



3D

Bioprinting from Lab to Industry

Edited by **Prosenjit Saha** • **Sabu Thomas**
Jinku Kim • **Manojit Ghosh**

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Contents

List of Contributors xv

Foreword xxi

Ajoy Kumar Ray

- 1 Introduction of 3D Printing and Different Bioprinting Methods** 1
Asmita Biswas, Baisakhee Saha, Hema Bora, Pravin Vasudeo Vaidya, Krishna Dixit, and Santanu Dhara
- 1.1 Introduction of 3D Printing: Principles and Utility 1
- 1.2 Ink Preparation and Printability 2
- 1.3 Methods of Bioprinting in Fabrication and Tissue Engineering 5
- 1.3.1 Laser-Based Printing 5
- 1.3.1.1 Types of Laser Printing 7
- 1.3.2 Extrusion-Based Printing 9
- 1.3.3 Droplet Printing 11
- 1.3.4 Inkjet-Based Printing 13
- 1.3.5 Stereolithography 3D Printing 15
- 1.4 Scaffold Modeling and G Coding 16
- 1.4.1 Scanning Technology 16
- 1.4.2 CT Imaging 16
- 1.4.3 MRI Scanning 17
- 1.4.4 Preferred Accuracy Parameters for Scanning 17
- 1.4.5 Biomodeling Process for RP 17
- 1.5 Applications and Utility in Large-Scale Manufacturing 18
- 1.5.1 Bone 18
- 1.5.2 Cartilage 19
- 1.5.3 Skin 19
- 1.5.4 Vascular Grafts 20
- 1.5.5 Heart 21
- 1.5.6 Lungs 21
- 1.5.7 Liver 22
- 1.5.8 Kidney and Urethra 22

1.5.9	Brain and Spinal Cord	23
1.5.10	Cornea	23
1.5.11	Therapeutics	23
1.6	Complications and Troubleshooting	25
1.6.1	Laser-Based Printing	25
1.6.2	Inkjet-Based Printing	26
1.6.3	Extrusion-Based Printing	26
1.6.4	Droplet Printing	26
1.6.5	Stereolithography 3D Printing	27
	References	27
2	Cellular Requirements and Preparation for Bioprinting	39
	<i>Shalini Dasgupta, Vriti Sharma, and Ananya Barui</i>	
2.1	Introduction	39
2.2	Types of Bioprinting	40
2.2.1	Inkjet-Assisted Printing	41
2.2.2	Extruder-Assisted Printing	42
2.2.3	Laser-Assisted Bioprinting	43
2.3	Features Required for Bioprinting with Cells	44
2.3.1	Sterility Parameters	44
2.3.2	Printing Speed and Pressure	45
2.3.3	pH and Osmotic Condition	46
2.3.4	Hydrogel Generation	47
2.3.4.1	Natural Polymers	48
2.3.4.2	Synthetic Polymers	51
2.3.5	Culture Duration and Conditions	54
2.3.6	Rheological Properties	54
2.4	Bioprinting Methodologies for Cell Expansion and Proliferation	55
2.5	The Impact of Bioprinting Process Conditions on Phenotype Alterations	57
2.5.1	Bioprinting Techniques for Stem Cell Differentiation	58
2.5.1.1	Bioprinting Strategies for Cellular Environment Alterations	59
2.5.1.2	Bioprinting Strategies for Cell Behavior Modulation	59
2.5.1.3	Bioprinting Strategies for Genetic Modulation and Transcriptomics Variation	62
2.5.2	Bioprinting Techniques for Tumorigenic Differentiation	64
2.5.2.1	Bioprinting Strategies for Oncogenic Cell Growth	65
2.5.2.2	Bioprinting Strategies for the Development of Tumor Models	67
2.6	Discussion	68
2.7	Conclusion	69
2.8	Future Prospects	69
	References	70

