Index

Abbreviations, xi–xiii
Acellular collagenous grafts, 295
Acetic acid, for collagen ELISA buffers, 198
ACL differentiation inducer, 196
ACL fibroblasts (ACLFs), in ACL tissue engineering, 195, 196. *See also* Anterior cruciate ligament (ACL); Human ACL fibroblasts (ACLFs)
Action potentials, of engineered heart tissue preparations, 282–284
Adenoviral gene transfer, in engineered heart tissue, 273, 274
Adhesion, cell, 444
Adipocytes, mesenchymal stem cell differentiation to, 29
Adipogenesis assay
  fixing mesenchymal stem cells for, 51–52
  staining mesenchymal stem cells with Nile Red for, 52–53
Adipogenic induction, in vitro, 50–51
Adipogenic induction medium (AIM), 50, 51
  solutions for, 31
ADMET (adsorption, distribution, metabolism, excretion, toxicity) drug testing, 423
Adrenal anlagen, SA cells from, 378
Adrenaline, 378
Adrenal medullary chromaffin cells, 377–378, 379
Adrenal medullary endothelial cells, 379
Adrenal medullary organoids, 379
Adrenergic cells, 378
Adventitial layer, of arteries, 297
Agarose, chondrogenic cells on, 174
Agarose culture model, for articular chondrocytes, 95
Aggrecan (Agg)
  in articular cartilage repair, 159, 160
  assays of, 347
  immunohistochemical staining for, 231–232
Aggregate culture model, for articular chondrocytes, 95
Aggregate cultures, for neuroendocrine/neuronal cells, 383–384
Aggregation, in chondrocyte cultures, 101–103
Albumin secretion, liver, 428, 429–432
Albumin secretion quantification, enzyme-linked immunosorbent assay for, 429–430
Alginate beads. *See also* Alginate encapsulation
  proteinase K digestion of, 182–183
  release of chondrocytes from, 174–175
Alginate culture model, for articular chondrocytes, 95, 97–98, 160
Alginate depolymerization solution, 87
Alginate dissolving buffer, preparation for articular cartilage cultures, 162–163
Alginate encapsulation for articular cartilage cultures, 162–163
  of chondrocytes, 172–173
Alginate-recovered chondrocyte (ARC) method, 173–176. *See also* ARC entries
Alginate solution, 87
  preparation for articular cartilage cultures, 162
Alkaline phosphatase (ALP) activity, determination of, 45–46
Alkaline phosphatase assays, 347
  biochemical, 46–47
  for bone constructs, 351–353
  cytochemical, 47–48
  solutions for, 30–31
Allen, Jared W., 417
Allogenic chondrocytes, 89
Allografts, 133
  osteochondral, 159
Allograft tendons, ACL replacement via, 193
Altman, Gregory H., 191

Culture of Cells for Tissue Engineering, edited by Gordana Vunjak-Novakovic and R. Ian Freshney

Amino acids, engineered blood vessels and, 300
Ammonia production, assessment of, 149
Analytical assays, bone engineering, 347–362
Angiogenesis, 76–77
ANOVA (analysis of variance), in ligament tissue engineering, 205
Anterior cruciate ligament (ACL), 193. *See also* ACL entries
  injury to, 193–194
Anterior cruciate ligament tissue engineering. *See also* ACL differentiation inducer
  background of, 194–195
  choice of cells for, 195
  knee function restoration via, 193
  silk fibroin as scaffold for, 205–207
  silk in, 195
Antibiotics, microbial contamination and, 16–17
Antibodies. *See also* SH2 antibodies
  desmin, 247
  labeling with fluorescent, 72, 73–74
  sarcomeric tropomyosin, 249, 250
Antigens, mesenchymal stem cell–specific, 335
Antithrombin III, 297
Applications. *See also* Clinical applications; In vitro applications
  of bone tissue engineering, 366–368
  of engineered heart tissue, 284–286
  of human skeletal muscle engineering, 239–257
  of lipid-mediated gene transfer, 124–125
  of liver tissue engineering, 457–461
ARC cartilage, collagen extraction from, 181–182. *See also* Alginate-recovered chondrocyte (ARC) method
ARC chondrocytes, culture in filter well inserts, 175–176
Arginine-glycine-aspartic acid (RGD) surface modification, of silk fibers, 200–201, 207–208. *See also* Silk-RGD
Arterial grafts, 294, 295–296
Arteries. *See also* Blood vessels
  structure of, 296–297
  tissue-engineered, 301
Articular cartilage. *See also* Articular chondrocytes
  collection of, 91
  human, 89–91
  media and reagent preparation for, 160–168
  repair capacity of, 159
  sources of materials for, 185–187
  tissue engineering of, 157–189
Articular cartilage tissue, harvesting, 168–169
Articular chondrocytes
  for cartilage engineering, 136
  for cartilage tissue engineering, 89–98
  monolayer culture of, 93–94
  monolayer culture under proliferating conditions, 171–172
  nonhuman, 91–92
  proliferation of, 92–93
  transfection of, 114
L-Ascorbate-2-phosphate stock, 88
Ascorbic acid (AA), engineered blood vessels and, 300
L-Ascorbic acid, in bone tissue engineering, 332–333
Ascorbic acid 2-phosphate, 30
Ascorbic acid stock, 86
Aspiration, of human bone marrow, 31–32
Assays
  for adipogenesis, 50–53
  of biomechanical properties, 184
  bone engineering, 347–362
  calcium, 49–50
  of cartilaginous tissue formation, 176–178
  for chondrocyte phenotypic modulation, 98
  collagen, 164–166, 178–180
  DNA, 166–168
  of engineered constructs, 148–149
  hepatocyte function, 428–435
  of human embryonic stem cells, 64
  of hydrogel-encapsulated cartilage, 226–227, 232–234
  for in vitro chondrogenesis, 103–104
  in ligament tissue engineering, 203–205
  myoblast purity, 247, 248–249
  for osteogenesis, 43–50
  for phenotypic potency, 27–28
  photoencapsulation and, 219
  proteoglycan, 163–164, 176–178 for reporter gene expression, 122–124
Atherosclerotic vascular disease, 294
Athletic disability, 193, 194
Authorization, with human culture material, 10
Autograft, 133
Autologous bone, harvesting of, 366
Autologous cells, 325
Autologous chondrocyte implantation (ACI) protocol, 92–93
Autologous chondrocytes, in articular cartilage repair, 159
Autologous grafts, 325
Autologous stem cells, in tissue engineering, 25
Autologous tendon grafts, ACL replacement via, 193
Autologous venous grafts, 294, 301
Avian bioartificial muscles, 240
Avian chondrocytes, transfection of, 115
Axons, dorsal root ganglia, 400–401

Bacterial infections, of cell cultures, 17
Basal medium, 88–89, 117
Basic fibroblast growth factor (bFGF, FGF-2), 18, 63
  in bioreactor cultivation, 146–147
  preparation of, 334
Bergstrom biopsy needle apparatus, 243–244

