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A review on 3D printing for cardiovascular regeneration: A focus on bioprinted cardiac valves

Devangini Sharma
Department of Mechatronics Engineering
Ganpat University- U V Patel College of Engineering

Mrunali Patel
Assistant Professor, Department of Biotechnology
Ganpat University- U V Patel college of Engineering

Jignesh Mevada
Professor, Department of Mechatronics Engineering
Ganpat University - U V Patel College of Engineering

Abstract

The emergence of 3D bioprinting has marked a pivotal advancement in regenerative medicine, particularly within the scope of cardiovascular repair. By enabling the creation of patient-specific valves that are anatomically and functionally tailored, 3D printing offers promising solutions to enhance treatment efficacy and long-term patient care. Furthermore, bioprinted valves hold the potential for regenerative capabilities, advancing the field of cardiovascular tissue engineering. Understanding these advancements is crucial for shaping future research directions and clinical applications aimed at improving cardiovascular health outcomes. This review meticulously examines the current landscape and prospective developments in 3D bioprinting applications for cardiovascular regeneration, with a focused analysis on bioprinted cardiac valves.

Bioprinting, a method for constructing complex tissue architectures through a layer-by-layer deposition process, offers unparalleled precision and customization in tissue engineering. This paper begins by elucidating the principles of bioprinting, its necessity in modern medicine, and the various bioprinter technologies, including inkjet, extrusion, and laser-assisted bioprinters. Each type presents distinct advantages that render them suitable for different cardiovascular tissue engineering applications. Hydrogels used as bioinks in bioprinting which play a crucial role in forming biocompatible and functional tissue structures. This review explores the different hydrogel types, emphasizing their properties and appropriateness for cardiac tissue engineering.

The core focus is the application of 3D bioprinting in fabricating cardiac valves. Bioprinted cardiac valves represent a significant advancement over traditional valve replacement therapies, offering tailored solutions that potentially mitigate the risks of rejection and reduce the dependence on lifelong medication. The paper also examines the potential of 3D bioprinting in creating cardiovascular assist devices (CVADs), which highlights its capabilities in addressing severe cardiovascular conditions and ponders upon the recent progress in the bioprinting of cardiac valves, discussing key studies and their implications for clinical application. It further identifies the current challenges and outlines future research directions necessary to overcome

Corresponding author. Tel.: 7567276276
E-mail address:jignesh.mevada@ganpatuniversity.ac.in

these obstacles and enhance the efficacy of 3D bioprinting in treating cardiovascular diseases.

In summary, 3D bioprinting presents transformative potential in cardiovascular medicine. By enabling the fabrication of patient-specific cardiac tissues and devices, it opens avenues for more effective and personalized therapeutic strategies for cardiovascular diseases. Ongoing advancements are anticipated to significantly improve the management and outcomes of cardiovascular conditions.

Keywords: 3D printing, regenerative medicine, extrusion based bioprinting, hydrogel, cardiovascular disease, cardiac valves, scaffold.

1. INTRODUCTION:

According to Srinath *et al.* (2005), cardiovascular diseases (CVDs) have become the leading cause of mortality in India since the start of the 21st century. As studied by (Joshi *et al.,* 2007) and (Xavier et al., 2008), cardiovascular disease (CVD) tends to appear about ten years earlier in Indians compared to individuals of European descent, predominantly affecting them during their most productive midlife years. Unfortunately, heart valves do not regenerate, and dysfunctional heart valves must be restored artificially by Jordan et al. (2012). While valves can sometimes undergo surgical repair when damage or disease is not severe, biological and mechanical prosthetic valves are the sole options for valve replacement. However, these valves are constrained by issues of thrombogenicity and calcification, with a specific concern for non-remodeling in pediatric patients as studied by (Basude *et al.,* 2012) and (Simonescu *et al.,*2012)]. Thus, a primary objective will be to prioritize the exploration of alternative tissue treatments that maintain natural tissue integrity while avoiding harmful side effects.

As stated by [(Murphy *et al.*, 2014) and (Adhikari *et al.*,2021)], recent advancements in three-dimensional (3D) printing and bioprinting show significant promise in the regeneration of organs, scaffolds, and blood vessels. These methods hold potential for partially or completely replacing natural organs within the human body.

Bioprinting was first introduced by (Klebe, 1988), and was initially termed as cytoscribing technology, to micro-position live cells and create 2D synthetic tissues.

As stated by (Ozbolat, 2016), bioprinting is a computer-assisted manufacturing process that enables the simultaneous deposition of live cells and biological materials in layered arrangements. This technology is utilized primarily in the fabrication of biologically engineered structures, particularly in applications such as soft-tissue engineering, scaffold fabrication, tissue regeneration, and similar fields.

According to (Norotte *et al.*, 2009), three-dimensional bioprinting is anticipated to be a highly promising method in tissue engineering due to its precise control over the geometry and quantity of biomaterials during the fabrication process. As studied by (Chimene *et al.*, 2020), this technique specifically enables cells to fully integrate into hydrogels, effectively replicating the microenvironment of the extracellular matrix (ECM). Additionally, it enables direct printing onto the targeted host location. As studied by (Mironov, 2003), the main concerns for a successfully bioprinted organ or tissue is cell survival, cell proliferation, cell differentiation, structural stability and structural stiffness, etc.

The primary types of 3D bioprinting methods include microextrusion, inkjet, pressure-assisted, laser-assisted, ITOP, and robotic bioprinting. This review paper focuses on Extrusion based bioprinting.

1.1 Extrusion-based Bioprinting:

As studied by (Roche *et al.*, 2020), (Li *et al.*, 2016), (Gudapati *et al.*, 2016) and (Hinton *et al.*, 2015)**,** **e**xtrusion-based bioprinting, which includes both semi-solid extrusion (SSE) and fused deposition modeling (FDM), extrudes biomaterials through a nozzle to create continuous filaments at room

temperature. This method is advantageous for creating complex structures with homogeneous cell distribution and precise 3D patterning, utilizing pneumatic motion, a plunger, or screw mechanisms to generate extrusion pressure.

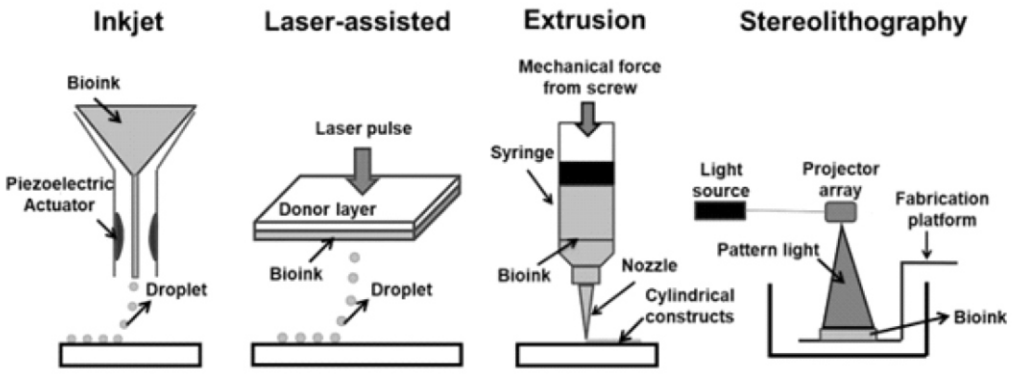


Figure 1: Types of 3D bioprinting technologies

According to (Yanagava *et al.,* 2016), in most instances, the critical determinant is the selection of suitable materials for each specific methodology. The printability is influenced not only by the physicochemical properties of pre-gel solutions (e.g. viscosity) but also by the gelation process.

