Contents

A Note to the Reader	
Foreword	
Donald E. Ingber	
Preface	
Acknowledgments	
63	
Introduction: Frailty, Thy Name Is Human	1
1. Printing Paradigms	3
Automated Printing	3
3D Printing—The Foundation of Bioprinting	4
3D Printing Techniques	5
Trailblazers of Bioprinting	7
Medical Successes of 3D Printing	9
Transitioning from 3D Printing to Bioprinting	10
Bioreactors—A Boarding School Where Bioprinted Constructs	
Go to Mature	13
Bioinks—The Write Stuff	14
Extracellular Matrices—The Places Cells Call Home	15
Rheology—It's Flow Time	15
Scaffolds	17
A Return to Paradigm Shifts	18
2. All About Cells	21
Mapping out Our Tour	21
Cell Surface Membrane	23
Cell Membrane Proteins	25
Cell Factories	27
Becoming a Cell—Stem Cells	29
Induced Pluripotent Stem Cells	30
Additional Sources of Stem Cells	31
Progenitor Cells	31
The Different Cells in Our Body	32
The Human Cell Atlas	35
Cells Live in Three Dimensions—Not Two Dimensions	35
Cells Talk	36
"When Is a Kidney a Kidney?"	37
Moore's Law	38

3.	Bioprinted Cartilage: The Dream and the Devilish Details Articular Cartilage—A Heavily Challenged Tissue	40 41
	Bioprinted Hydrogels with Articular Cartilage Progenitor Cells	43
	Bioprinting of Collagen Hydrogels for Cartilage Substitutes	47
	Bioprinted Ears from an Integrated Tissue–Organ Printer	50
	Decellularized Extracellular Matrix Bioink	52
	Decellularization: Re-creating a Native Extracellular Matrix with	
	Optimal Fidelity	55
4.	Vascularization: Getting Blood from Here to There, Everywhere	58
	The Vasculature of the Human Body	58
	Blood Vessels Aren't Just Pipes	61
	Microchannel Networks Created with Fugitive Ink	63
	Multi-nozzle Print Heads—It's About Time	64
	Creating Vasculature in Bioprinted Tissues	64
	The Thickness Dilemma of Vascularized Tissue	67
	Responding to the Thickness Dilemma in Bioprinted Tissue	67
	The Splendid Scheme	68
	Perfused Vasculature Brings the Gift of Life	72
	Response to the Thickness Dilemma, Take Two	73
5.	Innervation: The Body's Internet	77
	Nerve Growth Factor	78
	The Nervous System of the Human Body	80
	Bioprinting of a Cellular Nerve Guide	81
	Implanted Nerve Guides	83
	A Bioprinted Nerve Guide Using Growth Factors as Biochemical Cues	84
	Bioprinting Takes Aim at the Central Nervous System—Spinal Cord Tissue	89
	Nervous System Nomenclature	90
	Optimizing Bioink for Induced Pluripotent Stem Cell-Derived Neural	00
	Progenitor Cells	92
	Building Spinal Cord Scaffolds	93
	Cell Types Within Spinal Cord Scaffolds Calcium Imaging of Bioprinted Spinal Neurons	93 95
6.	Skin and Bones—and Muscle Too	98
	Give Me Some (Bioprinted) Skin	99
	Bioprinted Skin with Uniform Pigmentation Throw Me a (Bioprinted) Bone	100 104
	Morphing Cartilage to Bone: Emulating Embryonic Development	104
	A Succinct Sketch of Gene Therapy	108
	Gene Activated Bioinks	109
	Bioprinted Skeletal Muscle	1109
	The Twitch	113
	Bioprinted Neuromuscular Junctions—Inks from the Silkworm <i>Bombyx mori</i>	113
	Skeletal Muscle Alphabet Soup	116
	Cat's Cradle	116

7.	The Liver: Regenerative Tissue That Can Almost Bioprint Itself	119
	The Hectic Life of the White Rabbit	120
	Cytoarchitecture	121
	Liver Cells Are Swimming in Blood	122
	Your Liver Is Like a Plush Bath Towel	123
	Bioprinting of Human Pluripotent Stem Cells for the Generation of	
	Liver-Like Cells	124
	Searching for Cell Markers	125
	Patterning of Cell-Laden Hydrogels in the Form of Liver Tissue	126
	It's All Done with Mirrors	130
	Physiological Relevance	131
	Bioprinting Liver Tissue by Fusing Liver Bud Spheroids	133
	Caution: Road Work Ahead	134
	Human Liver Tissue Seeds	135
	A Historical Event: Organovo	136
8.	The Heart: Cardiac Patches, Calcium, and Contraction Forces	138
	Keeping a Sense of Perspective	139
	The Sistine Chapel Ceiling Pre-Michelangelo	140
	High-Resolution Bioprinted Heart Patch	140
	Heart Attack	142
	Back from the Abyss	144
	Goosebump Moment	145
	Who Is to Blame for the Pipe Water?	146
	Pre-Vascularized Bioprinted Heart Patch	147
	A Mini Noah's Ark	147
	Ejection Fraction and Echocardiograms	148
	Fractional Shortening	149
	Bioprinted Functional and Contractile Cardiac Tissue	150
	Hooke's Law	152
	Coming Back to Contractile Cardiac Tissue	152
9	Organs-on-a-Chip: Tissues for Testing	155
9.		
	A Lilliputian Landscape Donald Ingber's Voyage to Organs-on-a-Chip	155 158
	Tensegrity—Say What?	158
	Geometric Control of Cell Life and Death	159
	Miniaturized Plumbing	160
	Bioprinted Three-Tissue Organ-on-a-Chip Platform	162
	Flexing Tissue Module Muscles One at a Time	165
	Flexing Tissue Module Muscles Two at a Time: Liver- and Heart-on-a-Chip	100
	Interactive Tests	166
	Lung Module Characterization	167
	Three at a Time: Liver, Heart, and Lung Interactive Tests—Enter Sherlock	_01
	Holmes	167
	The Plot Thickens	170
	Bioprinting a Functional Airway-on-a-Chip	171

x Contents

	Asthmatic Airway Epithelium on the Bioprinted Airway-on-a-Chip Mucus Invasion of the Dust Mites Return Engagement Bioprinting Versus Manual Seeding Animal Testing	174 176 177 178 179
10.	The Kidney: The Ne Plus Ultra of Filters The Nephron Is the Kidney's Workhorse Keeping on an Even Keel: Maintaining Electrolyte and Fluid Homeostasis Proximal Convoluted Tubules—A Cherished Goal for Bioprinting Starting out on the Road to Proximal Convoluted Tubules on Perfusable Chips Printed Proximal Tubules Form a Polarized Epithelium Drug Toxicity Testing Vectorial Transport—A Directed Passage Healthy and Mature Phenotypes Active, Selective Reabsorption of Key Solutes Measuring Albumin Reabsorption Hyperglycemia	183 184 186 187 190 191 192 193 195 198 200
11.	What's in the Offing? A Thumbnail History of Polio Contemplating Bioprinting's Possible Future with a Sketch of Polio's History in Hand Vascularization and Scaling up to Human Size Aggregates of Pluripotent Stem Cells: Spheroids, Organoids, and Embryoid Bodies High Cellular Density with Vasculature via Embedded Printing A Slice May Suffice Organ Reserve A Portion Might Be the Prescription An Editorial Perspective	202 202 203 203 203 213 214 215 216
Epi	logue	219
Note Glos	ssary	221 259 279