β-adrenergic agonists, in cardiac myocyte- populated matrix, 262
β-glycerophosphate (BGP), 30
Bhatia, Sangeeta N., 417
Bile duct excretion, 428
Bioartificial muscles (BAMs), 240–241
  in extracellular matrix materials, 249–253
  in gene therapy, 254
  in muscle repair/replacement, 254–255
Biochemical alkaline phosphatase assays, 46–47
Biochemical analysis, of cartilage hydrogels, 222
Biochemical evaluation, of engineered constructs, 148–149
Biocompatibility, of silks, 327, 328
Biomaterial, silk as, 325–328
Biomaterial scaffolds, 138–139. *See also* Scaffolds
Biomechanical properties, assessment of, 184
Biopsies
  for human skeletal myoblasts, 244
  skeletal muscle needle, 242–244
Biopsy instruments, 243–244
Bioreactor cultivation, 146–149
  assessment of engineered constructs in, 148–149
  physical signals in, 147–148
Bioreactor culture, zonated CYP2B expression resulting from, 449–450
Bioreactor/flow circuit assembly, for hepatocyte micropatterning, 448–449
Bioreactors, 139–141, 336. *See also* Rotating wall vessel (RWV) bioreactors; Vascular cell bioreactor
  in bone tissue engineering, 343
  hepatocytes in, 421–422, 423
  for ligament tissue engineering, 202–203
  preparation for engineered blood vessels, 306, 307–308
  types of, 140–141
Bioreactor systems, for regulating hepatocyte zonal heterogeneity, 444–451
Biosafety, human culture material and, 10–11
Biphasic ceramics, 39
Blastocysts, 62
Blocking buffer, 242
  preparation for liver tissue engineering, 425
Blood vessels. *See also* Arteries; Capillary tube formation; Engineered blood vessels; Vascular entries; Venous grafts; Vessel formation
  structure of, 296–297
  tissue-engineered, 293–322
BM-cycline, 18
Bombyx mori silk, 200, 207, 327, 328, 336
Bone. *See also* Collagen entries; Osteoblasts; Osteogenesis
  pellet culture of, 342
  tissue engineering of, 323–373, 343–347
Bone constructs
  assay of alkaline phosphatase activity in, 351–353
  calcium assay in, 353–355
  fixation and paraffin embedding of, 363–365
  gene expression analysis in, 360–362
  glycosaminoglycan assay in, 355–357
  RNA extraction from, 357–360
  von Kossa staining of sections of, 364–365
Bone engineering, analytical assays related to, 347–362. *See also* Bone tissue engineering
Bone-like tissue, engineering of, 367
Bone marrow, 340. *See also* Marrow entries
  aspiration of, 31–32
  characterization of hMSCs from, 340–343
  chondroprogenitor cells from, 99, 100, 101
  collection of, 32
  isolation of hMSCs from, 337–338
  precursor cells from, 137
  stem cells in, 335
Bone marrow-derived mesenchymal stem cells, 217, 218
Bone marrow-derived stem cell isolation/expansion, 218–219
Bone marrow stromal cell adhesion, surface modification of silk fibers with RGD for, 200–201, 207–208
Bone marrow stromal cell culture medium (BMSC medium)
  for ligament tissue engineering, 197
  seeding silk matrix cords with, 201–202
Bone marrow stromal cells (BMSCs), in ACL tissue engineering, 195, 196, 200. *See also* Human bone marrow stromal cells (hBMSCs)
Bone morphogenic protein-2 (BMP-2). *See also* Osteogenic protein-1 (OP-1, BMP-7)
  assays of, 347, 348
  in bone tissue engineering, 332
  preparation of, 334–335
Bone sialoprotein (BSP)
  assays of, 347
  silk matrix design and, 207
Bone tissue engineering, 343–347. *See also* Bone engineering
  histology of, 363–365
  in vitro applications of, 366–368
  reagent and media preparation for, 332–335
  sources of materials for, 368–370
Bone tissue scaffolds, silk purification for, 328–329
Bovine articular cartilage, 91, 93, 95. *See also* Cartilage
  isolation of chondrocytes from, 169–170
Bovine articular cartilage digestion medium, 86
Bovine articular cartilage tissue, harvesting, 168–169
Bovine cartilage
  histologic sections of, 224–225, 226–227
  primary culture of, 119–120
Bovine cartilage cells, gene transfer to, 120–121
Bovine chondrocytes, photoencapsulation of, 224–228. *See also* Chondrocyte

Bovine knee joint, chondrocyte isolation from, 217–218
Brain, tissue constructs transplanted into, 393–394
Brain-derived neurotrophic factor (BDNF), 379
Bramano, Diah, 191
5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal), 122. *See also* X-Gal entries
Buffers, for scanning electron microscopy, 198

Calcium
  in cardiac myocyte-populated matrix, 262
  contractile heart tissue response to, 282, 283
Calcium- and bicarbonate-free Hanks' balanced salt solution with HEPES (CBFHH), 266
Calcium assay, 49–50
  in bone constructs, 353–355
Calcium chloride ($CaCl_2$) solution, 87
  preparation for articular cartilage cultures, 163
Calcium-magnesium-free phosphate-buffered saline (CMF-PBS, PBSA), for dorsal root ganglia cultures, 403. *See also* CMF; Invitrogen
Cameron, Don F., 375, 377, 390
Canine jugular vein endothelial cells, in venous grafts, 301
Canine model, venous grafts in, 301
Capillary tube formation, in vitro, 76
Caplan, Arnold I., 23
Cardiac constructs. *See also* Engineered heart tissues (EHTs)
  assessment of, 149
  physical signals of, 148
Cardiac myocyte-populated matrix (CMPM), 261–262
Cardiac myocytes, 134–135. *See also* Engineered heart tissues (ETHs)
  culturing of, 261–263
  in engineered heart tissues, 285
  neonatal, 268–269
  primary, 137
  TEM of, 280–282, 285
  use in tissue engineering, 138
Cardiac tissue engineering, 137. *See also* Engineered heart tissues (EHTs)
  gel-cell seeding of porous scaffolds for, 144–145
Cardiovascular disease, 294
Cartilage, 149. *See also* Chondrocytes; Chondrogenesis; Collagen entries
  body sites of, 84
  harvesting, 168–169
  histologic sections of, 224–225, 226–227
  pellet culture of, 343
  repairing lost, 84–85
  in tissue engineering, 134–135
  transfected, 122–123
  types of, 84
Cartilage constructs
  fixation and paraffin embedding of, 363–365
  gene expression analysis in, 360–362
  glycosaminoglycan assay in, 355–357
  Safranin-O staining of sections of, 365
Cartilage engineering, cells used for, 136
Cartilage extracellular matrices, 84
Cartilage hydrogels, 222–224
  biochemical analysis of, 222
  histologic analysis of, 223–224
  RT-PCR of, 222–223
Cartilage implants, tissue engineering from cells with chondrogenic potential, 104
Cartilage layers, isolation of, 224
Cartilage medium, for cell characterization, 334
Cartilage medium double concentration, preparation for bone tissue engineering, 334
Cartilage tissue engineering, 366. *See also* Lipid-mediated gene transfer
  articular chondrocytes for, 89–98
  cell sources for, 83–111
  dynamic seeding in spinner flasks for, 142–143
  media and reagent preparation for, 85–89
  photoencapsulation of bovine chondrocytes for, 224–228
  photoencapsulation of goat bone marrow-derived mesenchymal stem cells for, 228–235
  recombinant DNA technology in, 114
  sources of materials for, 105
Cartilaginous aggregates, 101–103
  Toluidine Blue metachromatic matrix of, 102
Cartilaginous tissue, fabrication by scaffold-free alginate-recovered chondrocyte method, 173–176
Cartilaginous tissue formation, criteria for evaluating, 176–185
Casting
  of circular engineered heart tissues, 276, 285–286
  of engineered rat heart tissue, 271–272
Casting molds
  for cardiac myocyte-populated matrix, 262
  for engineered heart tissue cultures, 267
  silicone rubber, 249, 250–251
Catecholaminergic cells, 377, 379
Cationic liposomes, transfection via, 115
cbfa1, assays of, 347
CD105, 335
Cell adhesion, 444
Cell-associated matrix (CM), 160
Cell-based therapies
  liver disease and, 419, 420
  to replace impaired myocardium, 260
Cell-based therapy development, liver tissue engineering for, 458–459
Cell-cell interactions, 435–438
  differentiation and, 19
Cell characterization, cartilage medium for, 334
Cell concentration, 13–14
Cell cultures. *See also* Cocultures; Cultures; Subculture