Table 1: Comparison of bio-printing methods

BIO-PRINTING METHODS	CHARACTERISTICS						
	VISCOSITY	CELL DENSITY	VIABLE CELLS (%)	RESOLUTION	PRINT SPEED	NOZZLE SIZE	REFERENCES
MICRO-EXTRUSION	6–30 × 10^7 mPa/s	High	40–80%	100 µm - millimeter	00 µm/s	20 µm-mm	[7, 19, 20, 21, 23, 24, 25, 26, 27]
INKJET	3.5 –12 mPa/s	Low < 10^6 cells/mL	85%	75 µm	–10000 drops/s	20–150 µm	[7, 19, 20, 23, 24, 26, 28, 29, 30]
ITOP	N/A	High	>90%	2–50 µm	N/A	50 µm	[32]
ROBOTIC BIOPRINTING	N/A	High	>90%	N/A	N/A	N/A	[33]

1.2 Requirements for Extrusion based Bioprinting:

According to Murphy *et al.* (2014), bioink in 3D bioprinting is a specialized material consisting of living cells and various biological substances that can be extruded through a 3D bioprinter. As studied by Groll *et al.* (2018), bioinks are generally characterized as formulations of cells that are compatible with automated biofabrication technologies, and may also incorporate biologically active components and biomaterials. According to Groll *et al.* (2016), bioinks must demonstrate essential qualities such as biocompatibility, appropriate viscoelasticity, robust mechanical integrity, suitable degradability, non-toxicity, and non-immunogenicity to be considered optimal. Furthermore, bioinks are engineered

to replicate the natural extracellular matrix (ECM), thus providing an optimal environment for cellular growth, proliferation, and differentiation. As studied by Ozbolat (2015), bioinks are composed of multiple components, including hydrogels, growth factors, and biomaterials, which collectively support the creation of intricate tissue structures and organs. As stated by (Nguyen *et al.,*2017), these components are pivotal in advancing applications in regenerative medicine, tissue engineering, and biomedical research.

2. TYPES OF BIOINKS

2.1 Natural Bioinks

According to (Skardal *et al.*, 2015), natural bioinks are derived from biological sources and are commonly used in bioprinting due to their biocompatibility and ability to promote cell attachment and proliferation.

As stated by (Hospodiuk *et al.*, 2017), these bioinks are typically composed of materials such as agarose, cellulose, collagen, gelatin, alginate, hyaluronic acid, etc. which are naturally occurring in the extracellular matrix of tissues. According to (Malda *et al.*, 2013), the advantage of natural bioinks lies in their ability to closely mimic the native cellular environment, facilitating cell differentiation and tissue regeneration.

However, their mechanical properties and printability can sometimes be less optimal compared to synthetic bioinks, necessitating modifications or the addition of other materials to enhance their functionality (Gu *et al.*, 2020).

Table 2: Types of natural bioinks and their characteristics.

Bioink	Type	Advantages	Disadvantages	Printing Methods	Tissues and Organs
Agarose	Linear polysaccharide derived from seaweed	Fast gelation; readily available at low cost [47, 48]	Poor cell adhesion and viability [49, 50]	Extrusion [51]	[52]
Alginate	Linear copolymer polysaccharide derived from brown algae [47]	Structural diffusivity [47], bioinert [53]	Requires modification for improved cell interaction [47]	Extrusion, inkjet, or laser-assisted [54]	Bone [55], cartilage [56]
Cellulose	Linear polysaccharide found in plants [57, 58]	Environmental response; easily processable [57, 58]	Typically blended because of poor intrinsic properties [57]	Extrusion [58]	Skin [59]
Collagen	Protein found in native ECM [60, 61]	Good cell adhesion [60, 61]	Lengthy processing time because of cross-linking requirement [62, 63]	Extrusion, inkjet [60, 61]	Skin [64], bone [65]
Decellularized ECM (dECM)	Varied structures derived from native ECM [75, 76]	Extremely high cell adhesion; excellent biocompatibility [75,76]	Poor mechanical properties [75, 77]	Extrusion [78]	Heart [78], liver [79]
Fibrin	Protein formed by enzymatic reaction between thrombin and fibrinogen [80]	Biodegradable [80]	Weak mechanical properties [80]	Extrusion, inkjet [81]	Blood vessels [82], skin [83]

Hyaluronic Acid	Glycosaminoglycan found in native ECM [84]	Very good cell adhesion [84]	Poor mechanical stability [85]	Extrusion [86]	Cartilage [87]
Silk	Protein derived from silkworms and spiders [88]	Exceptional cell permeability; extremely high tensile strength [88]	Lower cell viability [88]	Extrusion [89]	Bone [90]

2.2 Synthetic Bioinks

As stated by (Hospodiuk *et al.*, 2017), synthetic bioinks are engineered from artificial polymers and offer greater control over their chemical and mechanical properties compared to natural bioinks. As stated by (Malda *et al.*, 2013), common materials used in synthetic bioinks include polyethylene glycol (PEG), polycaprolactone (PCL), and polylactic acid (PLA). These bioinks provide consistent and reproducible properties, making them suitable for creating scaffolds with precise architectures and mechanical strength, as studied by (Skardal *et al.*, 2015). Additionally, synthetic bioinks can be tailored to degrade at specific rates, which is advantageous for applications requiring controlled tissue regeneration as stated by (Chimene *et al.*, 2016). Despite their advantages, synthetic bioinks may lack the inherent biological cues present in natural bioinks, potentially requiring the incorporation of bioactive molecules to enhance their biocompatibility and functionality as understood by (Ouyang *et al.*, 2016).

Table 3: Types of synthetic bioinks and their characteristics.

Bioink	**Type**	**Advantages**	**Disadvantages**	**Printing Methods**	**Tissues and Organs**
PEG (Polyethylene glycol)	Synthetic linear or multi-armed polymer [91]	Highly tunable mechanical properties [91]	Bioinert [91]	Inkjet, stereolithography [98]	Bone [91], cartilage [98]
PEGDA (Polyethylene Glycol Diacrylate)	Synthetic hydrogel [91], [99]	Highly tunable mechanical properties [91, 99]	Bioinert, requiring modification for cell interaction [91]	Inkjet, stereolithography [98]	Bone [91] cartilage [98]
Pluronic F127	Synthetic triblock copolymer [100]	Biocompatible; water soluble [100]	Unstable once printed [100]	Extrusion [101]	N/A
PVA (Polyvinyl Alcohol)	Synthetic hydrogel [102, 103]	Biocompatible; good mechanical properties [102, 103]	Limited cell adhesion [102]	Extrusion, stereolithography [103]	Cartilage, cardiac tissue [104]

2.3 Hybrid bioinks

As studied by (Gu *et al.*, 2020), hybrid bioinks combine the beneficial properties of both natural and synthetic bioinks, aiming to create a balanced material that offers biocompatibility, mechanical strength, and printability. By blending natural and synthetic components, hybrid bioinks can achieve the biological functionality of natural materials while maintaining the structural integrity provided by synthetic polymers [96]. This synergy allows for the creation of complex tissue structures with enhanced cell viability and mechanical properties [93]. Hybrid bioinks are particularly useful in

applications where both the biological environment and the mechanical demands are critical, such as in the engineering of bone and cartilage tissues [94].

