

Contents

Preface *xv*

1	3D Bioprinting, A Powerful Tool for 3D Cells Assembling	1
1.1	What Is 3D Bioprinting?	1
1.2	Evolution of 3D Bioprinting	3
1.3	Brief Classification of 3D Bioprinting	4
1.4	Evaluation of Bioinks	5
1.5	Outlook and Discussion	6
	References	8
2	Representative 3D Bioprinting Approaches	11
2.1	Introduction	11
2.2	Inkjet Bioprinting	13
2.2.1	Mechanisms of Droplet Formation	14
2.2.1.1	Continuous-Inkjet Bioprinting	14
2.2.1.2	Drop-on-Demand Inkjet Bioprinting	15
2.2.1.3	Electrohydrodynamic Jet Bioprinting	16
2.2.2	Hydrogel-Based Bioinks for Inkjet Bioprinting	17
2.2.2.1	Material Properties for Inkjet Bioprinting Applications	18
2.2.2.2	Commonly Used Hydrogels in Inkjet Bioprinting	19
2.2.3	Representative Cell Printing Applications	20
2.2.3.1	Bone and Cartilage Tissues	21
2.2.3.2	Organoids	22
2.2.3.3	Skin Tissues	22
2.2.3.4	Vascular Networks	22
2.2.4	Summary	22
2.3	Extrusion Bioprinting	23
2.3.1	Mechanisms of Extruding Biocompatible Materials	23
2.3.2	Primary Extrusion Bioprinting Strategies	24
2.3.3	Main Categories of Extrudable Biomaterials	25
2.3.3.1	Hydrogels	25
2.3.3.2	Micro-Carriers	26
2.3.3.3	Cell Aggregates	27

2.3.3.4	Decellularized Matrix Components	28
2.3.4	Summary	28
2.4	Light-Based Bioprinting	28
2.4.1	Laser-Assisted Bioprinting	28
2.4.1.1	Mechanism	28
2.4.1.2	Materials	30
2.4.1.3	Biomedical Applications	30
2.4.2	Stereolithography	32
2.4.2.1	Mechanism	32
2.4.2.2	Materials	33
2.4.2.3	Biomedical Applications	33
2.4.3	Multi-Photon Polymerization	34
2.4.3.1	Mechanism	34
2.4.3.2	Materials	35
2.4.3.3	Biomedical Applications	35
2.4.4	Digital Light Projection 3D Printing	35
2.4.4.1	Mechanism	36
2.4.4.2	Materials	37
2.4.4.3	Biomedical Applications	37
2.4.5	Computed Axial Lithography	37
2.4.5.1	Mechanism	37
2.4.5.2	Materials and Biomedical Applications	37
2.4.6	Summary	38
	References	38
3	Bioink Design: From Shape to Function	47
3.1	Significance of Bioink Design	47
3.2	Categories of Bioink	47
3.3	Three Evaluation Criteria of Bioink	48
3.3.1	Printability	48
3.3.2	Mechanical Properties	48
3.3.3	Biocompatibility	48
3.4	Strategies for Enabling the Printability	49
3.4.1	Optimization of Cross-linking Sequence	49
3.4.2	Support Material-Assisted Bioprinting	50
3.4.3	Microgel-Based Bioink	50
3.5	Strategies for Bioink Reinforcement	50
3.5.1	Composite Bioink Design	50
3.5.2	Microfiber-Assisted Reinforcement	51
3.6	Strategies for Improving the Biocompatibility	51
3.7	Representative Bioink Design Case: GelMA-Based Bioinks	52
3.7.1	Property Characterization of the GelMA Bioink	52
3.7.2	3D Bioprinting of GelMA Bioinks with Dual Cross-linking Strategy	53
3.7.3	3D Bioprinting of GelMA Bioinks with Nanoclay as Support	55
3.8	Commercial Bioink	57