characterization and validation of, 16–20
cryopreservation of, 14–16
of human marrow-derived mesenchymal stem cells, 26–27
isolation of cells for, 9–11
for ligament tissue engineering, 196
micropatterned, 435–444
neuroendocrine/neuronal, 375–415
thawing and maintenance of hMSC, 339
types of, 4–9
Cell cycle, senescence in, 12
Cell density, 13–14
Cell encapsulation, 225. *See also* Cellular photoencapsulation; Encapsulation; Photoencapsulation photopolymerization for, 216
Cell expansion, in undifferentiated state, 77–78. *See also* Cell isolation/expansion; Expansion entries
Cell-extracellular matrix interactions, 435–438
Cell-hydrogel constructs, 215
Cell isolation
for cellular photoencapsulation, 217–219, 225
in ligament tissue engineering, 199–200
Cell isolation/expansion, for cellular photoencapsulation, 228–230
Cell lines, 4
cross-contamination of, 16
growth cycle of, 12–14
life span of, 12
origin of, 6
Cell loading, of ceramic cubes, 40–41
Cell number and viability, determination of, 170
Cell pellets, preparation of, 349, 354
Cell recombination, 7, 8, 9
Cell replacement therapy, for Parkinson disease, 390–391
Cells
with chondrogenic differentiation potential, 98–100
preferential detachment and attachment of, 75
proliferation versus differentiation of, 4–7
in tissue engineering, 135–137
Cell–scaffold–bioreactor system, for tissue engineering, 135
Cell-seeded silk matrices, isolation of collagen from, 204–205
Cell seeding
of cryopreserved murine embryonic fibroblast cells, 66
of human mesenchymal stem cells, 33–34
of mitotically inactivated murine embryonic fibroblasts, 66–67
onto scaffolds, 78, 122
in 3D scaffolds, 142
Cell seeding/cultivation, in ligament tissue engineering, 201–202
Cell sources
for liver cell-based therapies, 419–420
liver tissue engineering for, 459
in tissue engineering, 136–137
Cell types
hand enrichment of, 75
isolation from cultures originating from ES cells, 72–75
Cellular micropatterning, on modified surfaces, 441–444. *See also* Micropatterning
Cellular photoencapsulation, 213–238
biochemical analysis in, 232–235
biochemical characterization in, 226–228, 230 of bovine chondroctyes, 224–228
cell isolation/expansion for, 228–230
engineered tissue analysis in, 221–224
histology and immunohistochemistry in, 229–230
in hydrogel scaffold, 219–221
media and reagent preparation for, 217
methodology principles in, 216
sources of materials for, 235–236
tissue harvest and cell isolation in, 217–219
Cellular polarity, 7, 19
Cell viability assay, in ligament tissue engineering, 203
Centrifugation, of hMSC cultures, 339
Ceramic cubes
implantation into SCID mice, 41–43
preparation and cell loading of, 39–40, 40–41
Characterization, cell culture, 16–20
Charged surfaces, as culture substrates, 9
Chen, Jingsong, 191
Chicken eggs/embryos, preparation for dorsal root ganglia cultures, 401–402. *See also* Embryonic chick entries
Chicken embryo dorsal root ganglia, 400–401
dissection and culture of, 404–408
Chief surgeon, in culture projects, 10
Chloramphenicol-acetyl transferase (CAT) gene, 122
Chondrocyte cultures
collagen content of, 178–180
proteoglycan content of, 176–178
Chondrocytes, 84–85. *See also* Articular chondrocytes; Cartilage
alginate culture for, 97–98
culture of, 89–98, 160, 171–173
encapsulation in alginate beads, 172–173
encapsulation of bovine, 224–228
in hydrogels, 215
isolation from bovine knee joint, 217–218
isolation from bovine or human articular cartilage, 169–170
isolation of, 89–98, 169–170
pellet culture for, 96–97
release from alginate beads, 174–175
transfection of, 114–115
use in tissue engineering, 137

Chondrogenesis
in pellet cultures, 100–101, 103–104
physical signals of, 147
of progenitor cells in vitro, 100–104
Chondrogenic differentiation, in pellet culture, 343
Chondrogenic differentiation potential, cells with, 98–100
Chondrogenic potential, tissue engineering cartilage implants from cells with, 104
Chondroprogenitor cells, 98–100
in articular cartilage repair, 159
in vitro chondrogenesis of, 100–104
Chromaffin cells, 377–378, 379
Ciliary neurotropic factor (CNF), 18
Ciprofloxacin, 18
Circular engineered heart tissues
casting and culture of, 276, 285–286
contractile properties of, 282, 283
Cirrhosis, 419
Citrate buffered acetone, 31
Citrate working solution, 30
Class II biosafety cabinet, 10
Clinical applications, tissue engineering human skeletal muscle for, 239–257
Clinical staff, in culture projects, 9–10
Clots, in engineered blood vessels, 297–299
CMF, 241. *See also* Calcium-magnesium-free phosphate-buffered saline (CMF-PBS, PBSA)
Coating buffer, preparation for liver tissue engineering, 424
Cocultures
hepatocytes in, 421, 423, 435–438
preparation of NT2 cells for, 398–399
Collagen, 84. *See also* Collagens
in arterial grafts, 295
in engineered blood vessels, 299–302
Collagenase, 117, 217
passaging human embryonic stem cells with, 67–68
preparation for liver tissue engineering, 424
Collagenase A, 85
Collagenase-dispase solution, 242
Collagenase-P digestion, 170
Collagenase-P digestion solution, preparation for articular cartilage cultures, 161
Collagenase solution, preparation for neuroendocrine/neuronal cell culture, 395
Collagenase Type II, 86
Collagen assays, for articular cartilage cultures, 164–166
Collagen content, of chondrocyte cultures, 178–180
Collagen culture model, for articular chondrocytes, 95
Collagen ELISA buffers, 198–199
Collagen extraction buffer, preparation for articular cartilage cultures, 166
Collagen fiber-poly(L-lactic acid) (PLA) composites, in ACL tissue engineering, 195
Collagen isolation, from cell-seeded silk matrices, 204–205
Collagenous extracellular matrix, of engineered blood vessels, 299–302
Collagen reconstitution buffer, preparation for articular cartilage cultures, 165
Collagens. *See also* Collagen
as biomaterials, 326
silks and, 327
Collagen type, determining, 180–182
Collagen type I, 232, 444
in ACL tissue engineering, 194–195
in articular cartilage repair, 159
assays of, 347
biodegradation of, 326
in bone, 325–326
for engineered heart tissue cultures, 266–267, 271
protein content of, 204
Collagen type II, 84, 122, 134, 226, 232
analysis of, 221–222
in articular cartilage repair, 159, 160
silk matrix design and, 207
Collette, Adam, 191
Complete hepatocyte culture medium (CHCM), preparation for liver tissue engineering, 425
Compressive properties, of filter well insert constructs, 184–185
Computer-aided design (CAD), in liver tissue engineering, 460
Confocal immunofluorescence studies, of engineered heart tissue, 274, 275
Confocal laser scanning microscopy (CLSM), of engineered heart tissues, 274, 278
Connexin 43 (Cx-43), 265
Construct cultivation, 141
Contaminant cells, quickly doubling populations of, 73
Contractile function, implanted engineered heart tissues and, 264–265
Contractile properties, of circular engineered heart tissues, 282, 283
Controlled studies of cells and tissues, 133
Control medium, preparation for bone tissue engineering, 333
Convective mixing, in bioreactors, 146
Coomassie Blue staining solution, 242
Coral-based ceramics, 39
Coverslips, for dorsal root ganglia cultures, 403
Coxsackievirus and adenovirus receptor (CAR), 273
Cross-contamination, 16
Cross-linked collagen scaffolds, 326
Cross-link formation
in engineered blood vessels, 300
in hydrogels, 215, 216
Cryopreservation, 14–16
of human mesenchymal stem cells, 37–38
Cryopreserved human mesenchymal stem cells (hMSCs), thawing, 38–39

Cryopreserved murine embryonic fibroblast cells, seeding, 66
Culture conditions, for chondrocytes, 160
Cultured hepatocytes
  cytochrome P450 1A1 activity assay in, 434–435
  urea synthesis assay in, 433
Cultured human marrow-derived mesenchymal stem cells, 26–27
Cultured mesenchymal stem cells (MSCs)
  calcium assay in, 49–50
  staining for mineralization in, 48–49
Culture geometry, attention to, 19–20
Culture materials, sources of, 20
Culture media
  complete, 265–266
  for PC12 cells, 380
  preparation for articular cartilage cultures, 160
  for skeletal muscle tissue engineering, 241
Culture methodology, for human mesenchymal stem cells, 335–340
Culture models, differentiation/redifferentiation, 95, 98
Cultures. *See also* Cell cultures; Subculture
  chondrocyte, 171–173
  hanging drop, 71
  histotypic, 7, 8
  of human skeletal myoblasts, 244–246
  microbial contamination of, 16–18
  organ, 7
  organotypic, 7–9
  proliferating monolayer, 171–172
  two-dimensional confluent, 71
  types of, 5
Culturing, as an explant, 407
Cyclic adenosine monophosphate (cAMP), 19
Cytochemical alkaline phosphatase assays, 47–48
Cytochrome P450 1A1 activity assay, in cultured hepatocytes, 434–435
Cytochrome P450 B2 (CYPB2), in liver metabolism, 446–447. *See also* Zonated CYP2B expression
Cytochrome P450 enzyme activity, measurement of, 428, 433–435
Cytokines
  in bone formation, 348
  in stem cell cultures, 78