Table 4: Types of hybrid bioinks and their characteristics.

Bioink	Advantages	Disadvantages	Printing Methods	Tissues and Organs	References
Alginate-Gelatin	Improved cell adhesion; good mechanical properties	Rapid degradation of gelatin; requires cross-linking	Extrusion	Cartilage	[105, 106, 107]
Alginate-PEG	Tunable mechanical properties; improved biocompatibility	Bioinert; needs modification for better cell interaction	Extrusion, inkjet	Bone	[108, 109]
Chitosan-PEG	Biocompatible; good mechanical properties	Requires cross-linking; potential for immunogenicity	Extrusion, inkjet	Skin	[110]
Gelatin-Methacryloyl (GelMA)	Excellent cell adhesion; tunable mechanical properties	Requires UV cross-linking; potential cytotoxicity	Extrusion, stereolithography	Cartilage, skin	[111]
Collagen-PEGDA	Enhanced mechanical properties; good cell adhesion	Requires UV cross-linking; potential cytotoxicity	Extrusion, stereolithography	Cardiac tissues	[112, 113]
Hyaluronic Acid-PEG	Excellent cell adhesion; tunable properties	Requires modification for stability; potential for rapid degradation	Extrusion, stereolithography	Cartilage	[114]
Fibrin-PEG	Biodegradable; good mechanical properties	Potential immunogenicity, requires cross-linking	Extrusion, inkjet	Cardiac tissues	[113, 115]
Silk-PEG	High tensile strength; good cell adhesion	Lower cell viability; requires cross-linking	Extrusion	Bone	[116]
Collagen-GelMA	Enhanced mechanical properties; good cell adhesion	Requires UV cross-linking; potential cytotoxicity	Extrusion, stereolithography	Cardiac tissues, skin	[117]

3. Bioprinting Cardiac Valves:

The human heart consists of four valves: the aortic, pulmonary, tricuspid, and mitral valves. The aortic valve is frequently implicated in valvular heart diseases, notably aortic stenosis and aortic regurgitation. As a result, tissue engineering efforts in cardiac valve regeneration predominantly concentrate on the aortic valve. This paper predominantly focuses on the bioprinting of Aortic Valve.

As studied by (Butcher *et al.*, 2011), the aortic valve's functional unit encompasses the aortic root and cusps. As stated by (Otto *et al.*, 1994) and (Rabkin *et al.*, 2001), the aortic root is located at the ascending aorta, initiating at the point of cusp attachment. Structurally, the aortic root wall comprises three layers: the inner intima, the middle media, and the outer adventitia. It contains blood vessels and is inhabited by endothelial cells, smooth muscle cells, and fibroblasts. The annulus, a parabola-shaped junction on the aorta muscle wall, connects the valve to the cusps, while the commissure refers to the junction between two adjacent leaflets. The sinus refers to the aortic muscle wall above the annulus. The annulus and commissure provide support for the cusps, which are suspended in the air. The cusps themselves are very thin, less than 1 mm in thickness, but are relatively thicker near the annulus and become progressively thinner toward the commissure. As stated by (Hara *et al.*, 2007) and (Hinton *et al.*, 2011), each cusp is composed of three stratified layers of connective fibers: fibrosa, spongiosa, and ventricularis. As understood by (Kershaw *et al.*, 2004) and (Sacks *et al.,* 2007), the fibrosa layer comprises circumferentially oriented collagen fibers (types I and III) providing mechanical strength to the cusps and bearing the entire workload. This layer also has minimal elastin and glycosaminoglycans. In the middle spongiosa layer, randomly oriented proteoglycan fibers are interspersed with collagen fibers, forming a cushion between the fibrosa and ventricularis layers, thereby offering compressibility and structural integrity to the valve. The ventricularis layer contains radially oriented elastic fibers, which facilitate the coiling and extension of the valve during systole and diastole, respectively. As studied by (Misfeld *et al.*, 2007), the orientation of cells and proteins within the cusp is crucial for its functionality. As understood by (Combs *et al.*, 2009), valvular interstitial cells are located within the cusps, while the outer layers consist of a monolayer of valvular endothelial cells. Although the aorta has a vascular system, the leaflets themselves are avascular until they sustain damage or become defective.

3.1 Advancements and applications of bioprinting in cardiac valve engineering: A case study analysis

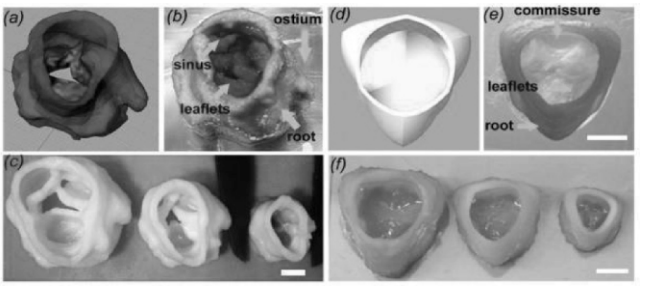


Figure 1: Printing Heterogeneous Valve and Scaled Valve Constructs. (A) Porcine aortic valve model, which was (B) printed with the root formed using 700 MW PEG-DA hydrogel, and the leaflets formed with 700/8000 MW PEG-DA hydrogels, retaining key features such as the coronary ostium and sinuses. (C) Scaffolds were printed with 700 MW PEG-DA at various scales for fidelity analysis, with inner diameters (ID) of 22, 17, and 12 mm. (D) Axisymmetric valve model, which was (E) printed using two blends of hydrogels (F) at 22-, 17-, and 12-mm ID. Scale bar = 1 cm. [(Hockaday et al., 2016)]

In a pivotal study conducted by (Hockaday *et al.*, 2016), they employed a 3D extrusion-based bioprinter (Fab@Home Model 1) to fabricate an aortic valve structure using PEGDA hydrogel supplemented with alginate. The team utilized an STL file derived from a micro-CT scan image of a porcine aortic valve, encompassing the sinus and cusps, to ensure anatomical precision. As studied by (Hockaday *et al.*, 2016), the addition of alginate to PEGDA enhanced the extrusion viscosity of the UV cross-linkable hydrogel. In this study conducted by (Hockaday *et al.*, 2016), sodium chloride was incorporated to maintain the pH of the hydrogel, aligning it with phosphate buffer solution, cross-linking was facilitated by a precursor (Igracure), and the mechanical properties of the hydrogel were

modulated based on the molecular weight of the components, crosslinker concentration, and crosslinking duration. This study successfully demonstrated the capacity to print valves with varied mechanical properties, with inner diameters ranging from 12 to 22 mm. As observed by (Hockaday *et al.*, 2016), larger structures exhibited superior shape fidelity, with resolution contingent upon extrusion rate and needle diameter. Furthermore, valvular interstitial cells sourced from porcine aortic valves were seeded onto the printed scaffolds, which maintained high viability (~100%) for over 21 days.