3.8.1	GelMA (EFL-GM Series)	58
3.8.2	Fluorescent GelMA (EFL-GM-F Series)	58
3.8.3	Porous GelMA (EFL-GM-PR Series)	60
3.8.4	HAMA (EFL-HAMA Series)	64
3.8.5	SilMA (EFL-SilMA Series)	64
3.8.6	PCLMA (EFL-PCLMA Series)	64
	References	66
4	Coaxial 3D Bioprinting	69
4.1	Introduction	69
4.1.1	Significance	69
4.1.2	Two Categories	72
4.1.2.1	Solid Fiber-Based Coaxial Bioprinting	72
4.1.2.2	Hollow Fiber-Based Coaxial Bioprinting	73
4.2	Printable Ink Materials	74
4.2.1	Forming Mechanism	74
4.2.2	Categories of Printable Bioinks	75
4.2.2.1	Alginate	75
4.2.2.2	Gelatin	78
4.2.2.3	GelMA	79
4.3	Representative Biomedical Applications	80
4.3.1	Morphology-Controllable Microfiber-Based Organoids	80
4.3.2	Vessel-on-a-Chip	81
4.4	Future Perspective	85
	References	86
5	Digital Light Projection-Based 3D Bioprinting	89
5.1	Introduction	89
5.1.1	Printing Process	89
5.1.2	Significance	89
5.2	Photocurable Biomaterials	91
5.2.1	Photo-Cross-Linking Mechanism	92
5.2.1.1	Conversion of Light Energy to Chemical Energy: Photoinitiator	92
5.2.1.2	Formation of Molecular Network: Monomer Polymerization	93
5.2.2	Typical Materials: Gelatin Methacryloyl (GelMA)	94
5.2.2.1	Composition and Synthesis	94
5.2.2.2	Substitution Degree	95
5.3	Printing Equipment	96
5.3.1	Optical Units	96
5.3.1.1	Image Forming: Digital Micromirror Devices	97
5.3.1.2	Objective Lens: Focusing System	97
5.3.1.3	Material Storage Units	98
5.3.1.4	Environment Controlling Systems	98
5.3.1.5	Ink Tank: Transparent and Non-stick Bottom	99
5.4	Mechanical Movement Units	99

5.4.1	Lifting Mechanism: Main Movement	99
5.4.2	Tilting Mechanism: Mixing and Separation	100
5.4.2.1	Printing Error Formation and Optimization Strategies	100
5.5	Optimization of Several Typical Structures	102
5.5.1	Printing Strategies of Solid Structures	103
5.5.2	Printing Strategies of Channel Structures	104
5.5.3	Printing Strategies of Conduit Structures	104
5.5.4	Printing Strategies of Thin-Walled Structures	105
5.5.5	Printing Strategies of Microcolumn Structures	105
5.6	Applications	107
5.6.1	DLPBP Structures with High Precision	107
5.6.2	Customized Physical Properties Bioprinting	107
5.6.3	Regenerative and Biomedical Applications	108
	References	110
6	Direct Ink Writing for 3D Bioprinting Applications	113
6.1	Introduction	113
6.2	Printable Bioinks in DIW	114
6.2.1	Supporting Mechanisms and Representative Bioinks	115
6.2.1.1	Rapid Solidification-Induced Mechanical Stiffness Improvement	115
6.2.1.2	Yield-stress Additive-Induced Self-Supporting Capacity	119
6.2.2	Design Criteria of Bioinks for Direct Writing Applications	121
6.2.2.1	Rheological Properties	122
6.2.2.2	Cross-linking Capacity	122
6.2.2.3	Biocompatibility and Biodegradation	123
6.2.2.4	Mechanical Properties	124
6.3	Technical Specifics in Direct Ink Writing	124
6.3.1	Investigation on Printability of Bioinks	124
6.3.2	Different Printing Strategies in Rapid Solidification-Induced 3D Printing Approach	126
6.3.2.1	Printing of Thermal Cross-linkable Biomaterials	126
6.3.2.2	Printing of Ionic Cross-linkable Biomaterials	127
6.3.2.3	Printing of Photo Cross-linkable Biomaterials	128
6.3.2.4	Printing of Enzyme Cross-linkable Biomaterials	129
6.3.3	3D Structure Printing Using Self-Supporting Material-Assisted 3D Printing Approach	130
6.3.3.1	Internal Scaffold Additive-Assisted 3D Printing	130
6.3.3.2	Microgel Additive-Assisted 3D Printing	132
6.4	Representative Biomedical Applications	132
6.4.1	Aortic Valve Printing	132
6.4.2	Bone and Cartilage Tissue Printing	133
6.4.3	Cardiac Tissue Printing	134
6.4.4	Liver Tissue Printing	135
6.4.5	Lung Tissue Printing	135
6.4.6	Neural Tissue Printing	135