3	3D Bioprinting: Materials for Bioprinting Bioinks Selection	85
	<i>Mona Moaness and Mostafa Mabrouk</i>	
3.1	Introduction	85
3.2	Bioprinting Materials	87
3.2.1	Biomaterials	87
3.2.2	Cells	87
3.2.3	Biomolecules or Additive Molecules	89
3.2.4	Hydrogels	89
3.3	Bioinks Selectivity Guide	90
3.3.1	Printability of Materials	90
3.3.2	Material Biocompatibility	91
3.3.3	Structural Properties	92
3.3.4	Materials Degradation	92
3.3.5	Biomimicry	93
3.4	Classification of Bioprinting Materials	94
3.4.1	According to Material Type	94
3.4.1.1	Polymers	94
3.4.1.2	Nanocomposites	97
3.4.1.3	Nanoparticles	98
3.4.2	According to Cell Dependence	98
3.4.2.1	Cell-based Bioinks	98
3.4.2.2	Cell-Free Bioinks (Biomaterial Inks)	99
3.5	3D Bioprinting Methods According to the Type of the Bioinks	100
3.5.1	Extrusion-Based 3D Bioprinting	100
3.5.2	Inkjet 3D Bioprinting	100
3.5.3	Stereolithography 3D Bioprinting	101
3.5.4	Laser-Based 3D Bioprinting	101
3.5.5	Bioplotting	101
3.6	Bioinks Selection According to Biomedical Application	102
3.7	Multicomponent Bioinks	106
3.8	Future Prospects	107
	References	107
4	Printed Scaffolds in Tissue Engineering	119
	<i>Thara Tom, Samanta Sam, Josmin P. Jose, M.S. Sreekala, and Sabu Thomas</i>	
4.1	Introduction	119
4.2	Biomedical Application of 3D Printing	120
4.2.1	Implants and Scaffolds	122
4.2.2	Drug Delivery/Drug Modeling Application	124
4.2.3	Applications of 3D Printed Scaffolds During COVID-19	124
4.3	Tissue Engineering: Emerging Applications by 3D Printing	128

- 4.3.1 Cartilage Tissue Engineering by Printed Scaffolds 130
- 4.3.2 Liver Tissue Engineering by Printed Scaffolds 130
- 4.3.3 Nerve Tissue Engineering by Printed Scaffolds 132
- 4.3.4 Cardiac Tissue Engineering by Printed Scaffolds 134
- 4.4 Conclusions 136
- References 136

5 Printability and Shape Fidelity in Different Bioprinting Process 143

Prajisha Prabhakar, Aiswarya Sathian, and Sabu Thomas

- 5.1 Introduction 143
- 5.2 Fundamentals of Printability 144
- 5.3 Bioprinting Techniques and Printability 146
 - 5.3.1 Extrusion-Based Bioprinting 146
 - 5.3.2 Inkjet-Based Bioprinting 148
 - 5.3.3 Stereolithography-Based Bioprinting (SL) 150
- 5.4 Shape Fidelity 152
 - 5.4.1 Shape Fidelity in Planar Structures 153
 - 5.4.2 Shape Fidelity in Multilayered Structures 153
 - 5.4.3 Characterization Approaches 157
 - 5.4.3.1 Rheological Characterization 157
 - 5.4.3.2 Mechanical Characterization 158
 - 5.4.3.3 Swelling Test 158
 - 5.4.3.4 Viability Characterization 159
 - 5.4.3.5 Bioprinting Procedure 159
- 5.5 Case Studies and Applications 161
- 5.6 Conclusion 163
- References 163

6 Advancements in Bioprinting for Medical Applications 169

Kevin Y. Wu, Maxine Joly-Chevrier, Laura K. Gorwill, Michael Marchand, and Simon D. Tran

- 6.1 Introduction 169
- 6.2 Bioprinting for Drug Development and Testing 170
 - 6.2.1 Overview 170
 - 6.2.2 3D Bioprinted Organoids 171
 - 6.2.3 Organ-on-a-Chip/Microfluidic Systems 173
 - 6.2.4 Bioprinted Models for Cancer Research 176
 - 6.2.5 3D Bioprinting for Immunotherapy and Cell Therapy 181
- 6.3 Bioprinting in Tissue Engineering, Regenerative Medicine, and Organ Transplantation 183
 - 6.3.1 Ocular Tissue Engineering 183
 - 6.3.1.1 Retina 183
 - 6.3.1.2 Cornea 184