Building on the previous work, (Duan *et al.*, 2013) utilized the Fab@Home Model 1 printer to fabricate an aortic heart valve using alginate-gelatin hydrogels. An STL file from a micro-CT scan image of a porcine aortic valve was employed. The hydrogel mixture comprised alginate and gelatin, prepared in sodium chloride solution or phosphate-buffered saline to ensure cellular viability. Porcine aortic valve interstitial cells and smooth muscle cells were separately encapsulated within the hydrogel mix. In this study conducted by (Duan *et al.*, 2013), the printing parameters were set with a print-head speed of 5 mm/s, strand width of 800 μm, and strand height of 700 μm. Post-printing, the hydrogel structure was incubated in calcium chloride, facilitating cross-linking, though the mechanical properties diminished over time due to calcium ion diffusion. As observed by (Duan *et al.*, 2013), the stiffness of the cell-laden hydrogel remained stable after 7 days, with cell survival approximating 82%.

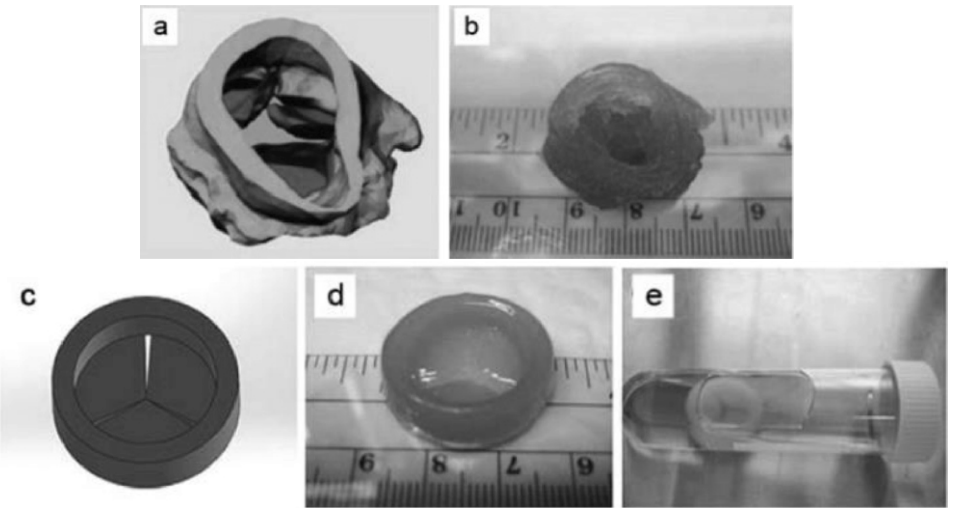


Figure 2: Bioprinting of Aortic Valve. (A) Micro-CT scanned image model of a porcine aortic valve, with green indicating the valve root and red indicating the valve leaflets. (B) Printed version of the scanned image using an alginate-gelatin hydrogel. (C) Designed heart valve conduit model. (D) Bioprinted heart valve conduit. (E) The valve conduit after 7 days of culture. [(Duan *et al.*, 2013) and (Duan *et al.*, 2014)]

4. Conclusion

3D bioprinting is revolutionizing cardiovascular medicine, particularly in the fabrication of patient-specific cardiac valves and assistive devices. This technology offers promising solutions to enhance the precision and personalization of treatments, reducing risks of rejection and lifelong medication dependency. By leveraging advanced bioprinter technologies and biocompatible bioinks, 3D bioprinting holds the potential to transform cardiovascular repair and regeneration. While challenges remain, ongoing research and development are expected to significantly improve therapeutic strategies and clinical outcomes in cardiovascular care.

References

[1] Srinath Reddy K, Shah B, Varghese C, Ramadoss A. Responding to the threat of chronic diseases in India. Lancet. 2005; 366:1744–1749. Doi: 10.1016/S0140-6736(05)67343-6.

[2] Joshi P, Islam S, Pais P, Reddy S, Dorairaj P, Kazmi K, Pandey MR, Haque S, Mendis S, Rangarajan S, Yusuf S. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. JAMA. 2007; 297:286–294. doi: 10.1001/jama.297.3.286.

[3] Xavier D, Pais P, Devereaux PJ, Xie C, Prabhakaran D, Reddy KS, Gupta R, Joshi P, Kerkar P, Thanikachalam S, Haridas KK, Jaison TM, Naik S, Maity AK, Yusuf S; CREATE registry investigators. Treatment and outcomes of acute coronary syndromes in India (CREATE): a prospective analysis of registry data. Lancet. 2008; 371:1435–1442. doi: 10.1016/S0140-6736(08)60623-6.

[4] Jordan, J.E., Williams, J.K., Lee, S.J., Raghavan, D., Atala, A., Yoo, J.J., 2012. Bioengineered self-seeding heart valves. J. Thorac. Cardiovasc. Surg. 143, 201–208.

[5] Basude, S., Hein, C., Curtis, S.L., Clark, A., Trinder, J., 2012. Low-molecular-weight heparin or warfarin for anticoagulation in pregnant women with mechanical heart valves: what are the risks? A retrospective observational study. BJOG 119, 1008–1013.

[6] Simionescu, D.T., Chen, J., Jaeggli, M., Wang, B., Liao, J., 2012. Form follows function: advances in trilayered structure replication for aortic heart valve tissue engineering. J. Healthc. Eng. 3, 179–202.

[7] Murphy SV, Atala A. 3D bioprinting of tissues and organs. Nat Biotechnol. 2014 Aug;32(8):773-85. doi: 10.1038/nbt.2958. PMID: 25093879.

[8] Adhikari, J.; Roy, A.; Das, A.; Ghosh, M.; Thomas, S.; Sinha, A.; Kim, J.; Saha, P. Effects of Processing Parameters of 3D Bioprinting on the Cellular Activity of Bioinks. Macromol. Biosci. 2021, 21, e2000179.

[9] Klebe RJ. Cytoscribing: A method for micropositioning cells and the construction of two- and three-dimensional synthetic tissues. Experimental Cell Research. 1988;179(2):362–373.

[10] Ozbolat IT. 3D Bioprinting: Fundamentals, Principles and Applications, 2016. s.356

[11] Norotte, C.; Marga, F.S.; Niklason, L.E.; Forgacs, G. Scaffold-free vascular tissue engineering using bioprinting. Biomaterials 2009, 30, 5910–5917.

[12] Chimene, D.; Kaunas, R.; Gaharwar, A.K. Hydrogel Bioink Reinforcement for Additive Manufacturing: A Focused Review of Emerging Strategies. Adv. Mater. 2020, 32, 1902026.

[13] Roche CD, Brereton RJL, Ashton AW, Jackson C, Gentile C. Current challenges in three-dimensional bioprinting heart tissues for cardiac surgery. Eur J Cardiothorac Surg. 2020 Sep 1;58(3):500-510. doi: 10.1093/ejcts/ezaa093. PMID: 32391914; PMCID: PMC8456486.

[14] Li J, Chen M, Fan X, Zhou H. Recent advances in bioprinting techniques: approaches, applications and future prospects. J Transl Med. 2016 Sep 20; 14:271. doi: 10.1186/s12967-016-1028-0. PMID: 27645770; PMCID: PMC5028995.