Dacron mesh, in engineered blood vessels, 300–301, 306
Davol Ultrafoam™, 139
Decellularized collagen matrices, in engineered blood vessels, 301
Decontamination, 17
  of mycoplasma infection, 17–18
Defective cardiac tissue, tissue engineering for, 286–288
Defined cell structures, mechanical isolation of, 75
DelTatto, Michael, 239
Dermis, chondroprogenitor cells from, 100
Desmin, 247
Detoxification, liver, 428
Dexamethasone (dex), 29, 31, 104, 378
  in bone tissue engineering, 332
  human MSC response to, 45
  PC12 cells and, 381
Dexamethasone stock, 88
Differentiated cells, seeding onto scaffolds, 78
Differentiation. *See also* Three-dimensional neuroendocrine/neuronal differentiation; Undifferentiated entries
  cell, 4–7
  cell culture, 18–20
  EHT cardiac myocyte, 285, 287
  human embryonic stem cell, 62–63
  induction in embryonic stem cells, 69–72
  mesenchymal stem cell, 26
  of NT2 cells into NT2N neurons, 392–393
  stem cell, 335, 337
Differentiation medium, 86, 241
Differentiation/redifferentiation culture models, 95, 98
Digestion medium
  for bovine articular cartilage, 86
  for human articular cartilage, 85
Digestion solutions, preparation for articular cartilage cultures, 161–162
Digestive enzyme solutions, for skeletal muscle tissue engineering, 242
Dilution scheme, proteoglycan, 177
Dimethylmethylene Blue (DMMB) dye reagent, preparation for articular cartilage cultures, 163. *See also* DMMB reagent
Dimethyl sulfoxide (DMSO), 14–16, 31
Direct perfusion, in bioreactors, 146
Disaggregation, 11
  tissue, 4
Dissection medium, 85
Dissection tools, for dorsal root ganglia cultures, 403
DMMB reagent, in collagen content assays, 178. *See also* Dimethylmethylene Blue (DMMB) dye reagent
DNA (deoxyribonucleic acid)
  fluorometric assay of, 183
  plasmid, 116–117
DNA analysis
  of hydrogel-encapsulated cartilage, 226–227, 232–234
  proteinase K digestion of alginate beads for, 182–183
  silk matrix design and, 207
DNA assay
  for articular cartilage cultures, 166–168
  of cells cultured on scaffolds or as pellets, 348–350
DNA content, determining, 182–183

DNase stock solution
preparing for engineered heart tissues, 266
preparing for neuroendocrine/neuronal cell culture, 396
DNA standard solution, preparation for articular cartilage cultures, 167
DNA transfer, 114, 115, 116
Donor organs, 419
Donor tissues for transplantation, lack of, 132
Dopamine (DA), 390, 393
Dopamine-β-hydroxylase (DBH), 378
Dopaminergic phenotype, retention by tissue constructs transplanted into the brain, 393–394
Dorsal root ganglia (DRG), 400
dissection and culture of, 404–408
Dorsal root ganglia cultures, 400–408
egg and embryo preparation for, 401–402
Doubling time, 12, 13, 14, 15, 37
Dragline silk, 326
Drug development, liver tissue engineering for, 458
Drug screening, bioartificial muscles in, 255
Dulbecco's modified Eagle's medium (DMEM), 241, 242. *See also* Dulbecco's modified Eagle's medium with 1 g/l glucose (DMEM-LG)
for blood vessel engineering reagents, 302, 303
concentrated, 266
for engineered heart tissue cultures, 267
for engineered heart tissues, 265–266
Dulbecco's modified Eagle's medium with 1 g/l glucose (DMEM-LG), 33, 37, 38–39. *See also* High-glucose DMEM
Dynamic seeding, in spinner flasks, 142–144

EC markers, immunostaining for, 311–312. *See also* Endothelial cells (ECs)
EHT preparations, action potentials of, 282–284. *See also* Engineered heart tissues (EHTs)
Elastase solution, for collagen ELISA buffers, 199
Elastic cartilage, 84
Elastin, in engineered blood vessels, 299, 302
ELISA buffers, collagen, 198–199
Elisseeff, Jennifer, 213
Elson, E. L., 261
Embryoid bodies (EBs)
formation in hanging drop cultures, 71
formation in methylcellulose, 70
formation in nonadhesive dishes, 70–71
formation of, 69–71
human, 69–70
Embryoid body cell media, 65
Embryonic chick cardiac myocytes, culturing of, 261–262. *See also* Chicken eggs/embryos
Embryonic chick fibroblasts, culturing of, 261
Embryonic chick limb bud system, mesenchymal cells from, 26
Embryonic fibroblast feeder cells, 63
Embryonic stem (ES) cells, 62–63. *See also* ES entries; Human embryonic stem cells
characteristics of, 62–63
differentiation in three-dimensional cultures on polymer scaffolds, 72
differentiation in two-dimensional confluent cultures, 71
expansion and passaging of, 67–68
future perspectives on, 78–79
induction of differentiation in, 69–72
isolating cell types from cultures from, 72–75
for Parkinson disease, 391
protocols for using, 78
scale-up of, 77–78
sources of materials related to, 79
Encapsulation, of hepatocytes, 421, 422. *See also* Cell encapsulation; Cellular encapsulation; Cellular photoencapsulation; Photoencapsulation
Endoglin, 335
Endothelial cell isolation/culture, for vascular tissue engineering, 304–305
Endothelial cell layer, of arteries, 296. *See also* Endothelium
Endothelial cells (ECs), 303, 304. *See also* EC markers
in culture, 316
luminal seeding into vascular cell bioreactor, 310–311
separating from embryoid bodies by immunostaining and flow sorting, 74
Endothelial markers, expression of, 75–76
Endothelial precursor cells, isolated, 75–77
Endothelium, of engineered blood vessels, 297–299. *See also* Endothelial cell layer; Endothelial cells (ECs)
Engineered blood vessels
bioreactor preparation for, 307–308
characterization of, 311–316
collagenous extracellular matrix in, 299–302
elastin in, 299, 302
endothelium of, 297–299
genetic manipulation in, 316–318
mechanical measurements of, 314–316
media and reagent preparation for, 302–303
transmission electron microscopy of, 312–313
Engineered cardiac tissue, 137. *See also* Engineered heart tissues (EHTs)
Engineered cartilage, after bioreactor cultivation, 124
Engineered grafts, 133
Engineered heart tissues (EHTs), 262–265. *See also* Cardiac entries; EHT preparations; Engineered cardiac tissue
advantages and problems of, 287–288
cardiac function and, 265
casting and culture of, 276
confocal immunofluorescence of, 275
contractile properties of, 282, 283
conventional histology of, 273–274

force measurement in, 272–273
gene transfer in, 273, 274
heart contractile function and, 264–265
histologic procedures in, 273–276
histology of, 276–277
immunoconfocal characterization of, 277–279
improving contractile function and tissue formation in, 263
in vitro applications of, 284–286
isolation and culture methodology for, 266–273
reagent preparation for, 265–266
to replace impaired myocardium, 260
representative tissue culture study of, 276–284
sources of materials for, 288
survival in rats, 263–264
transmission electron microscopy of, 275–276
ultrastructural characterization of, 279–282, 285
vascularization and integration of, 264
Engineered ligament culture medium (ligament medium), for ligament tissue engineering, 197
Engineered liver tissue development, hepatocyte microenvironment regulation for, 422–424
Engineered rat heart tissue, preparing casts for and culturing, 271–272
Engineered tissue analysis, in cellular photoencapsulation, 221–224
Engineered tissue constructs, for transplantation, 391
Engineered tissues, in vitro cultivation of, 132–135
Engineered vessels, scanning electron microscopy of, 313
Enhanced green fluorescent protein (EGFP) expression, 317
Enzyme-linked immunosorbent assay (ELISA). *See also* ELISA buffers
for albumin secretion quantification, 429–430
silk matrix surface modification and, 208
Enzymes
engineered blood vessels and, 300
preparation for articular cartilage cultures, 161–162
Epidermal keratinocytes, 19
Epinephrine (E), 378, 379
Equine articular cartilage, 91
ES cell medium, 65
Eschenhagen, T., 259
Escherichia coli β-galactosidase marker gene, 116–117, 122
Ethics, human culture material and, 10–11
Ethylenediaminetetracetic acid (EDTA), Krebs-Ringer buffer with, 424
Expansion culture, of articular chondrocytes, 92–93
Expansion medium, preparation for bone tissue engineering, 333
Explant culture, 407. *See also* Primary explant entries
Exponential growth, of cell lines, 12, 13
Expression plasmid vectors, 116–117
Extracellular matrix (ECM), 134
in ligament tissue engineering, 207
protein formation in, 204
silk in, 328
Extracellular matrix materials, tissue engineering of human skeletal myoblasts with, 249–253