6.4.7	Eye and Ear Printing	136
6.4.8	Pancreas Printing	137
6.4.9	Skin Tissue Printing	137
6.4.10	Blood Vessel Printing	138
6.5	Conclusions and Future Work	138
	References	139
7	Liquid Support Bath–Assisted 3D Bioprinting	149
7.1	Introduction	149
7.2	Liquid Support Bath Materials	150
7.2.1	Support Bath Materials Based on Different Supporting Mechanisms	151
7.2.1.1	Unrecoverable Matrix Materials	151
7.2.1.2	Buoyant Support Fluids	151
7.2.1.3	Reversibly Self-Healing Hydrogels	153
7.2.1.4	Yield-Stress Fluids	154
7.2.2	Preparation Methods	156
7.2.2.1	Microparticle Aggregation	156
7.2.2.2	Homogenous Suspensions with Micro/Nanostructures	157
7.2.2.3	Chemical Synthesis	158
7.2.2.4	Other Methods	158
7.2.3	Design Criteria for Ideal Liquid Support Bath Material	158
7.2.3.1	Rheological Properties	158
7.2.3.2	Chemical Stability	159
7.2.3.3	Physical Stability	159
7.2.3.4	Biocompatibility	161
7.2.3.5	Hydrophilicity and Hydrophobicity	161
7.2.3.6	Others	161
7.3	Scientific Issues During Liquid Support Bath–Assisted 3D Printing	162
7.3.1	Effects of Operating Conditions on Filament Formation in Support Bath	162
7.3.2	Effects of Support Bath Materials on Filament Morphology	162
7.3.2.1	Rheological Properties of Support Bath Materials	162
7.3.2.2	Diffusion of Ink Materials into Surrounding Support Bath	163
7.3.2.3	Interfacial Tension–Induced Filament Deformation	165
7.3.3	Effects of Nozzle Movement on the Printed Structure	165
7.3.4	Path Design in Liquid Support Bath–Assisted 3D Printing	166
7.4	Post-treatments for Liquid Support Bath–Assisted 3D Printing	167
7.4.1	Post-treatments in e-3DP	167
7.4.2	Post-treatments in Support Bath–Enabled 3D Printing	169
7.5	Representative Biomedical Applications	169
7.5.1	Organ Printing	169
7.5.2	Lab-on-a-Chip	171
7.5.3	Other Bio-Related Applications	173
7.6	Conclusions and Future Directions	173
	References	175