6.3.2	Neural Tissue	187
6.3.3	Skin	190
6.3.3.1	Disease and Pharmaceutical Studies	190
6.3.3.2	Wound Healing	191
6.3.3.3	Reconstructive Surgery	195
6.3.4	Cartilage and Bone	196
6.3.4.1	Cartilage Printing Modalities	196
6.3.4.2	Cartilage Regeneration	196
6.3.5	Vascular Tissue	200
6.3.6	Cardiac Tissue Engineering	201
6.3.7	Pancreas	204
6.3.7.1	Modulating Bioink Formulation to Enhance Tissue Viability	204
6.3.7.2	Controlling Other Printing Parameters to Enhance Tissue Viability	205
6.3.7.3	Using Printed Models to Study Pancreatic Cancer	205
6.3.8	Liver	206
6.3.8.1	Developing Suitable In Vitro Models	206
6.3.9	Lungs	207
6.3.9.1	Developing Suitable In Vitro Models	207
6.3.9.2	Application of 3D Construct	209
6.3.10	Renal/Kidney	210
6.3.10.1	Printing Parameters Affecting the Viability of Printed Model	210
6.3.10.2	Applications of 3D-Printed Model	211
6.3.11	Composite Tissues	211
6.3.12	Other Tissues	213
6.4	Bioprinting in Tissue: Challenges, Barriers to Clinical Translation, and Future Directions	215
6.4.1	Introduction	215
6.4.1.1	Current Challenges in Organ Transplantation	215
6.4.1.2	Potential of Bioprinted Organs for Transplantation	215
6.4.1.3	Challenges and Limitations in Bioprinting Tissues and Organs	216
6.4.2	Insight on Barriers to Clinical Translation of Bioprinting Technology	216
6.4.3	Future Directions	217
6.5	Conclusions	218
	Acknowledgments	218
	References	219
7	4D-Printed, Smart, Multiresponsive Structures and Their Applications	231
	<i>Jinku Kim, D.A. Gouripriya, and Prosenjit Saha</i>	
7.1	Introduction	231
7.2	4D-Printing Technologies	232
7.3	Biomaterials for 4D Bioprinting	234

7.3.1	Water-Responsive Polymers	235
7.3.2	Temperature-Responsive Polymers (Hydrogels)	236
7.3.3	Electrical/Magnetic-Responsive Polymers	237
7.4	Biomedical Applications for 4D Bioprinting	239
7.4.1	Limitations of 3D Bioprinting	240
7.4.2	Biomedical Applications of 4D Printing	240
7.4.3	Scaffold Preparation	241
7.4.4	Drug Delivery	241
7.4.5	Sensors	242
7.4.6	Medical Devices	242
7.4.7	Tissue Engineering and Organ Regeneration	243
7.5	Future Perspectives	244
	References	246
8	Toxicity Aspects and Ethical Issues of Bioprinting	251
	<i>Noura Al Hashimi and Sanjairaj Vijayavenkataraman</i>	
8.1	Introduction	251
8.2	Toxicity Issues in Bioprinting	253
8.2.1	Cell Harvesting and Culture	253
8.2.2	Aseptic Techniques in Bioprinting	254
8.3	Ethical Issues in Bioprinting	255
8.3.1	Purpose	255
8.3.2	Cell Source	256
8.3.3	Data and Consent	257
8.3.4	Safety	258
8.3.5	Cost and Equity	258
8.3.6	Reproductive Organs	259
8.4	Issues in Clinical Trials	259
8.4.1	Personalized Treatment	260
8.4.2	Inability to Withdraw or Access Alternate Treatments	261
8.5	Legal Issues in Bioprinting	262
8.5.1	Intellectual Property Rights and Product Classification	262
8.5.2	Lack of Regulatory Guidelines	263
8.6	Conclusion	265
	References	266
9	Planning Bioprinting Project	273
	<i>Anish Deb, Prosenjit Saha, and Debashis Sarkar</i>	
9.1	Introduction	273
9.2	Background: Image Capturing and Solid Model Preparation of Virtual Anatomical Model for 3D Printing	275
9.2.1	Other Imaging Techniques	284
9.2.2	Digital Process for STL Generation	284