[15] Gudapati H, Dey M, Ozbolat I. A comprehensive review on droplet-based bioprinting: Past, present and future. Biomaterials. 2016 Sep; 102:20-42. doi: 10.1016/j.biomaterials.2016.06.012. Epub 2016 Jun 7. PMID: 27318933.

[16] Hinton TJ, Jallerat Q, Palchesko RN, Park JH, Grodzicki MS, Shue HJ, Ramadan MH, Hudson AR, Feinberg AW. Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. Sci Adv. 2015 Oct 23;1(9): e1500758. doi:

10.1126/sciadv.1500758. PMID: 26601312; PMCID: PMC4646826.

[17] What are the types of 3D bioprinting technologies? – faCellitate

[18] Yanagawa F, Sugiura S, Kanamori T, Hydrogel microfabrication technology toward three-dimensional tissue engineering. Regenerative Therapy. 2016; 3:45–57.

[19] Ma X, Qu X, Zhu W, *et al.*, 2016, Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting. Proceedings of the National Academy of Sciences, vol.113(8):2206–2211.http://dx.doi.org/10.1073/pnas.1524510113

[20] 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 Jul;98(1):160-70. doi: 10.1002/jbm.b.31831. Epub 2011 Apr 18. PMID: 21504055; PMCID: PMC3772543.

[21] Smith CM, Stone AL, Parkhill RL, Stewart RL, Simpkins MW, Kachurin AM, Warren WL, Williams SK. Three-dimensional bioassembly tool for generating viable tissue-engineered constructs. Tissue Eng. 2004 Sep-Oct;10(9-10):1566-76. doi: 10.1089/ten.2004.10.1566. PMID: 15588416.

[22] Groll, J., Boland, T., Blunk, T., Burdick, J. A., Cho, D. W., Dalton, P. D., ... & Mironov, V. A. (2016). Biofabrication: reappraising the definition of an evolving field. *Biofabrication*, 8(1), 013001.

[23] Kim, J.D., Choi, J.S., Kim, B.S., Choi, Y.C., & Cho, Y.W. (2010). Piezoelectric inkjet printing of polymers: Stem cell patterning on polymer substrates. *Polymer, 51*, 2147-2154.

[24] Duan B. State-of-the-Art Review of 3D Bioprinting for Cardiovascular Tissue Engineering. Ann Biomed Eng. 2017 Jan;45(1):195-209. doi: 10.1007/s10439-016-1607-5. Epub 2016 Apr 11. PMID: 27066785.

[25] Dababneh, A., & Ozbolat, I.T. (2014). Bioprinting Technology: A Current State-of-the-Art Review. *Journal of Manufacturing Science and Engineering-transactions of The Asme, 136*, 061016.

[26] Wüst S, Müller R, Hofmann S. Controlled Positioning of Cells in Biomaterials-Approaches Towards 3D Tissue Printing. J Funct Biomater. 2011 Aug 4;2(3):119-54. doi: 10.3390/jfb2030119. PMID: 24956301; PMCID: PMC4030943.

[27] Suntornnond, R., An, J., & Chua, C. K. (2017). Roles of support materials in 3D bioprinting - Present and future. International journal of bioprinting, 3(1), 006. https://doi.org/10.18063/IJB.2017.01.006]

[28] Evolution of Bioinks and Additive Manufacturing Technologies for 3D Bioprinting Rod R. Jose, Maria J. Rodriguez, Thomas A. Dixon, Fiorenzo Omenetto, and David L. Kaplan ACS Biomaterials Science & Engineering 2016 *2* (10), 1662-1678 DOI: 10.1021/acsbiomaterials.6b00088

[29] Campbell, P. G., Miller, E. D., Fisher, G. W., Walker, L. M., & Weiss, L. E. (2005). Engineered spatial patterns of FGF-2 immobilized on fibrin direct cell organization. *Biomaterials*, *26*(33), 6762–6770. https://doi.org/10.1016/j.biomaterials.2005.04.032

[30] Duarte Campos, D. F., Blaeser, A., Buellesbach, K., Sen, K. S., Xun, W., Tillmann, W., & Fischer, H. (2016). Bioprinting Organotypic Hydrogels with Improved Mesenchymal Stem Cell Remodeling and Mineralization Properties for Bone Tissue Engineering. *Advanced healthcare materials*, 5 (11), 1336–1345. https://doi.org/10.1002/adhm.201501033

[31] Sobral, J. M., Caridade, S. G., Sousa, R. A., Mano, J. F., & Reis, R. L. (2011). Three-dimensional plotted scaffolds with controlled pore size gradients: Effect of scaffold geometry on mechanical performance and cell seeding efficiency. *Acta biomaterialia*, *7*(3), 1009–1018. https://doi.org/10.1016/j.actbio.2010.11.003

[32] Kang, H. W., Lee, S. J., Ko, I. K., Kengla, C., Yoo, J. J., & Atala, A. (2016). A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nature biotechnology*, *34*(3), 312–319. https://doi.org/10.1038/nbt.3413

[33] Mironov, V., Visconti, R. P., Kasyanov, V., Forgacs, G., Drake, C. J., & Markwald, R. R. (2009). Organ printing: tissue spheroids as building blocks. *Biomaterials*, *30*(12), 2164–2174. https://doi.org/10.1016/j.biomaterials.2008.12.084

[34] Mironov, V., 2003. Printing technology to produce living tissue. Expert. Opin. Biol. Ther. 3, 701–704.

[35] Butcher, J. T., Mahler, G. J., & Hockaday, L. A. (2011). Aortic valve disease and treatment: the need for naturally engineered solutions. *Advanced drug delivery reviews*, *63*(4-5), 242–268. https://doi.org/10.1016/j.addr.2011.01.008

[36] Otto, C. M., Kuusisto, J., Reichenbach, D. D., Gown, A. M., & O'Brien, K. D. (1994). Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and immunohistochemical studies. *Circulation*, *90*(2), 844–853. https://doi.org/10.1161/01.cir.90.2.844

[37] Rabkin, E., Aikawa, M., Stone, J. R., Fukumoto, Y., Libby, P., & Schoen, F. J. (2001). Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodeling in myxomatous heart valves. *Circulation*, *104*(21), 2525–2532. https://doi.org/10.1161/hc4601.099489

[38] Hara, H., Pedersen, W. R., Ladich, E., Mooney, M., Virmani, R., Nakamura, M., Feldman, T., & Schwartz, R. S. (2007). Percutaneous balloon aortic valvuloplasty revisited: time for a renaissance?. *Circulation*, *115*(12), e334–e338. https://doi.org/10.1161/CIRCULATIONAHA.106.657098

[39] Hinton, R. B., & Yutzey, K. E. (2011). Heart valve structure and function in development and disease. *Annual review of physiology*, *73*, 29–46. https://doi.org/10.1146/annurev-physiol-012110-142145

[40] Kershaw, J. D., Misfeld, M., Sievers, H. H., Yacoub, M. H., & Chester, A. H. (2004). Specific regional and directional contractile responses of aortic cusp tissue. *The Journal of heart valve disease*, *13*(5), 798–803.