8	Bioprinting Approaches of Hydrogel Microgel	179
8.1	Introduction	179
8.2	Auxiliary Dripping	179
8.2.1	Inkjet Printing	180
8.2.1.1	Piezoelectric Inkjet	180
8.2.1.2	Thermal Bubble Inkjet	183
8.2.2	Laser-Assisted Printing	184
8.2.3	Electrohydrodynamic Printing	185
8.3	Diphase Emulsion	195
8.3.1	Nonaqueous Liquid Stirring	195
8.3.2	Air-Assisted Atomization	197
8.3.3	Microfluidic Technology	198
8.4	Lithography Technology	202
8.4.1	Replica Mold	202
8.4.2	Discrepant Wettability	203
8.4.3	Photomask Film	206
8.4.4	Digital Light Processing	208
8.5	Bulk Crushing	208
	References	211
9	Biomedical Applications of Microgels	213
9.1	Introduction	213
9.1.1	Tiny Size	213
9.1.2	Hydrogel Network	213
9.1.3	Complex Mechanical Properties	214
9.2	In Vitro Model	214
9.3	Cell Therapy	216
9.4	Drug Delivery	219
9.5	Cell Amplification	223
9.6	Single-Cell Capture	227
9.7	Supporting Matrices	229
9.8	Secondary Bioprinting	232
	References	235
10	Microfiber-Based Organoids Bioprinting for In Vitro Model	237
10.1	Introduction	237
10.1.1	Significance and Challenge	237
10.1.2	Hydrogel Materials	238
10.2	Coaxial Bioprinting of Bioactive Cell-laden Microfiber	238
10.2.1	Microfluidic Coaxial Bioprinting	239
10.2.2	Coaxial Nozzle-Assisted Bioprinting	240
10.3	Heteromorphic/Heterogeneous Microfiber Bioprinting	241
10.3.1	Heteromorphic Microfiber	242
10.3.2	Heterogeneous Microfiber	244

10.4	3D Assembly of Microfibers	245
10.4.1	3D Bioweaving	245
10.4.2	3D Bioprinting	245
10.5	Microfiber-Based Organoids Bioprinting for In Vitro Mini Tissue Models	247
10.5.1	Vascular Organoid	247
10.5.2	Myocyte Fiber	248
10.5.3	Nerve Fiber	248
10.5.4	Cardiomyocyte Fiber	249
10.5.5	Co-cultured Multi-organoids Interactions	249
10.6	Discussion and Outlook	250
	References	251
11	Large Scale Tissues Bioprinting	257
11.1	Introduction	257
11.1.1	Challenges in Bioprinting Large Scale Tissues	257
11.1.2	Strategies in Bioprinting Large Scale Tissue with Nutrient Networks	258
11.1.2.1	Porous Network Printing	258
11.1.2.2	Hollow Channel Network Printing	259
11.1.2.3	Advanced Bioprinting Techniques-Enabled Printing Highly Biomimetic Vascular Network	259
11.2	Large Scale Cell-laden Porous Structures Printing	259
11.2.1	Independent Porous Structure Printing	259
11.2.2	Interconnected Porous Structure Printing	261
11.2.2.1	Directly Cell-laden Scaffold Printing	261
11.2.2.2	Synchronous Bioprinting (Bioink and Sacrificial Ink Half and Half)	261
11.2.3	Heterogeneous Independent/Interconnected Porous Structure Printing	262
11.2.4	Long-term Perfusion Culture on a Chip	265
11.2.5	Discussions (Properties, Pros, Cons, etc.)	265
11.3	Large Scale Cell-laden Structures with Vascular Channel Printing	266
11.3.1	Sacrificial Bioprinting	266
11.3.2	Coaxial Bioprinting	267
11.4	One-step Coaxial/Sacrificial Printing of Large Scale Vascularized Tissue Constructs	268
11.4.1	Mechanism	268
11.4.2	Freeform Structure with Vascular Channels Printing	269
11.4.3	Heterogeneous Structure with Vascular Channels Printing	270
11.4.4	Long-term Perfusion Culture on a Chip	272
11.4.5	Discussion (Properties, Pros and Cons, etc.)	272
11.5	Advanced Bioprinting Technique-Enabled Printing Highly Biomimetic Tissues	273
11.5.1	Support Bath-Assisted Bioprinting	273
11.5.2	Light-Based Bioprinting	273