9.2.3	Blueprint Modeling	286
9.2.4	CAD-Based Systems Characteristics	286
9.2.5	Image-Based Systems	287
9.2.6	Freeform Systems	288
9.2.7	Designs Using Implicit Surfaces	288
9.2.8	Space-Filling Curves	289
9.2.9	Planning of Toolpath for Bioprinting	289
9.2.10	Cartesian Form Toolpath Planning	290
9.2.11	Parametric Form in Toolpath Planning	292
9.2.12	Bioprinting Methods	292
9.2.12.1	Extrusion Bioprinting	292
9.2.12.2	Inkjet Printing	294
9.2.12.3	Laser-Assisted Printing	295
9.3	Conclusion	296
	References	297
10	Computational Engineering for 3D Bioprinting: Models, Methods, and Emerging Technologies	301
	<i>Vidyapati Kumar, Ankita Mistri, Varnit Jain, and Manojit Ghosh</i>	
10.1	Introduction	301
10.2	Fundamentals of Numerical Methods in Bioprinting	306
10.2.1	Finite Element Analysis	306
10.2.2	Computational Fluid Dynamics	307
10.2.3	Agent-Based Modeling	308
10.2.4	Lattice Boltzmann Method	309
10.2.5	Molecular Dynamics	310
10.3	Application of Machine Learning for 3D Bioprinting	312
10.4	Summary	315
	References	317
11	Controlling Factors of Bioprinting	323
	<i>Mridula Sreedharan, D.A. Gouripriya, Ankita Deb, Yves Grohens, Nandakumar Kalarikkal, Prosenjit Saha, and Sabu Thomas</i>	
11.1	Introduction	323
11.2	Factors Influencing the Printability of Hydrogel Bioink	324
11.2.1	Extrudability	325
11.2.2	Filament Type	326
11.2.3	Shape Fidelity	327
11.2.4	Optimization of Printing Parameters	327
11.3	Bioink Formulation	327
11.4	Influence of Printing Process on Cell Behavior	328
11.5	Importance of Patterning and Surface Topography	330
11.5.1	Importance of Patterning with 3D Bioprinting	330

11.5.2	Effects of Patterning	331
11.5.3	Challenges of Patterning	334
11.5.4	Influence of Surface Topography with 3D Bioprinting	334
11.5.5	Effects of Surface Topographical Features	334
11.6	Contact Guidance and Directional Growth of Cells	337
11.6.1	2D Topographic Cues	338
11.6.2	3D Topographic Cues	339
11.7	Cell Viability and Mitigation Process	339
11.7.1	Inkjet-Based Bioprinting	340
11.7.2	Extrusion-Based Bioprinting	340
11.7.3	Laser-Based Bioprinting	342
11.7.4	Stereolithography-Based Bioprinting	342
11.8	Possible Mitigation Techniques	342
11.9	Conclusion	342
	References	343
12	In Situ Bioprinting	347
	<i>Mina Mina, Kevin Y. Wu, Ananda Kalevar, and Simon D. Tran</i>	
12.1	Introduction	347
12.2	Advantages of In Situ Bioprinting	348
12.2.1	Precision in Cellular Deposition	348
12.2.2	Enhanced Integration with Host Tissue	348
12.2.3	High-Fidelity Replication of the Extracellular Environment	349
12.2.4	Reduced Immune Response	350
12.2.5	Regulatory Requirements	350
12.2.6	Potential for Personalized Medicine	351
12.3	In Situ Bioprinting Technologies	351
12.3.1	Brief Review of 3D Bioprinting Methods	351
12.3.2	Adaptations for In Situ Applications	353
12.3.2.1	Automated In Situ Bioprinters	353
12.3.2.2	Semiautomated In Situ Bioprinters	358
12.3.2.3	Handheld In Situ Bioprinters	360
12.4	Biopinks and Biomaterials for In Situ Bioprinting	362
12.4.1	Overview	362
12.4.2	Engineering Consideration	363
12.4.2.1	Photocrosslinking	363
12.4.2.2	Biocompatibility and Biomimicry	363
12.4.2.3	Mechanical Properties	363
12.5	In Situ Approaches for Tissue Regeneration	364
12.5.1	Cartilage and Bone Regeneration	364
12.5.2	Skeletal Muscle Regeneration	369

- 12.5.3 Skin and Wound Regeneration 373
- 12.5.4 Dental Pulp Regeneration 375
- 12.6 Future Directions 379
- 12.6.1 4D Bioprinting 379
- 12.6.2 Open-Source Collaboration 380
- 12.6.3 Integration with Machine Learning 380
- 12.7 Conclusion 381
- Acknowledgments 382
- References 382

- 13 Importance of Machine Learning in 3D Bioprinting 391**
Shohreh Vanaei, Saeedeh Vanaei, Michèle Kanhonou, Sofiane Khelladi, Abbas Tcharkhtchi, and Hamid Reza Vanaei
- 13.1 Introduction 391
- 13.2 3D Bioprinting 392
- 13.2.1 Fundamentals of 3D Bioprinting 392
- 13.2.2 Materials and Technologies in 3D Bioprinting 393
- 13.2.2.1 Different Materials in 3D Bioprinting 393
- 13.2.2.2 Different Technologies/Techniques in 3D Bioprinting 397
- 13.2.3 Application of 3D Bioprinting 398
- 13.3 Machine Learning in 3D Bioprinting 399
- 13.3.1 3D Bioprinting Process Using ML 400
- 13.3.2 Application of ML in 3D Bioprinting 401
- 13.4 Challenges in 3D Bioprinting Process Using ML 404
- 13.5 Future Outlook 405
- 13.6 Summary and Conclusion 406
- References 407

