteochondral tissue bioprinting. Biofabrication 2014;6(3):035004. doi: 10.1088/1758-5082/6/3/035004.
44. Skardal A, Mack D, Kapetanovic E, *et al*. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. Stem Cells Transl Med 2012;1(11):792-802. doi: 10.5966 /sctm.2012-0088.
45. Schiele NR, Chrisey DB, Corr DT. Gelatin-based laser direct-write technique for the precise spatial patterning of cells. Tissue Eng Part C Met 2011;17(3):289-298. doi: 10.1089/ten.TEC.2010.0442.
46. Fan R, Piou M, Darling E, Cormier D, Sun J, Wan J. Bio-printing cell-laden Matrigel–agarose constructs. J Biomater Appl 2016;31(5):684-692. doi: 10.1177/0885328216669238.
47. Gurkan UA, El Assal R, Yildiz SE, *et al*. Engineering Anisotropic Biomimetic Fibrocartilage Microenvironment by Bioprinting Mesenchymal Stem Cells in Nanoliter Gel Droplets. Mol Pharm 2014;11(7):2151-2159. doi: 10.1021/mp400573g.
48. Gao G, Yonezawa T, Hubbell K, Dai G, Cui X. Inkjet-bioprinted acrylated peptides and PEG hydrogel with human mesenchymal stem cells promote robust bone and cartilage formation with minimal printhead clogging. Biotechnol J 2015;10(10):1568-1577. doi: 10.1002/biot.201400635.
49. Grayson WL, Fröhlich M, Yeager K, *et al*. Engineering anatomically shaped human bone grafts. Proc Natl Acad Sci U S A 2010;107(8):3299-3304. doi: 10.1073/pnas.0905439106.
50. Alan H, Unlu G. Graft materials used in oral and maxillofacial surgery. J Int Dent Med Res 2011;4(3):150-154.
51. Adepu S, Dhiman N, Laha A, Sharma CS, Ramakrishna S, Khandelwal M. Three-dimensional bioprinting for bone tissue regeneration. Curr Opin Biomed Eng 2017;2:22-28. doi: 10.1016/j.cobme.2017.03.005.
52. Rosser J, Thomas DJ. Bioreactor processes for maturation of 3D bioprinted tissue. 3D Bioprinting Reconstr Surg January 2018:191-215. doi: 10.1016/B978-0-08-101103-4.00010-7.
53. Kang H-W, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. Nat Biotechnol 2016;34(3):312-319. doi: 10.1038/nbt.3413.
54. Herberg S, Kondrikova G, Periyasamy-Thandavan S, *et al*. Inkjet-based biopatterning of SDF-1β augments BMP-2-induced repair of critical size calvarial bone defects in mice. Bone 2014;67:95-103. doi: 10.1016/j.bone.2014.07.007.
55. Das S, Pati F, Choi Y-J, *et al*. Bioprintable, cell-laden silk fibroin–gelatin hydrogel supporting multilineage differentiation of stem cells for fabrication of three-dimensional tissue constructs. Acta Biomater 2015;11:233-246. doi: 10.1016/j.actbio.2014.09.023.
56. Raja N, Yun H. A simultaneous 3D printing process for the fabrication of bioceramic and cell-laden hydrogel core/shell scaffolds with potential application in bone tissue regeneration. J Mater Chem B 2016;4(27):4707-4716. doi: 10.1039/C6T B00849F.
57. Loozen LD, Wegman F, Öner FC, Dhert WJA, Alblas J. Porous bioprinted constructs in BMP-2 non-viral gene therapy for bone tissue engineering. J Mater Chem B 2013;1(48):6619. doi: 10.1039/c3tb21093f.
58. Norotte C, Marga FS, Niklason LE, Forgacs G. Scaffold-free vascular tissue engineering using bioprinting. Biomaterials 2009;30(30):5910-5917. doi: 10.1016/j.biomaterials.2009.06.034.

59. Evinger AJ, Jeyakumar JM, Hook LA, Choo Y, Shepherd BR, Presnell SC. Osteogenic Differentiation of Mesenchymal Stem/Stromal Cells Within 3D Bioprinted Neotissues. FASEB J 2013;27(1_supplement):193.2-193.2. doi: 10.1096/faseb j.27.1_supplement.193.2.
60. Orciani M, Fini M, Di Primio R, Mattioli-Belmonte M. Biofabrication and Bone Tissue Regeneration: Cell Source, Approaches, and Challenges. Front Bioeng Biotechnol 2017;5:17. doi: 10.3389/fbioe.2017.00017.
61. Stegen S, van Gastel N, Carmeliet G. Bringing new life to damaged bone: the importance of angiogenesis in bone repair and regeneration. Bone 2015;70:19-27. doi: 10.1016/j.bon e.2014.09.017.
62. Tang D, Tare RS, Yang L-Y, Williams DF, Ou K-L, Oreffo ROC. Biofabrication of bone tissue: approaches, challenges and translation for bone regeneration. Biomaterials 2016;83:363-382. doi: 10.1016/j.biomaterials.2016.01.024.
63. O'Brien FJ. Biomaterials & scaffolds for tissue engineering. Mater Today 2011;14(3):88-95. doi: 10.1016/S13 69-7021(11)70058-X.
64. Lakatos É, Magyar L, Bojtár I. Material Properties of the Mandibular Trabecular Bone. J Med Eng 2014;2014:470539. doi: 10.1155/2014/470539.
65. Odin G, Savoldelli C, Bouchard P-O, Tillier Y. Determination of Young's modulus of mandibular bone using inverse analysis. doi: 10.1016/j.medengphy.2010.03.009>.