[41] Sacks, M. S., & Yoganathan, A. P. (2007). Heart valve function: a biomechanical perspective. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *362*(1484), 1369–1391. https://doi.org/10.1098/rstb.2007.2122

[42] Misfeld, M., & Sievers, H. H. (2007). Heart valve macro- and microstructure. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *362*(1484), 1421–1436. https://doi.org/10.1098/rstb.2007.2125

[43] Combs, M. D., & Yutzey, K. E. (2009). Heart valve development: regulatory networks in development and disease. *Circulation research*, *105*(5), 408–421. https://doi.org/10.1161/CIRCRESAHA.109.201566

[44] Ozbolat, I. T. (2015). Bioprinting scale-up tissue and organ constructs for transplantation. *Trends in Biotechnology*, 33(7), 395-400.

[45] Nguyen, D., Hägg, D. A., Forsman, A., Ekholm, J., Widholm, T., Cartner, S., ... & van Blitterswijk, C. A. (2017). Cartilage tissue engineering by the 3D bioprinting of cells embedded in nanocellulose-based bioinks. *Scientific Reports*, 7(1), 658.

[46] Groll J, Burdick JA, Cho D, Derby B, Gelinsky M, Heilshorn SC, Jüngst T, Malda J, Mironov VA, Nakayama K, Ovsianikov A, Sun W, Takeuchi S, Yoo JJ, Woodfield TBF (2018) A definition of bioinks and their distinction from biomaterial inks. Biofabrication 11:013001. https:// doi.org/10.1088/1758-5090/aaec52

[47] Lee, K. Y., & Mooney, D. J. (2012). Alginate: properties and biomedical applications. *Progress in polymer science*, *37*(1), 106–126. https://doi.org/10.1016/j.progpolymsci.2011.06.003

[48] Hsieh, F. Y., Lin, H. H., & Hsu, S. H. (2015). 3D bioprinting of neural stem cell-laden thermoresponsive biodegradable polyurethane hydrogel and potential in central nervous system repair. *Biomaterials*, *71*, 48–57. https://doi.org/10.1016/j.biomaterials.2015.08.028

[49] Chang, C., Peng, N., Zhang, L. (2010). Aggregation of suspensions of cellulose nanocrystals and their derivatives. *Journal of Polymer Science Part B: Polymer Physics*, 48(22), 2361-2368.

[50] You, Fu & Wu, Xia & Chen, Xiongbiao. (2016). 3D Printing of Porous Alginate/gelatin Hydrogel Scaffolds and Their Mechanical Property Characterization. International Journal of Polymeric Materials and Polymeric Biomaterials. 66. 10.1080/00914037.2016.1201830.

[51] Axpe, E., & Oyen, M. L. (2016). Applications of Alginate-Based Bioinks in 3D Bioprinting. *International journal of molecular sciences*, *17*(12), 1976. https://doi.org/10.3390/ijms17121976

[52] Markstedt, K., Mantas, A., Tournier, I., Martínez Ávila, H., Hägg, D., & Gatenholm, P. (2015). 3D Bioprinting Human Chondrocytes with Nanocellulose-Alginate Bioink for Cartilage Tissue Engineering Applications. *Biomacromolecules*, *16*(5), 1489–1496. https://doi.org/10.1021/acs.biomac.5b00188

[53] Augst, A. D., Kong, H. J., & Mooney, D. J. (2006). Alginate hydrogels as biomaterials. *Macromolecular bioscience*, *6*(8), 623–633. https://doi.org/10.1002/mabi.200600069

[54] Wüst, S., Müller, R., & Hofmann, S. (2011). Controlled Positioning of Cells in Biomaterials-Approaches Towards 3D Tissue Printing. *Journal of functional biomaterials*, *2*(3), 119–154. https://doi.org/10.3390/jfb2030119

[55] Xu, T., Binder, K. W., Albanna, M. Z., Dice, D., Zhao, W., Yoo, J. J., & Atala, A. (2013). Hybrid printing of mechanically and biologically improved constructs for cartilage tissue engineering applications. *Biofabrication*, *5*(1), 015001. https://doi.org/10.1088/1758-5082/5/1/015001

[56] Cui, X., Breitenkamp, K., Finn, M. G., Lotz, M., & D'Lima, D. D. (2012). Direct human cartilage repair using three-dimensional bioprinting technology. *Tissue engineering. Part A*, *18*(11-12), 1304–1312. https://doi.org/10.1089/ten.TEA.2011.0543

[57] Bodin, A., Concaro, S., Brittberg, M., & Gatenholm, P. (2007). Collagen-based tissue engineering materials. *Materials Science and Engineering: C*, 27(1), 37-43.

[58] Bhattacharya, M., Malinen, M. M., Lauren, P., Lou, Y. R., Kuisma, S. W., Kanninen, L., Lille, M., Corlu, A., GuGuen-Guillouzo, C., Ikkala, O., Laukkanen, A., Urtti, A., & Yliperttula, M. (2012). Nanofibrillar cellulose hydrogel promotes three-dimensional liver cell culture. *Journal of controlled release : official journal of the Controlled Release Society*, *164*(3), 291–298. https://doi.org/10.1016/j.jconrel.2012.06.039

[59] Mertaniemi H, Escobedo-Lucea C, Sanz-Garcia A, *et al*. Human stem cell decorated nanocellulose threads for biomedical applications. *Biomaterials*. 2016; 82:208-220. doi: 10.1016/j.biomaterials.2015.12.020

[60] Yuan, T., Zhang, L., Li, K., Fan, H., & Fan, Y. (2004). Evaluation of collagen scaffold crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide for corneal tissue engineering. *Advances in Biomedical Materials*, 4(1), 37-46.

[61] Zhang, W., Feng, C., Yang, G., Li, G., Tang, T., Cui, W., & Li, Y. (2016). 3D-printed scaffolds for osteochondral repair combined with marrow stimulation and sustained release of transfection complexes. *Biofabrication*, 8(4), 045012.

[62] Lee CH, Singla A, Lee Y. Biomedical applications of collagen. *Int J Pharm*. 2001;221(1-2):1-22. doi:10.1016/s0378-5173(01)00691-3

[63] Glowacki J, Mizuno S. Collagen scaffolds for tissue engineering. *Biopolymers*. 2008;89(5):338-344. doi:10.1002/bip.20871

[64] You F, Eames BF, Chen X. Application of Extrusion-Based Hydrogel Bioprinting for Cartilage Tissue Engineering. *Int J Mol Sci*. 2017;18(7):1597. Published 2017 Jul 23. doi:10.3390/ijms18071597

[65] Kim BS, Lee JS, Gao G, Cho DW. Direct 3D cell-printing of human skin with functional transwell system. *Biofabrication*. 2017;9(2):025034. Published 2017 Jun 6. doi:10.1088/1758-5090/aa71c8

[66] Hockaday LA, Kang KH, Colangelo NW, *et al.* Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds. *Biofabrication*. 2012;4(3):035005. doi:10.1088/1758-5082/4/3/035005

[67] Duan B, Hockaday LA, Kang KH, Butcher JT. 3D bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. *J Biomed Mater Res A*. 2013;101(5):1255-1264. doi:10.1002/jbm.a.34420

[68] Duan, B., Kapetanovic, E., Hockaday, L. A., & Butcher, J. T. (2014). Three-dimensional printed trileaflet valve conduits using biological hydrogels and human valve interstitial cells. *Acta biomaterialia*, *10*(5), 1836–1846. https://doi.org/10.1016/j.actbio.2013.12.005

[69] Mizuno T, Takewa Y, Sumikura H, *et al*. Preparation of an autologous heart valve with a stent (stent-biovalve) using the stent eversion method. *J Biomed Mater Res B Appl Biomater*. 2014;102(5):1038-1045. doi:10.1002/jbm.b.33086

[70] Robinson, P.S., Johnson, S.L., Evans, M.C., Barocas, V.H., Tranquillo, R.T., 2008. Functional tissue-engineered valves from cell-remodeled fibrin with commissural alignment of cell-produced collagen. Tissue Eng. A 14, 83–95.