F12H medium, for dorsal root ganglia cultures, 402
F12HS10 medium, for dorsal root ganglia cultures, 402
Factors, from murine embryonic fibroblast (MEF) cells, 63
Fast Violet stain, 30
Fat, chondroprogenitor cells from, 100
Felix, Jennifer, 417
Ferland, Paulette, 239
Fetal bovine serum (FBS)
selecting for mesenchymal stem cells, 53–55
selection of, 28–29
Fetal dopaminergic neuron transplantation, for Parkinson disease, 390
Fetal stem cells, for Parkinson disease, 391
Fibrin culture model, for articular chondrocytes, 95
Fibroblast culture medium (FCM), preparation for liver tissue engineering, 425
Fibroblast growth factor, in articular chondrocyte cultures, 95
Fibroblasts (FBs)
in ACL tissue engineering, 195, 196
in arterial grafts, 295–296
in engineered blood vessels, 300–301
of engineered heart tissues, 281, 282
human ACL, 200
Fibrocartilage, 84, 104
Fibroin, in silk, 327
Fibrous scaffolds, 139, 143–144
Filter well insert constructs, compressive properties of, 184–185
Filter well inserts, 8, 9
culture of ARC chondrocytes and associated matrix in, 175–176
determining collagen content of chondrocyte cultures from, 178–180
determining proteoglycan content of chondrocyte cultures from, 176–178
Finite cell lines, 12
Flasks, 140–141
Flat plate systems, 421, 422
Flow cytometric adipogenesis assay
fixing mesenchymal stem cells for, 51–52
staining mesenchymal stem cells with Nile Red for, 52–53
Flow cytometry, solutions for, 31
Flow sorting, separating endothelial cells from embryoid bodies by, 73–74
Fluorescence-activated cell sorting (FACS), 11
following immunostaining, 72–74
of hMSCs, 340, 341–342

Fluorescence microscopy, for microbial contamination, 17
Fluorescent antibodies, labeling with, 72, 73–74
Fluorometric assay, of DNA, 183
Force measurement, in engineered heart tissue, 272–273
Freezing, of cell cultures, 14–16
Freezing medium (FM)
  for ligament tissue engineering, 197
  for NT2 cells, 395
Freshney, R. Ian, 3
FuGENE 6, 115–116, 117, 124
Fuller, Jason, 61
Functional tissue assembly, 134
Functional tissue engineering, 133
Functions, of liver, 419, 420, 421, 428–435, 435–444, 444–451, 457–458
Fungal infections, of cell cultures, 17
Fungizone™, 85
Further-removed matrix (FRM), 160

Gallo, Gianluca, 375, 377, 400
Gel-cell seeding, of porous scaffolds, 144–146
Gene expression analysis
  in bone and cartilage constructs, 360–362
  by RT-PCR of cartilage hydrogels, 222–223
Gene expression profiles, for cartilage-specific proteins, 226
Generation number, 15
Genes, for stem cell properties, 77–78
Gene therapy
  skeletal muscle tissue engineering in, 254
  for tissue-engineered blood vessels, 316–318
Genetically engineered selectable markers, 74–75
Genetic manipulation, in tissue-engineered blood vessels, 316–318
Gene transfer, 114
  to bovine cartilage cells, 120–121
  in engineered heart tissue, 273, 274
  lipid-mediated, 113–127
Glass, as culture substrate, 9
Glucocorticoids, 378, 379
  in bone tissue engineering, 332
Glucose consumption, assessment of, 149
Glutaraldehyde fixative, preparing for engineered heart tissues, 266
Glycerol, 14
Glycerophosphate, in bone tissue engineering, 332
Glycosaminoglycan (GAG) assay
  in cartilage and bone constructs, 355–357
  of hydrogel-encapsulated cartilage, 226–227, 233, 234
Glycosaminoglycan (GAG)-rich proteoglycan, 134
Goat bone marrow-derived mesenchymal stem cells, photoencapsulation of, 228–235
Grafts
  engineered, 133
  synthetic, 294–295
  vascular, 294–295, 296–297, 300–301
  venous, 301
Graft vasoconstriction tests, 313, 314
Green fluorescent protein (GFP), 317
Growth factors, in bioreactor cultivation, 146–147
Growth factor supplementation strategies, in stem cell cultures, 78
Growth medium, 86–87, 117
Guanidine hydrochloride (GuHCl)
  in collagen content assays, 178
  preparation for articular cartilage cultures, 164

Hand enrichment, of cell types, 75
Hanging drop cultures, formation of embryoid bodies in, 71
Hanks' balanced salt solution (HBSS), 85
Hanks' balanced salt solution with HEPES, calcium- and bicarbonate-free (CBFHH), 266
Harbor Extracellular Matrix (ECM), for engineered heart tissue cultures, 266–267, 270
Heart tissue, engineered, 259–291
Hematopoietic stem cells, in bone marrow, 335
Hennessey, James, 239
Heparin sulfate proteoglycans (HSPGs), 19
Hepatocellular constructs, liver tissue engineering for, 459
Hepatocyte culture medium
  complete, 425
  serum-free, 425
Hepatocyte/fibroblast cocultures, micropatterning of, 441–443
Hepatocyte function assays, 428–435
Hepatocyte medium, hormonally defined, 425
Hepatocyte microenvironment, regulation of, 422–424
Hepatocyte micropatterning, bioreactor/flow circuit assembly for, 448–449
Hepatocytes, 419, 420. *See also* Liver entries; Primary hepatocytes
  cytochrome P450 1A1 activity assay in, 434–435
  primary rat, 425–428
  urea synthesis assay in, 433
  zonated gene expression in, 447
Hepatocyte zonal heterogeneity, bioreactor system for regulating, 444–451
Hepatotoxicity risk assessment, liver tissue engineering for, 458
HepG2 cells, 419–420
HepG2 culture medium, preparation for liver tissue engineering, 425
HepLiu cells, 419–420
Heterogeneity, of embryonic stem cell-derived cultures, 72
Heterotypic 3-D cell-cell interactions, 378
Heterotypic hepatocyte-nonparenchymal interactions, 421, 423, 435–438