- 14 Advanced Bioprinting for the Future 411**
D.A. Gouripriya, Soumyadeep Bera, Jaideep Adhikari, Poonam Debnath, Prosenjit Saha, and Sabu Thomas
- 14.1 Introduction 411
- 14.2 Electrospinning and Bioprinting 412
- 14.3 4D Printing 413
- 14.4 5D and 6D Printing 418
- 14.5 Organ Printing 421
- 14.6 Vascularized Organ on a Chip 424
- 14.7 Multimaterial Bioprinting 426
- 14.8 Printing in Microgravity 429
- 14.9 In Vivo Bioprinting 430
- 14.10 Biohybrid Robots 432
- 14.11 Conclusion and Future Perspectives 434
- References 435

15 Nanomaterials for Designing Functional Properties of Bioinks 441

Laila Hussein, Mostafa Mabrouk, Mohamed G. Farahat, and Hanan H. Beherei

- 15.1 3D-Bioprinting 441
 - 15.1.1 Bioinks for 3D-Bioprinting 441
 - 15.1.2 Designing of Functional Bioinks 442
 - 15.1.2.1 Desirable Properties of Bioinks 442
 - 15.1.2.2 Limitations of Bioinks 443
- 15.2 Designing Functional Bioinks Using Nanoscale Biomaterials 443
 - 15.2.1 Types of Nano-Biomaterials 444
 - 15.2.1.1 Ceramic NPs (CNs) 445
 - 15.2.1.2 Carbon Nano-Biomaterials 447
 - 15.2.1.3 Polymer Nano-Biomaterials 452
 - 15.2.2 Smart Multifunctional Nanobioinks 453
- 15.3 Synthesis and Tailoring the Properties of Nanobioinks 456
 - 15.3.1 Choice of Bioink 457
 - 15.3.2 Porosity 458
 - 15.3.3 Surface Properties 458
- 15.4 Nanobioinks and Tissue Engineering 460
 - 15.4.1 Tissue Engineering of Bone and Cartilage 461
 - 15.4.2 Tissue Engineering of Nerves 461
 - 15.4.3 Tissue Engineering in Cardiovascular Disease 462
- 15.5 Future Outlook 462
- References 463

16 3D Bioprinting from Lab to Industry 475

Saeedeh Vanaei, Shohreh Vanaei, Michèle Kanhonou, Abbas Tcharkhtchi, and Hamid Reza Vanaei

- 16.1 Introduction 475
- 16.2 3D Bioprinting and Its Historical Point of View 477
- 16.3 Potential of 3D Bioprinting from Lab to Industry 478
- 16.4 The Diversity of 3D Bioprinting 479
 - 16.4.1 Medical Devices 479
 - 16.4.2 Drug Development 482
 - 16.4.3 Tissue Engineering 484
- 16.5 3D Bioprinting and Human Hearts 486
- 16.6 3D Bioprinting and Microfluidic Organ-on-a-Chip Models 488
- 16.7 Future Developments 490
- References 490

Index 493

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Foreword

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Tissue damage due to disease or injury has been a major concern for the entire global population, and its remediation poses serious challenges to the healthcare providers, biomedical researchers, and professionals across the globe. The concern becomes manifold since some organs and tissues have very limited self-renewal or regenerative capacity. Thus for restoring such injured or degraded tissues, we need to regenerate functional living tissue or even the whole organ artificially. This has resulted in an extremely important and emerging field of tissue engineering and regenerative medicine, which offers the potential to provide solutions for bio-fabrication of functional tissues.

Bioprinting, the process of creating complex, living tissues using 3D printing technology has received paramount importance in recent times. The interdisciplinary technology, lying at the intersection of engineering and biology, is the backbone of regenerative medicine and tissue engineering and points to the future of modern medicine.

Using this technology, it is important to precisely position multiple cell types layer by layer using computer-aided additive manufacturing techniques. Bioprinting is essentially a computer-aided transfer process for assembling biologically relevant materials including biomolecules, cells, tissues, and biodegradable biomaterials, resulting in the formation of an engineered bio-functional construct. Indeed it is a revolutionary concept in the regeneration or repair of damaged tissues by automating the layer-by-layer hierarchical fabrication of cell-laden structures both *in vitro* and *in vivo*.