11.5.3	Discussion (Properties, Pros and Cons, etc.)	275
11.6	Representative Biomedical Applications	275
	References	276
12	3D Printing of Vascular Chips	281
12.1	Introduction	281
12.2	Construction Process of Hydrogel-Based Vascular Chips	282
12.2.1	Damage-Free Demolding Process Based on Soft Fiber Template	282
12.2.1.1	Damage-Free Demolding Process	283
12.2.1.2	Comparative Analysis of Damage-Free and Conventional Demolding Processes	283
12.2.2	Hydrogel Bonding Strategy Based on Twice-Cross-linking Mechanism	286
12.2.2.1	Manufacturing Process of Hydrogel-Based Microfluidic Chips	287
12.2.2.2	Mechanism Study	287
12.2.2.3	Material Selection	288
12.2.2.4	Feasible Domain	289
12.2.2.5	Bonding Results	289
12.2.3	Multi-Scale 3D Printing Process	291
12.2.3.1	Mechanism of Multi-Scale 3D Printing Process	291
12.2.3.2	Printing Parameters	292
12.2.4	Construction Process of Hydrogel-Based Vascular Chips	293
12.3	Characterization of Vascular Chips	295
12.3.1	Fundamental Characterization of Vascular Chips	295
12.3.1.1	Characterization of Endothelium Function of Channels	295
12.3.1.2	Characterization of Endothelial Cells Viability	295
12.3.1.3	Characterization of Endothelial Cells Morphology	296
12.3.1.4	Characterization of Endothelium Channel	297
12.3.2	Morphology Characterization of Hydrogel-Based Vascular Chips	298
12.3.2.1	Multi-Level Bifurcated Channel Network Structure	298
12.3.2.2	Multi-Scale Vascular Model	299
12.3.2.3	Biomimicking Vascular Model	299
12.3.3	Characterization of Vascular Function	302
12.3.3.1	Nutrition Supply Function	302
12.3.3.2	Expression of Key Functional Proteins in Endothelial Cells	302
12.3.3.3	Simulation of Vascular Inflammation Reaction	303
12.3.3.4	Characterization of Vascular Barrier Function	304
12.4	Conclusion	307
	References	308
13	3D Printing of In Vitro Models	311
13.1	Introduction	311
13.2	Typical 3D Bioprinting Technologies and Common Target Tissue/Organ Demand	312
13.2.1	Inkjet-Based Bioprinting	313

13.2.2	Extrusion-Based Bioprinting	314
13.2.3	Light-Assisted Bioprinting	315
13.3	Developing Process of In Vitro Models	316
13.3.1	Mini-Tissue in 3D Growth State	316
13.3.1.1	Sphere Mini-Tissue Model	316
13.3.1.2	Fiber Mini-Tissue Model	317
13.3.1.3	Array Mini-Tissue Model	318
13.3.1.4	Limitations	319
13.3.2	Organ-on-a-Chip with Multiplex Microenvironment	319
13.3.2.1	Integrated Organ-on-a-Chip	321
13.3.2.2	Modular Microfluidic System	322
13.3.2.3	Multiple-Organ System	323
13.3.2.4	Limitations	325
13.3.3	Tissue/Organ Construct with Biomimicking Property	325
13.3.3.1	Vascular Construct	326
13.3.3.2	Vascularized Tissue Construct	328
13.3.3.3	Limitations	330
13.4	3D Printing of In Vitro Tumor Models	330
13.4.1	Tumor Cell-Laden Construct	330
13.4.2	Multi-Cell Tumor Sphere	331
13.4.3	Tumor Metastasis Model with Angiogenesis	332
13.5	Summary and Prospect	334
13.5.1	Key Virtue and Comparison	334
13.5.2	Outlook	334
13.5.2.1	3D Bioprinting Technology	335
13.5.2.2	Individual Differences	335
13.5.2.3	Systematic Interaction	335
13.5.2.4	Industrialization	335
13.6	Conclusions	336
	References	336
14	Protocol of Typical 3D Bioprinting	339
	Reference	343
	Index	345