High Aspect Ratio Vessels (HARVs), 141, 383, 392, 393, 394
  generation of PC12 organoids in, 385–387
High-glucose DMEM, 86. *See also* Dulbecco's modified Eagle's medium with 1 g/l glucose (DMEM-LG)
High-performance liquid chromatogaphy (HPLC), 393
  in collagen content assays, 178–180
High-salt tris buffer, preparation for articular cartilage cultures, 166
Histologic analysis, of cartilage hydrogels, 223–224, 224–225, 226–227
Histologic assessment, of engineered constructs, 148
Histologic fixative, for engineered heart tissues, 266
Histologic procedures, in engineered heart tissue, 273–276
Histology
  of bone tissue engineering, 363–365
  in cellular photoencapsulation, 229–232
  of engineered heart tissues, 276–277
Histotypic cultures, 7, 8
Hoechst 33258 dye buffer, preparation for articular cartilage cultures, 167
Hoechst 33258 dye solution, preparation for articular cartilage cultures, 167–168
Hofmann, Sandra, 323
Hollow fiber devices, 421, 422
Homotypic 3-D cell-cell interactions, 378
Homotypic hepatocyte-hepatocyte interactions, 421, 435–438
Horan, Rebecca, 191
Hormonally defined hepatocyte medium (HDHM), preparation for liver tissue engineering, 425
Hormones, in bone formation, 348
hTRT gene, 12
Human ACL fibroblasts (ACLFs), 200. *See also* ACL fibroblasts (ACLFs)
  silk matrix surface modification and, 207–208
Human articular cartilage, 89–91
Human articular cartilage chondrocytes, isolation of, 90–91, 169–170
Human articular cartilage predigestion/digestion media, 85
Human articular cartilage tissue, harvesting, 169
Human bioartificial muscles (HBAMs), 240–241
  in extracellular matrix materials, 249–253
  in gene therapy, 254
  in muscle repair/replacement, 254–255
Human bone marrow, aspiration and collection of, 31–32. *See also* Bone marrow
Human bone marrow stromal cells (hBMSCs). *See also* Bone marrow stromal cells (BMSCs)
  mechanical stimulation and, 208–209
  primary culture of, 199
  silk matrix design and, 206–207
  silk matrix surface modification and, 207–208
Human culture material, biosafety and ethics related to, 10–11
Human embryoid bodies (hEBs), 69–70
Human embryonic stem (hES) cell culture
  seeding mitotically inactivated murine embryonic fibroblasts for, 66–67
  for tissue engineering, 61–82
Human embryonic stem cells
  induction of differentiation in, 69–72
  maintenance and expansion of, 63–68
  for Parkinson disease, 391
  passaging with collagenase, 67–68
  passaging with trypsin, 68
  pluripotency of, 62–63, 63–64
  preparation of media and reagents for, 64–65
Human insulin-like growth factor-I (IGF-I), 124–125
  in bioreactor cultivation, 146–147
Human ligament, primary explant culture of, 200
Human marrow-derived mesenchymal stem cells (hMSCs), isolation of, 31–35
Human mesenchymal stem cell culture
  methodology of, 335–340
  on silk-RGD scaffolds in spinner flasks, 344–347
  thawing and maintenance of, 339
Human mesenchymal stem cells (hMSCs) assays of, 347
  characterization of, 340–343
  cryopreservation of, 37–38
  expansion of, 339–340
  FACS analysis of, 341–342
  isolation and culture methodology of, 335–340
  isolation and seeding of, 33–34
  osteogenic differentiation in, 44–45
  selective differentiation of, 337
  subculture of, 35–36, 37
  thawing, 38–39
Human skeletal muscle. *See also* Human skeletal myoblasts
  tissue engineering and maintenance of, 251–253
  tissue engineering for clinical applications, 239–257
Human skeletal myoblasts
  characterization of, 246–249
  isolation and culture of, 244–246
  tissue engineering with extracellular matrix materials, 249–253
Hyaline cartilage, 84
Hyaluronidase, 116, 118
Hydrodynamic environment, in bioreactor cultivation, 146
Hydrogel microstructures, 452
  multilayer, 453, 456
Hydrogels, 215. *See also* Cartilage hydrogels
  cellular photoencapsulation in, 213–238
  in vitro cultivation of, 229
  methods for forming, 215–216
  photoinitiated, 221
  photopatterned three-dimensional, 451
  photopolymerization in, 216
Hydrogel scaffold, cell photoencapsulation in, 219–221

Hydroxyapatite crystal formation, 348
Hydroxyproline content, determining, 180
Hydroxyproline derivatizing solution, preparation for articular cartilage cultures, 164–165
Hydroxyproline drying solution, preparation for articular cartilage cultures, 164
Hydroxyproline HPLC eluent A, preparation for articular cartilage cultures, 165–166
Hydroxyproline HPLC eluent B, preparation for articular cartilage cultures, 166
Hydroxyproline HPLC standard, preparation for articular cartilage cultures, 165

Immature chondrocyte monolayers, 174
Immediate functionality, 133
Immortality, of embryonic stem cells, 62
Immune response, of implanted engineered heart tissues, 264
Immunochemical assays, of human embryonic stem cells, 64
Immunoconfocal characterization, of engineered heart tissues, 277–279
Immunofluorescence assay
  of human embryonic stem cells, 64
  for intracellular albumin detection, 430–431
Immunofluorescence studies, confocal, 274, 275
Immunohistochemistry, in cellular photoencapsulation, 229–230
Immunohistochemistry assay, for intracellular albumin detection, 431–432
Immunolabeling, of engineered heart tissues, 278
Immunosorting, by magnetizable beads, 11
Immunostaining
  followed by cell fluorescence-activated cell sorting, 72–74
  separating endothelial cells from embryoid bodies by, 73–74
  for SMC and EC markers in vascular constructs, 311–312
Implantable liver systems, design of, 460
Incubation medium (IM), preparation for neuroendocrine/neuronal cell culture, 395
Indicator cultures, 17
Insulin, 31
Insulin-like growth factor-I (IGF-I), 124–125
  in bioreactor cultivation, 146–147
Insulin, transferrin, and selenous acid (ITS), 85
Integration, of implanted engineered heart tissues, 264
Intercellular adhesion molecule-I (ICAM-I), 316
Intracellular albumin detection, 429–432
  immunofluorescence assay for, 430–431
  immunohistochemistry assay for, 431–432
In vitro adipogenic induction, 50–51
In vitro applications
  of bone tissue engineering, 366–368
  of engineered heart tissues, 284–286
  of liver tissue engineering, 457–458
In vitro chondrogenesis, 103
  of progenitor cells, 100–104
In vitro cultivation, of engineered tissues, 132–135
In vitro drug screening, bioartificial muscles in, 255
Invitrogen, 117. *See also* Calcium-magnesium-free phosphate-buffered saline (CMF-PBS, PBSA)
In vitro osteogenic induction, 43–45
  solutions for, 29–30
In vitro stabilization, of primary hepatocytes, 420–422
In vitro tube formation, 76
In vivo assays, for osteogenesis, 39–43
In vivo differentiation, seeding undifferentiated embryonic stem cells for, 78
In vivo elastin formation, in engineered blood vessels, 302
In vivo vessel formation, 76–77
Isocratic reverse-phase high-performance liquid chromatography, in collagen content assays, 178–180
Isolated endothelial precursor cell, characterization of, 75–77
Isolation
  of embryonic stem cell types from cultures, 72–75
  of human mesenchymal stem cells, 335–340
Isolation/culture methodology, for engineered heart tissues, 266–273
Isolation medium, preparation for articular cartilage cultures, 160
Isoprenaline, contractile heart tissue response to, 282, 283, 284–285

Johnstone, Brian, 83
Juvenile bovine cartilage, histologic sections of, 224–225, 226–227

Kaplan, David L., 191, 323
Karnovsky fixative stock solution, for scanning electron microscopy, 198
Keratinocyte growth medium (KGM), 11
Khademhosseini, Ali, 61
Khetani, Salman R., 417
Kim, Tae-Gyun, 213
Klinger, Rebecca Y., 293
Knee function. *See also* Bovine knee joint
  restoration of, 193, 194
  silk matrix design in restoring, 205–207
Koç. Omer, 32
Krebs-Henseleit solution, for blood vessel engineering reagents, 303
Krebs-Ringer buffer (KRB)
  with EDTA, 424
  preparation for liver tissue engineering, 424