Some of the early models of 3D bioprinting technology used computer-controlled ink-jet printer or graphics plotter and were used for precisely positioning the cells on a 2D substrate. This work provided the fundamental platform for

3D bioprinting. From those early efforts in mid-1980s, the interdisciplinary endeavor in 3D bioprinting and regenerative medicine technology has progressed substantially over the past three and a half decades, making personalized medicine a reality.

Since the original native tissues of a patient have their own distinct complex architecture as well as 3D organization and distribution of cells and extracellular matrix, it remains a significant challenge to precisely understand the complexity of native tissues from a structural and functional perspective. It holds endless promise for revolutionizing healthcare and various industries. From regenerative medicine to pharmaceutical testing and beyond, the possibilities offered by bioprinting are vast and continually expanding. However, the growth in this field also holds the numbers of existing challenges that need to be addressed by the scientific community.

In this context, the book *3D Bioprinting from Lab to Industry*, edited by Prosenjit Saha, Sabu Thomas, Jinku Kim, and Manojit Ghosh, has opened up the exciting world of bioprinting and its modern industrial applications.

The book, written by some of the esteemed scientists in this field, will embark on a voyage through the intricate landscapes of bioprinting. The contributors of each chapter have shared their experiences to present the fundamental challenges along with the solutions that lie in each area. From the first chapter, where the authors explain the fundamental principles of 3D bioprinting, the readers will enjoy a journey through the diverse facets of bioprinting – controlling this translational technology to the design, fabrication, and applications of biomaterials and bioinks at industrial scale, the book covers all the important aspects of 3D bioprinting.

The broad overlap of additive manufacturing with tissue engineering has transcended the boundaries of conventional medicine, presenting enormous potential for a future where organs can be printed on demand at laboratory, customized to individual needs with precision. It holds endless promise for revolutionizing healthcare for various industries. From regenerative medicine to pharmaceutical testing and beyond, the possibilities offered by bioprinting are vast and continually expanding. However, the growth in this field also holds the numbers of existing challenges that need to be addressed by the scientific community.

Because of its affordability, commercial viability, and capability to fabricate complex and hollow constructs, the extrusion-based bioprinting, one of the most common techniques in bioprinting field, has been employed to print living cells, tissue constructs, organ modules, and even organ on-a-chip devices. In this book, the extrusion-based 3D bioprinting has been covered in adequate details for the readers. Bioinks are formulation of materials suitable for processing by an automated biofabrication technology. With the advancement of technology – especially the introduction of extrusion-based printing techniques that use a

small-diameter nozzle to deposit bioinks for the fabrication of complex constructs – individual cells and cell aggregates lack the ability to withstand the shear stress.

Starting with an introduction of the fundamental principles of bioink, the authors have presented here the translational technology for the design, fabrication, and applications of biomaterials and bioinks at industrial scale.

Furthermore, the book presents the industrial applications of bioprinting, showcasing how this technology can be used in personalized medicine, from planning to implementation, through case studies and laboratory-based research outcomes. The profound impact of AI and machine learning on the design of 3D bioprinting has also been included here. The processing parameters on the design of scaffolds, organoids, and cell/tissue models during printing process have also been discussed in detail by the authors.

With a lucid introduction to the realm of bio printing, followed by cellular requirements and preparation for bio printing, the authors have discussed various materials used for bioprinting. Bioprinting in regenerated organs, 4D bioprinted multiresponsive structure, the controlling factors in bioprinting, in situ bioprinting, machine learning, and in particular deep learning techniques in 3D bioprinting, nanomaterial and design of scaffolds, organoids, and cell/tissue models during printing process have been discussed in detail by the authors.

How to plan a bioprinting project and how it moves from laboratory to industry are two very important issues, which have been discussed here. The industrial applications of bioprinting, showcasing how this technology is utilized in personalized medicine from planning to implementation, have been discussed here through case studies and lab-based research outcomes. Finally, toxicity issues hold paramount importance in 3D bioprinting, and along with this comes the most pertinent ethical issues involved in this area. These have also been adequately discussed in this book.

For researchers, industry professionals, students, or anyone curious about the present and the future of 3D bioprinting and tissue engineering, this book will provide valuable insights into this rapidly evolving field. I hope that the book inspires and informs readers about the remarkable possibilities presented by 3D printing in shaping the future of modern medicine and healthcare industry.