[71] Syedain ZH, Meier LA, Reimer JM, Tranquillo RT. Tubular heart valves from decellularized engineered tissue. *Ann Biomed Eng*. 2013;41(12):2645-2654. doi:10.1007/s10439-013-0872-9

[72] Lueders C, Jastram B, Hetzer R, Schwandt H. Rapid manufacturing techniques for the tissue engineering of human heart valves. *Eur J Cardiothorac Surg*. 2014;46(4):593-601. doi:10.1093/ejcts/ezt510

[73] 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

[74] Kosair Children's Hospital. (2021). 3D printed heart model for pediatric heart surgery.

Retrieved from www.louisville.edu

[75] Crapo, P. M., Gilbert, T. W., & Badylak, S. F. (2011). An overview of tissue and whole organ decellularization processes. *Biomaterials*, *32*(12), 3233–3243. https://doi.org/10.1016/j.biomaterials.2011.01.057

[76] Badylak, S. F., Freytes, D. O., & Gilbert, T. W. (2009). Extracellular matrix as a biological scaffold material: Structure and function. *Acta biomaterialia*, *5*(1), 1–13. https://doi.org/10.1016/j.actbio.2008.09.013

[77] Singelyn JM, Christman KL. Injectable materials for the treatment of myocardial infarction and heart failure: the promise of decellularized matrices. *J Cardiovasc Transl Res*. 2010;3(5):478-486. doi:10.1007/s12265-010-9202-x

[78] Pati, F., Jang, J., Ha, D. H., Won Kim, S., Rhie, J. W., Shim, J. H., Kim, D. H., & Cho, D. W. (2014). Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nature communications*, *5*, 3935. https://doi.org/10.1038/ncomms4935

[79] Jaramillo, M., Lyons, L. P., & Lorenzana, J. (2018). Decellularized extracellular matrix as a 3D scaffold for liver bioengineering and transplantation: Recent advances. *Acta Biomaterialia*, 86, 15-22.

[80] Ahmed, T. A., Dare, E. V., & Hincke, M. (2008). Fibrin: A versatile scaffold for tissue engineering applications. *Tissue Engineering Part B: Reviews*, 14(2), 199-215.

[81] Du, M., Chen, B., Liu, Y., & Deng, J. (2017). Biofabrication of human endothelial cells with fibrin for 3D vascular constructs. *Frontiers in Bioengineering and Biotechnology*, 5, 22.

[82] Yamamoto, M., Yamamoto, Y., Inoue, N., Sumitomo, Y., Yamamoto, M., & Nagase, T. (2017). Development of a new tissue-engineered vascular graft: Cell-free grafts recellularized in vivo by circulating blood-derived cells. *Acta Biomaterialia*, 51, 331-343.

[83] Bayer IS. Advances in Fibrin-Based Materials in Wound Repair: A Review. *Molecules*. 2022;27(14):4504. Published 2022 Jul 14. doi:10.3390/molecules27144504

[84] Gupta RC, Lall R, Srivastava A, Sinha A. Hyaluronic Acid: Molecular Mechanisms and Therapeutic Trajectory. *Front Vet Sci*. 2019;6:192. Published 2019 Jun 25. doi:10.3389/fvets.2019.00192

[85] Lee, H. Y., Hwang, C. H., Kim, H. E., & Jeong, S. H. (2018). Enhancement of bio-stability and mechanical properties of hyaluronic acid hydrogels by tannic acid treatment. *Carbohydrate polymers*, *186*, 290–298. https://doi.org/10.1016/j.carbpol.2018.01.056

[86] Petta, D., D'Amora, U., Ambrosio, L., Grijpma, D. W., Eglin, D., & D'Este, M. (2020). Hyaluronic acid as a bioink for extrusion-based 3D printing. *Biofabrication*, *12*(3), 032001. https://doi.org/10.1088/1758-5090/ab8752

[87] Flégeau, K., Puiggali-Jou, A., & Zenobi-Wong, M. (2022). Cartilage tissue engineering by extrusion bioprinting utilizing porous hyaluronic acid microgel bioinks. *Biofabrication*, *14*(3), 10.1088/1758-5090/ac6b58. https://doi.org/10.1088/1758-5090/ac6b58

[88] Vepari, C., & Kaplan, D. L. (2007). Silk as a Biomaterial. *Progress in polymer science*, *32*(8-9), 991–1007. https://doi.org/10.1016/j.progpolymsci.2007.05.013

[89] Chen, N., Zhang, X., Lyu, J., Zhao, G., Gu, K., Xia, J., Chen, Z., & Shao, Z. (2022). Preparation of a novel regenerated silk fibroin-based hydrogel for extrusion bioprinting. *Soft matter*, *18*(38), 7360–7368. https://doi.org/10.1039/d2sm00984f

[90] Wang, Y., Rudym, D. D., Walsh, A., Abrahamsen, L., Kim, H. J., Kim, H. S., Kirker-Head, C.,

& Kaplan, D. L. (2008). In vivo degradation of three-dimensional silk fibroin scaffolds. *Biomaterials*, *29*(24-25), 3415–3428. https://doi.org/10.1016/j.biomaterials.2008.05.002

[91] Bryant, S. J., & Anseth, K. S. (2001). The effects of scaffold thickness on tissue engineered cartilage in photocrosslinked poly(ethylene oxide) hydrogels. *Biomaterials*, *22*(6), 619–626. https://doi.org/10.1016/s0142-9612(00)00225-8

[92] Skardal, A., & Atala, A. (2015). Biomaterials for integration with 3-D bioprinting. *Annals of biomedical engineering*, *43*(3), 730–746. https://doi.org/10.1007/s10439-014-1207-1

[93] Hospodiuk, M., Dey, M., Sosnoski, D., & Ozbolat, I. T. (2017). The bioink: A comprehensive review on bioprintable materials. *Biotechnology advances*, *35*(2), 217–239. https://doi.org/10.1016/j.biotechadv.2016.12.006

[94] Malda, J., Visser, J., Melchels, F. P., Jüngst, T., Hennink, W. E., Dhert, W. J., Groll, J., & Hutmacher, D. W. (2013). 25th anniversary article: Engineering hydrogels for biofabrication. *Advanced materials (Deerfield Beach, Fla.)*, *25*(36), 5011–5028. https://doi.org/10.1002/adma.201302042

[95] Gu, Z., Fu, J., Lin, H., & He, Y. (2020). Development of 3D bioprinting: From printing methods to biomedical applications. *Asian journal of pharmaceutical sciences*, *15*(5), 529–557. https://doi.org/10.1016/j.ajps.2019.11.003

[96] Chimene, D., Lennox, K. K., Kaunas, R. R., & Gaharwar, A. K. (2016). Advanced Bioinks for 3D Printing: A Materials Science Perspective. *Annals of biomedical engineering*, *44*(6), 2090–2102. https://doi.org/10.1007/s10439-016-1638-y

[97] Ouyang, L., Yao, R., Zhao, Y., & Sun, W. (2016). Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells. *Biofabrication*, 8(3), 035020.