Lactate production, assessment of, 149
Lag period, 12, 13
Laminin (LN), for dorsal root ganglia cultures, 403
Langer, Robert, 61
Lelkes, Peter I., 375, 377
Lennon, Donald P., 23
Leukemia inhibitory factor (LIF), 18–19, 63
Levenberg, Shulamit, 61
Ligament differentiation inducer, mechanical stimulation as, 202, 208–209
Ligament medium, for ligament tissue engineering, 197
Ligament tissue engineering, 191–211. *See also* Anterior cruciate ligament tissue engineering
  ACL replacement via, 193–194
  analytical assays for, 203–205
  bioreactor for, 202–203
  cell seeding and cultivation in, 201–202
  context of, 193
  hydrogels and, 215–216
  methodology principles for, 194–196
  reagent preparation for, 197–199
  representative studies related to, 205–209
  silk matrices and RGD surface modification in, 200–201, 207–208
  sources of materials for, 209–210
  tissue harvest and cell isolation in, 199–200
Link protein, immunohistochemical staining for, 231–232
Lipid-based transfection, 114. *See also* Lipid- mediated gene transfer
  advantages and shortcomings of, 115
  efficient, 115–116
Lipid-mediated gene transfer, 113–127. *See also* Lipid-based transfection
  applications of, 124–125
  principle of, 114–115
  protocols for, 119–123
  reagent and media preparation for, 116–118
  sources of materials related to, 125
LipofectAmine®, 115, 116
Lipofectin®, 115, 116
Liposome-DNA complexes, 115
Liposomes, 114–115
Liu Tsang, Valerie, 417
Liver cell-based therapies, cell sources for, 419–420. *See also* Hepatocyte entries
Liver disease, cell-based therapies and, 419, 420
Liver donors, 419
Liver regeneration, 460–461
Liver tissue engineering, 417–471
  clinical applications of, 458–461
  hepatocyte function assays, 428–435
  in vitro applications of, 457–458
  isolation of primary rat hepatocytes, 425–428
  micropatterned cell cultures, 435–444
  photopatterned three-dimensional hydrogels, 451–457
  reagent preparation for, 424–425
  sources of materials for, 461–462
Liver transplantation, 419
Liver zonation, 444, 445, 449–451
Living cells, photopatterned three-dimensional hydrogels containing, 451–457
Log phase, 12, 13
Low-density lipoprotein (LDL), incorporation of, 76
Lubricin, tissue formation and, 184
Luminal seeding, of endothelial cells into vascular cell bioreactor, 310–311
Lung carcinoma, 5

Macdonald, Mara, 61
Madry, Henning, 113
Magnetic sorting, 74
Magnetizable beads (MACS), immunosorting by, 11
Maintenance medium (MM), preparation for neuroendocrine/neuronal cell culture, 395
Mallory–Heidenhain staining, 43, 44
Markers
  endothelial, 75–76
  genetically engineered selectable, 74–75
  immunostaining for, 311–312
Marrow, mesenchymal stem cell enrichment from, 32–34. *See also* Bone marrow entries
Marrow-derived mesenchymal stem cells, 26
Masuda, Koichi, 157
Materials sources. *See also* Suppliers List
  for bone tissue engineering, 368–370
  for cellular photoencapsulation, 235–236
  for engineered heart tissues, 288
  for ligament tissue engineering, 209–210
  for liver tissue engineering, 461–462
  MSC-related, 55–56
  for neuroendocrine/neuronal cell culture, 409–411
  related to articular cartilage, 185–187
  related to cartilage tissue engineering, 105
  related to embryonic stem cells, 79
  related to lipid-mediated gene transfer, 125
  related to tissue engineering, 150
  for skeletal muscle tissue engineering, 255–256
  for tissue-engineered blood vessels, 318–319
Matrigel®, for engineered heart tissue cultures, 266–267, 270, 271
Matrix (matrices), 9. *See also* Cardiac myocyte-populated matrix (CMPM)
  extracellular, 134
Matrix mineralization, in bone formation, 348
Matrix seeding, in ligament tissue engineering, 202
MCDB 153, 11
McGuire, Sharon, 239
Mechanical analysis, in ligament tissue engineering, 203

Mechanical properties
of scaffold biomaterials, 326
of silk, 328
Mechanical stimulation
as ACL differentiation inducer, 196
in ligament differentiation inducer, 208–209
Mechanical strain, in cardiac myocyte-populated matrix, 261–262
Mechanical strength, of engineered blood vessels, 299–302. *See also* Strength; Tensile strength
Mechanical testing flow system, 315
Media, tissue engineering, 137–138
Medial layer, of arteries, 296–297
Media preparation
for articular cartilage cultures, 160–168
for bone tissue engineering, 332–335
for cartilage tissue engineering, 85–89
for cellular photoencapsulation, 217
for engineered blood vessels, 302–303
for human embryonic stem cells, 64–65
for lipid-mediated gene transfer, 116–118
for neuroendocrine/neuronal cell culture, 379–380, 395–396
for skeletal muscle tissue engineering, 241–242
Medium additives, for dorsal root ganglia cultures, 402
Meinel, Lorenz, 323
Mesenchymal stem cells (MSCs), 23–59. *See also* Cultured mesenchymal stem cells (MSCs); MSC photoencapsulation; Osteogenesis
bone marrow-derived, 26, 217, 218, 335, 336, 337–338
bone tissue engineering from, 332, 366
characterization of, 336
differentiation of, 26, 29
enrichment from human marrow, 32–34
fixing for flow cytometric adipogenesis assay, 51–52
goat bone marrow-derived, 228–235
isolation of, 31–35, 337–338
phenotypic potency assays of, 27–28
primary cultures of, 34–35
propagation of, 35–37
selecting fetal bovine serum for, 53–55
silks and, 327
sources of materials related to, 55–56
staining with Nile Red for flow cytometric adipogenesis assay, 51, 52–53
in tissue engineering, 25, 137
Mesengenic process, 26
Mesh surface treatment solution, polyglycolic acid, 303
Metabolism, liver, 428
Methylcellulose, formation of embryoid bodies in, 70
Mice. *See* SCID mice
Microbial contamination, 16–18
Microcapillaries, 8, 9
Microfabrication, of substrates, 438–441
Microgravity, effects on bioartificial muscle tissue, 240, 255
Micromass cultures, for differentiated chondrocytes, 101–103
Micropatterned cell cultures, in liver tissue engineering, 435–444
Micropatterning, 423
of hepatocyte/fibroblast cocultures, 441–443
Microscopy. *See also* Fluorescence microscopy; Phase-contrast light microscopy; Scanning electron microscopy (SEM); Transmission electron microscopy (TEM)
of human embryonic stem cells, 64
of osteogenesis, 43, 44
Mineralization, staining for, 48–49
Mitogen-activated protein kinase (MAPK) pathway, 208
Mitomycin, 64–65
Mitotically inactivated murine embryonic fibroblasts, seeding for human embryonic stem culture, 66–67
Mixed flasks, 140
Modified surfaces, cellular micropatterning on, 441–444
Monolayer culture
of articular chondrocytes, 93–94
of articular chondrocytes under proliferating conditions, 171
Monolayers, 4
immature chondrocyte, 174
Moreau, Jodie, 191
Morphology analysis, in ligament tissue engineering, 203–204
mRNA assays, 347
MSC photoencapsulation, 229
MTT solution, for ligament tissue engineering, 197
MTT staining, in ligament tissue engineering, 203, 207
Multiple seeding, in ligament tissue engineering, 202
Murine embryonic fibroblast (MEF) cells
preparation of, 66
in preventing human embryonic stem cell differentiation, 63
Murine embryonic fibroblast medium, 65
Muscarinic agonists, in cardiac myocyte-populated matrix, 262
Muscle, tissue engineering of, 239–257. *See also* Cardiac myocytes; Human skeletal muscle; Smooth muscle entries
Muscle cells. *See also* Human skeletal myoblasts; Myoblasts; Myocytes
in arterial grafts, 295–296
seeding into vascular cell bioreactor, 308–310
Muscle repair/replacement, skeletal muscle tissue engineering in, 254–255
Mycoplasma, in cell cultures, 17
Mycoplasma Removal Agent (MRA), 18

Myoblast purity, 247, 248–249
Myoblasts. *See also* Myocytes
  human skeletal, 244–246, 246–249, 249–253
  in tissue engineering, 240
Myocardial infarction, engineered heart tissues and, 265
Myocardium, 149
  replacing impaired, 260
  in tissue engineering, 134–135
Myocytes, neonatal cardiac, 268–269. *See also* Bioartificial muscles (BAMs); Engineered heart tissues (ETHs); Human bioartificial muscles (HBAMs); Muscle cells; Myoblasts; Neonatal rat cardiac myocytes
Myofibers, 249
  in tissue engineering, 240