Happy reading!

1

Introduction of 3D Printing and Different Bioprinting Methods

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1.1 Introduction of 3D Printing: Principles and Utility

3D printing (3DP), also known as additive manufacturing (AM), solid-freeform (SFF), and rapid prototyping (RP), is a fabrication technique using model data, where 3D structures are fabricated using controlled layer-by-layer deposition [1]. It was first described by Charles Hull in 1986, followed by production and commercialization by S. Scott Crump and his company Stratasys [2]. The basic subcategories of 3DP are stereolithography, fused deposition modeling, selective laser melting, electronic beam melting, and laminated object manufacturing [3]. 3DP involves scaffold construction by material addition, with high geometric precision reducing material waste. The primary procedure comprises data acquisition and synthesis of meshed 3D computer models in computer-aided design (CAD), followed by surface tessellation language (STL) file creation. This is followed by the slicing of mesh data into multiple 2D layer files and transferring them to a 3DP machine for fabrication [4]. Manufacture of complex designs, low cost, ease of access, and rapid and environment-friendly procedures are some of the advantages of 3DP in industrial, research, healthcare, and biomedical sectors.

1.2 Ink Preparation and Printability

The choice of the base material as well as the recipe of its preparation to cater to the need for 3DP are of utmost importance. Bioink is the material used to produce either engineered or artificial living tissue using 3DP. It is the cell-trapping milieu composed of a multicomponent aqueous mixture that usually forms gels. This sol-gel transition of bioink is offered either by ionic bonds, covalent bonds, hydrogen bonds, or van der Waals interactions. Bioinks may be hydrogels, decellularized extracellular matrix, cell pellets, or tissue spheroids, of which hydrogels are the most common [5] due to their cell adhesion, growth, and proliferation capability since they absorb and retain large amounts of water. Ink for 3D bioprinting can be subdivided into two categories: cell-laden inks called bioink and cell-free inks called biomaterial ink. The bioinks usually consist of hydrogel precursors and are directly printed into Petri dishes filled with media and antibodies, whereas the biomaterial inks are usually utilized to print 3D scaffolds wherein the cells can be seeded on the scaffold under controlled conditions [6].

An ideal bioink should provide mechanical stability, stiffness, viscosity, surface tension, structural integrity, and biological ability – biocompatibility and biodegradability [7]. Many natural polymers like alginate, agarose, gelatin, chitosan, collagen, fibrin, and hyaluronic acid and synthetic polymers like polylactic acid (PLA), poly-D,L-Lactic acid (PDLLA), polylactic-co-glycolic acid (PLGA), polyvinyl alcohol (PVA), acrylonitrile butadiene styrene (ABS), polyethylene glycol (PEG), polyether ketone (PEEK), polycaprolactone (PCL), polybutylene terephthalate, and polyurethane (PU) [8] are used as bioinks for 3DP in the form of single or multicomponent.

Printability: The term “printability” is the ability of a bioink to form a 3D structure with accurate fidelity and integrity as per the design and the geometry. However, the terminology modulates itself according to the printing approach. For extrusion printing, the bioink must be able to form continuous filament; for the inkjet technique, it should form well-defined droplets while for laser printing, a prominent jet is required. The different printability indices [5] are the following:

- 1) *Extrudability:* The minimum extrusion pressure essential for printing at the desired flow rate.
- 2) *Strand printability:* Comparison of the diameter of printed strands with the CAD-generated parameters.
- 3) *Integrity factor:* Comparison of the thickness of printed scaffolds with designed geometries.
- 4) *Pore printability:* Comparison of the printed pores with designed internal geometry.
- 5) *Irregularity:* Comparison of outer geometry of scaffolds with designed parameters in X, Y, and Z directions.