[98] Gao, G., Yonezawa, T., Hubbell, K., Dai, G., & Cui, X. (2015). Inkjet-bioprinted acrylated peptides and PEG hydrogel with human mesenchymal stem cells promote robust bone and cartilage formation with minimal printhead clogging. *Biotechnology journal*, *10*(10), 1568–1577. https://doi.org/10.1002/biot.201400635.

[99] Yuan Y, Tyson C, Szyniec A, *et al*. Bioactive Polyurethane-Poly(ethylene Glycol) Diacrylate Hydrogels for Applications in Tissue Engineering. *Gels*. 2024;10(2):108. Published 2024 Jan 29. doi:10.3390/gels10020108

[100] Gioffredi, Emilia & Boffito, Monica & Calzone, Stefano & Giannitelli, Sara & Rainer, Alberto & Trombetta, Marcella & Mozetic, Pamela & Chiono, Valeria. (2016). Pluronic F127 Hydrogel Characterization and Biofabrication in Cellularized Constructs for Tissue Engineering Applications. Procedia CIRP. 49. 125-132. 10.1016/j.procir.2015.11.001.

[101] Fu Z, Angeline V, Sun W. Evaluation of Printing Parameters on 3D Extrusion Printing of Pluronic Hydrogels and Machine Learning Guided Parameter Recommendation. *Int J Bioprint*. 2021;7(4):434. Published 2021 Oct 15. doi:10.18063/ijb.v7i4.434

[102] Chen, Ying & Li, Jie & Lu, Jiawei & Ding, Meng & Chen, Yi. (2022). Synthesis and properties of Poly(vinyl alcohol) hydrogels with high strength and toughness. Polymer Testing. 108. 107516. 10.1016/j.polymertesting.2022.107516.

[103] Liu P, Chen W, Liu C, Tian M, Liu P. A novel poly (vinyl alcohol)/poly (ethylene glycol) scaffold for tissue engineering with a unique bimodal open-celled structure fabricated using supercritical fluid foaming. *Sci Rep*. 2019;9(1):9534. Published 2019 Jul 2. doi:10.1038/s41598-019-46061-7

[104] Dattola E, Parrotta EI, Scalise S, *et al*. Development of 3D PVA scaffolds for cardiac tissue

engineering and cell screening applications. *RSC Adv*. 2019;9(8):4246-4257. Published 2019 Feb 14. doi:10.1039/c8ra08187e

[105] Chung JHY, Naficy S, Yue Z, Kapsa R, Quigley A, Moulton SE, Wallace GG (2013) Bio-ink properties and printability for extrusion printing living cells. Biomater Sci 1:763. https://doi.org/10.1039/c3bm00012e

[106] Balakrishnan, B., Joshi, N., Jayakrishnan, A., & Banerjee, R. (2014). Self-crosslinked oxidized alginate/gelatin hydrogel as injectable, adhesive biomimetic scaffolds for cartilage regeneration. *Acta biomaterialia*, *10*(8), 3650–3663. https://doi.org/10.1016/j.actbio.2014.04.031

[107] Sonaye, S.Y.; Ertugral, E.G.; Kothapalli, C.R.; Sikder, P. Extrusion 3D (Bio)Printing of Alginate-Gelatin-Based Composite Scaffolds for Skeletal Muscle Tissue Engineering. *Materials* 2022, *15*, 7945. https://doi.org/10.3390/ma15227945

[108] Cui, X., Li, J., Hartanto, Y., Durham, M., Tang, J., Zhang, H., Hooper, G., Lim, K., & Woodfield, T. (2020). Advances in Extrusion 3D Bioprinting: A Focus on Multicomponent Hydrogel-Based Bioinks. *Advanced healthcare materials*, *9*(15), e1901648. https://doi.org/10.1002/adhm.201901648

[109] Xia Y, Mei F, Duan Y, Gao Y, Xiong Z, Zhang T, Zhang H. 2012. Bone tissue engineering using bone marrow stromal cells and an injectable sodium alginate/gelatin scaffold. J Biomed Mater Res Part A 2012:100A:1044–1050.

[110] Lazaridou, M., Bikiaris, D. N., & Lamprou, D. A. (2022). 3D Bioprinted Chitosan-Based Hydrogel Scaffolds in Tissue Engineering and Localised Drug Delivery. *Pharmaceutics*, *14*(9), 1978. https://doi.org/10.3390/pharmaceutics14091978

[111] Piao, Y., You, H., Xu, T., Bei, H. P., Piwko, I. Z., Kwan, Y. Y., & Zhao, X. (2021). Biomedical applications of gelatin methacryloyl hydrogels. *Engineered Regeneration*, *2*, 47-56.

[112] Munoz-Pinto, D. J., Jimenez-Vergara, A. C., Gharat, T. P., & Hahn, M. S. (2015). Characterization of sequential collagen-poly(ethylene glycol) diacrylate interpenetrating networks and initial assessment of their potential for vascular tissue engineering. *Biomaterials*, *40*, 32–42. https://doi.org/10.1016/j.biomaterials.2014.10.051

[113] Alonzo, M., AnilKumar, S., Roman, B., Tasnim, N., & Joddar, B. (2019). 3D Bioprinting of cardiac tissue and cardiac stem cell therapy. *Translational research : the journal of laboratory and clinical medicine*, *211*, 64–83. https://doi.org/10.1016/j.trsl.2019.04.004

[114] Wang, M., Deng, Z., Guo, Y., & Xu, P. (2022). Designing functional hyaluronic acid-based hydrogels for cartilage tissue engineering. *Materials today. Bio*, *17*, 100495. https://doi.org/10.1016/j.mtbio.2022.100495

[115] Gamboa-Martínez, Tatiana & Luque, Victoria & Gonzalez-Garcia, Cristina & Gomez Ribelles, José Luis & Gallego Ferrer, Gloria. (2014). Crosslinked fibrin gels for tissue engineering: Two approaches to improve their properties: Crosslinked Fibrin Gels for Tissue Engineering. Journal of Biomedical Materials Research Part A. 103. 10.1002/jbm.a.35210.

[116] Cui, Xiaoliang & Zhang, Jun & Qian, Yan & Chang, Siqi & Allardyce, Benjamin & Rajkhowa, Rangam & Wang, Hui & Zhang, Ke-Qin. (2024). 3D Printing Strategies for Precise and Functional Assembly of Silk-based Biomaterials. Engineering. 10.1016/j.eng.2023.09.022.

[117] Fonseca, A. C., Melchels, F. P., Ferreira, M. J., Moxon, S. R., Potjewyd, G., Dargaville, T. R., ... & Domingos, M. (2020). Emulating human tissues and organs: a bioprinting perspective toward personalized medicine. *Chemical Reviews*, *120*(19), 11093-11139.