66. Tevlin R, McArdle A, Atashroo D, *et al*. Biomaterials for craniofacial bone engineering. J Dent Res 2014;93(12):1187-1195. doi: 10.1177/0022034514547271.
67. Motamedian SR, Hosseinpour S, Ahsaie MG, Khojasteh A. Smart scaffolds in bone tissue engineering: A systematic review of literature. World J Stem Cells 2015;7(3):657-668. doi: 10.4252/wjsc.v7.i3.657.
68. Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. Trends Biotechnol 2012;30(10):546-554. doi: 10.1016/j.tibtech.2012.07.005.
69. Turnbull G, Clarke J, Picard F, *et al*. 3D bioactive composite scaffolds for bone tissue engineering. Bioact Mater 2018;3(3):278-314. doi: 10.1016/J.BIOACTMAT.2017.10.001.
70. Hung BP, Naved BA, Nyberg EL, *et al*. Three-Dimensional Printing of Bone Extracellular Matrix for Craniofacial Regeneration. ACS Biomater Sci Eng 2016;2(10):1806-1816. doi: 10.1021/acsbiomaterials.6b00101.
71. Kuss MA, Harms R, Wu S, *et al*. Short-term hypoxic preconditioning promotes prevascularization in 3D bioprinted bone constructs with stromal vascular fraction derived cells. RSC Adv 2017;7(47):29312-29320. doi: 10.1039/c7ra04372d.
72. Khadka A, Hu J. Autogenous grafts for condylar reconstruction in treatment of TMJ ankylosis: current concepts and considerations for the future. Int J Oral Maxillofac Surg 2012;41(1):94-102. doi: 10.1016/j.ijom.2011.10.018.
73. Schek R, Taboas J, Hollister S, Krebsbach P. Tissue engineering osteochondral implants for temporomandibular joint repair. Orthod Craniofacial Res 2005;8(4):313-319. doi: 10.1111/j.1601-6343.2005.00354.x.
74. Needleman I, Worthington H V, Giedrys-Leeper E, Tucker R. Guided tissue regeneration for periodontal infra-bony defects. Cochrane Database Syst Rev April 2006. doi: 10.1002/1465 1858.CD001724.pub2.
75. Obregon F, Vaquette C, Ivanovski S, Hutmacher DW, Bertassoni LE. Three-Dimensional Bioprinting for Regenerative Dentistry and Craniofacial Tissue Engineering. J Dent Res 2015;94(9_suppl):143S-152S. doi: 10.1177/0022 034515588885.
76. Carlo Reis EC, Borges APB, Araújo MVF, Mendes VC, Guan L, Davies JE. Periodontal regeneration using a bilayered PLGA/calcium phosphate construct. Biomater 2011;32(35):92 44-9253. doi: 10.1016/j.biomateria ls.2011.08.040.
77. Park CH, Kim K-H, Lee Y-M, Seol Y-J. Advanced Engineering Strategies for Periodontal Complex Regeneration. Mater (Basel, Switzerland) 2016;9(1). doi: 10.3390/ma9010057.
78. Lee CH, Hajibandeh J, Suzuki T, Fan A, Shang P, Mao JJ. Three-dimensional printed multiphase scaffolds for regeneration of periodontium complex. Tissue Eng Part A 2014;20(7-8):1342-1351. doi: 10.1089/ten.TEA.2013.0386.
79. Nakao K, Morita R, Saji Y, *et al*. The development of a bioengineered organ germ method. Nat Met 2007;4(3):227-230. doi: 10.1038/nmeth1012.
80. Ikeda E, Morita R, Nakao K, *et al*. Fully functional bioengineered tooth replacement as an organ replacement therapy. Proc Natl Acad Sci U S A 2009;106(32):13475-13480. doi: 10.1073/pnas.0902944106.
81. Athirasala A, Lins F, Tahayeri A, *et al*. A Novel Strategy to Engineer Pre-Vascularized Full-Length Dental Pulp-like Tissue Constructs. Sci Rep 2017;7(1):3323. doi: 10.1038/s41598-017-02532-3.
82. Ng WL, Wang S, Yeong WY, Naing MW. Skin Bioprinting: Impending Reality or Fantasy? Trends Biotechnol 2016;34(9):689-699. doi: 10.1016/j.tibtech.2016.04.006.
83. He P, Zhao J, Zhang J, *et al*. Bioprinting of skin constructs for wound healing. Burn trauma 2018;6:5. doi: 10.1186/s41038-017-0104-x.
84. Koch L, Deiwick A, Schlie S, *et al*. Skin tissue generation by laser cell printing. Biotechnol Bioeng 2012;109(7):1855-1863. doi: 10.1002/bit.24455.
85. Michael S, Sorg H, Peck C-T, *et al*. Tissue Engineered Skin Substitutes Created by Laser-Assisted Bioprinting Form Skin-Like Structures in the Dorsal Skin Fold Chamber in Mice. Slominski AT, ed. PLoS One 2013;8(3):e57741. doi: 10.137 1/journal.pone.0057741.
86. Gao B, Yang Q, Zhao X, Jin G, Ma Y, Xu F. 4D Bioprinting for Biomedical Applications. Trends Biotechnol 2016;34(9):746-756. doi: 10.1016/j.tibtech.2016.03.004.
87. Li Y-C, Zhang YS, Akpek A, Shin SR, Khademhosseini A. 4D bioprinting: the next-generation technology for biofabrication enabled by stimuli-responsive materials. Biofabrication 2016;9(1):012001. doi: 10.1088/1758-5090/9/1/012001.