Rheological properties and gelation kinetics determine the printability of a bioink, which is again dictated by the type of bioprinting. Low-viscosity bioinks are preferred in inkjet bioprinting; rapidly crosslinkable, shear-thinning bioinks are desirable for extrusion bioprinting and photo-crosslinkable bioinks are favorable for stereolithographic printing [9]. Rheological properties like viscosity, viscoelasticity, yield stress, shear thinning, elastic recovery, and viscoelastic shear moduli affect the printability of bioinks. The rheological properties of the crosslinked bioink must facilitate scaffold remodeling to mimic the ECM environment. This process provides physicochemical cues to the cells, promoting their spreading and proper distribution. For instance, substrates that mimic the mechanical properties and Young's modulus (~ 12 kPa) of native skeletal muscles offer better myogenic differentiation [10]. The key rheological parameters for a "good" bioink are described below:

Viscosity: Viscosity is the ratio of shear stress to shear rate and is governed by the molecular weight and concentration of the polymer. High-viscosity inks are preferable for high-fidelity printing but may limit cell growth within the substrate due to higher shear stress. This shear stress can be overcome by either using hydrogel inks having shear-thinning properties or using pre-gel solutions with lower viscosities. For e.g., alginate-based bioinks are directly extruded into calcium solution leading to ionic crosslinking. Due to higher surface tension, viscous bioinks prevent droplet formation without any merger of the columns with one another. Hence, crosslinking agents come into the picture with the caution of appropriate concentration so as to avoid phase separation and phase change [11]. Temperature-dependent hydrogen bonding or hydrophobic interactions may be exploited, as in case of gelatin, Pluronic, etc. Colloidal-like suspensions of densely packed microgels or jammed gels also prevent exposure of cells to high shear stress [12].

Viscoelasticity and yield stress: Viscoelasticity is the property of retaining elastic shape while allowing viscous flow. It is guided by three parameters – storage modulus (G'), viscous modulus (G''), and yield stress. $\tan(\delta)$, the ratio between G' and G'' , gives information about the rheological characteristics of the bioink. Yield stress is the stress limit beyond which deformation occurs. The parameters of G' and yield stress are governed by the number of crosslinks within the bioink. These crosslinks offer resistance to shape change within the yield stress. Paradoxically, though yield stress of the bioink renders shape and stiffness to the substrate, it can also deter cell encapsulation and further growth. Hence, additives like carrageenan, gellan gum, and hyaluronan are added to the bioinks to improve yield stress [13, 14]. However, in stereolithography and light-assisted bioprinting, low-viscosity bioink is required for easy flow and for each layer to be crosslinked with each other.

Shear thinning: Shear thinning is the phenomenon where increase in shear rate results in the decrease in viscosity. Partially crosslinked hydrogels, colloidal suspensions, polymer melts, or polymer solutions above certain critical concentrations show shear-thinning properties with shape preservation. Shear thinning leads to decrease in viscosity in the extrusion phase, but rise in viscosity after extrusion results in shape preservation. For e.g., shape retention in printed calcium phosphate cement is due to high zero-shear viscosity [15]. PCL and PLA melts, used in polymer-based fused deposition modeling, possess intrinsic shear thinning properties due to shear-induced disentangling and alignment of long polymeric chains [16]. High resting viscoelasticity of pastes, solid suspensions, and colloidal dispersion bioinks arises due to the restoration of interaction between the suspended particles, which had been disrupted due to the shear-thinning process [17]. Hydrogels demonstrate non-Newtonian fluid behavior with shear-thinning features. So, the random polymer chains align themselves in one direction under shear force and become suitable for extrusion process.

Surface tension: Due to surface tension, there is an attraction between the liquid molecules, which ensures a contact angle between each printed strand. When the substrate has a higher surface energy than the surface tension of the bioink, the ink spreads; conversely, lower surface energy results in less spread [18]. For e.g., shape fidelity in printed constructs of ceramic slurries is reduced by both surface tension and gravity. It has been observed that a reduction in surface energy leads to droplet formation instead of a cuboidal structure [19].

Elastic recovery: This property explains how the bioink recovers its original solid-like property without any distortion after undergoing deformation or transition from liquid to solid state [20]. Due to this feature, multi-layered structures can be built up. Elastic recovery is the combination of both viscous flow and elastic recovery where the viscous modulus, G'' , explains the fluid-like behavior of bioinks, and elastic modulus, G' , defines the solid-like behavior of bioinks imparting elastic shape recovery. While the former allows mixing of cells and extrusion, the latter allows suspension of cells. Often, these moduli vary under different conditions of temperature, stress, and shear rate.

The recovery of solid-like behavior after extrusion through a needle must be fast to ensure good shape fidelity. The rheological evaluation of a bioink is done on the basis of the kinetics of yield stress and elastic recovery. The first step is to evaluate the effect of increasing shear stress and filament-forming capability, followed by the measurement of viscosity as a function of shear rate to evaluate the shear thinning property. Then, recovery tests are done to investigate whether the materials can restore their elastic properties on exposure to alternating low and high shear stress [17].