Nackman, Martin, 239
Naphthol AS-MX phosphate, 48
Native cartilage, collagen extraction from, 181–182
Native silkworm silk, in ACL tissue engineering, 195, 200–201, 201–202
Natural polymers, in ACL tissue engineering, 195
Natural scaffolds, chondrogenic cells on, 174
Neomycin resistance, 74–75
Neonatal cardiac myocytes, isolation of, 268–269
Neonatal rat cardiac myocytes, culturing of, 261
Neoteny, 378
Nephila claviceps silk, 326
Nerve growth factor (NGF), 379
  for dorsal root ganglia cultures, 402–403
  PC12 cells and, 381
Neurodegenerative disease, Sertoli-NT2N tissue constructs to treat, 390–400
Neuroendocrine/neuronal cell cultures
  dorsal root ganglia cultures, 400–408
  media and reagent preparation for, 395–396, 379–380
  sources of materials for, 409–411
  for tissue engineering, 375–415
Neuroendocrine/neuronal differentiation, of PC12 pheochromocytoma cells, 377–390
Neuronal precursor cells, NT2, 391
Neurotrophic factors, PC12 cells and, 381
Niklason, Laura E., 293
Nile Red staining, of mesenchymal stem cells, 51, 52–53
Nile Red working solution, 31
p-Nitrophenol, 30
Nonadhesive dishes, formation of embryoid bodies in, 70–71
Nonhematopoietic mesenchymal stem cells, in bone marrow, 335
Nonhuman articular chondrocytes, isolation of, 91–92
Nonliposomal lipid-mediated transfection, 115
Non-small cell lung carcinoma, 5
Noradrenaline, 378
Noradrenergic cells, 378
Norepinephrine (NE), 378, 379
NT2 medium (NT2M), preparation for neuroendocrine/ neuronal cell culture, 395
NT2N dopaminergic neurons, 391
  differentiation of NT2 cells into, 392–393
NT2 (NTera-2/clone D1) neuronal precursor cells, 391
  for coculturing, 398–399
  differentiation into NT2N neurons, 392–393
  freezing medium (FM) for, 395
  preparation of, 397–399
  propagating, 398
  Sertoli cell effects on, 394

OPD substrate buffer, preparation for liver tissue engineering, 424
Opti-MEM I reduced-serum medium, 85, 86
Optical density (OD), cell number and, 203
Organ culture, 7
Organ donors, 419
Organoids
  adrenal medullary, 379
  3D PC12, 383, 384–385, 385–387
Organotypic culture, 7–9
Organ transplantation, 325
Osmotic shock, 15
Osteoarthritis, 84
Osteoblasts, bone tissue engineering from, 332
Osteocalcin, 348
Osteochondral allografts, in articular cartilage repair, 159
Osteochondral plugs, 326
Osteoconductive scaffolds, 325
Osteogenesis
  in vitro osteogenic induction and assays for, 43–50
  in vivo assay for, 39–43
Osteogenic differentiation
  in human mesenchymal stem cells, 44–45
  in pellet culture, 342
Osteogenic induction, in vitro, 43–45
Osteogenic medium, preparation for bone tissue engineering, 333
Osteogenic medium double concentration, preparation for bone tissue engineering, 333–334
Osteogenic protein-1 (OP-1, BMP-7), tissue formation and, 183. *See also* Bone morphogenic protein-2 (BMP-2)
Osteoinductive growth factors, 325
Osteopontin (OP), assays of, 347, 348
Outgrowth, 4, 5
Oxygen (O_2) concentration, in liver metabolism, 445–446, 447–449
Oxygen gradients, steady-state, 447–449

Pancreatic elastase, preparation for articular cartilage cultures, 162
Papain enzyme digestion solution, preparation for articular cartilage cultures, 161–162
Paraffin embedding, of bone and cartilage constructs, 363–365
Paraformaldehyde, 198
Parkinson disease (PD), 379, 390, 394
Passage number, 15
Passaging
  of human embryonic stem cells, 67–68
  of undifferentiated PC12 cells, 382–383
PC12 organoids
  dynamic formation of, 383, 384–385
  generation in HARVs without beads, 385–387
  generation in STLVs with beads, 387–390
  generation of, 383
PC12 pheochromocytoma cells. *See also* Undifferentiated PC12 cells
  aggregate cultures of, 383–384
  culture media for, 380
  maintenance of, 380–383
  source of, 380
  three-dimensional neuroendocrine/neuronal differentiation of, 377–390
pcDNA3.1(−), 117
pcDNA3.1/Zeo(+), 117, 124
pCMVβgal gene, 116–117
PECAM1$^+$ cells, 77
Pellet culture
  of bone, 342
  of cartilage, 343
  chondrogenic differentiation in, 343
  osteogenic differentiation in, 342
Pellet-cultured cells, DNA assay of, 348–350
Pellet culture model
  for articular chondrocytes, 95, 96–97
  for differentiated chondrocytes, 100–101, 103–104
Penicillin/streptomycin, 85
Pepsin solution, for collagen ELISA buffers, 199
Pepsin stock solution, preparation for articular cartilage cultures, 162
Percoll density gradient, preparation of, 29
Perfusion
  in bioreactors, 146
  of porous scaffolds, 145–146
Perfusion beds/scaffolds, 421, 422, 423
Perfusion cartridges, 140, 141
Perichondreal layers, in chondrocyte cultures, 101–103
Perichondrium, in articular cartilage repair, 159
Periosteum
  in articular cartilage repair, 159
  chondroprogenitor cells from, 99
Periportal region, liver, 445
Perivenous region, liver, 445
PGA fibers, in scaffolds, 139, 143–144
Pharmacological responses, in cardiac myocyte-populated matrix, 262
Phase-contrast light microscopy, in morphology analysis, 203
Phenotypic potency assays, 27–28
Phenylethanolamine-*N*-methyltransferase (PNMT), 378, 390
Phenylisothiocyanate (PITC), preparation for articular cartilage cultures, 164–165
Phenylisothiocyanate derivatization, in collagen content assays, 178–180
Pheochromocytoma, 377. *See also* PC12 pheochromocytoma cells
Phorbol myristate acetate (PMA), 19
Phosphate-buffered saline (PBSA)
  calcium- magnesium-free, 403
  in mechanical testing of engineered vessels, 314–316
Phosphoenolpyruvate carboxykinase (PEPCK), in liver metabolism, 446
Photinus pyralis luciferase gene, 122
Photoencapsulation, 219–220. *See also* Cellular photoencapsulation
  of bovine chondrocytes, 224–228
  of goat bone marrow-derived mesenchymal stem cells, 228–235
Photoinitiated hydrogels, polymer-chondrocyte preparation with, 221
Photoinitiators, for cell photoencapsulation, 219
Photoinitiator toxicity, WST-1 analysis of, 219, 220–221
Photolithographic patterning, of surface modifications, 438–439
Photopatterned three-dimensional hydrogels, containing living cells, 451–457
Photopatterning
  of hydrogel with living cells, 452–455, 456
  in liver tissue engineering, 459–460
Photopolymerization, for cell encapsulation, 216
Piranha cleaning solution, preparation for liver tissue engineering, 425
Plasmid vectors, 116–117
Plastics, for neuroendocrine/neuronal cell culture, 379–380
Plateau phase, 12–13
Platelet-derived growth factor (PDGF), 6, 18
Pluripotency, of embryonic stem cells, 62–63, 63–64
Polarity, cellular, 7, 19
Poly(ethylene glycol) (PEG)-based hydrogels, 451. *See also* Polyethylene glycol diacrylate (PEGDA)
  encapsulating chondrocytes in, 224–228
Polyethylene glycol diacrylate (PEGDA), in goat bone marrow MSC encapsulation, 229, 231, 234
Polyethylene oxide-diacrylate, 225
Polyethylene oxide (PEO) photopolymerization, 216
Polyglycolic acid (PGA) mesh surface treatment solution, 303

Poly(glycolic acid)-PLA copolymers, in ACL tissue engineering, 195
Polyglycolic acid scaffolds
for arterial grafts, 296, 303
assembly of, 305–306
chondrogenic cells on, 173–174
Polylactic acid (L-PLA), as biomaterial, 326
Poly(lactic acid-co-glycolic acid) (PLGA) scaffolds, 77
in bone tissue engineering, 366
Polylactic acid scaffolds, chondrogenic cells on, 173–174
Polylactide (PLA) films, silks and, 327
Poly-L-lactic acid (PLLA) scaffolds, 77
Polylysine (PL), for dorsal root ganglia cultures, 403
Polymerase chain reactions (PCRs), real-time reverse transcriptase, 204. *See also* Real-time reverse transcriptase-polymerase chain reaction (RT-PCR); Reverse transcriptase- polymerase chain reaction (RT-PCR)
Polymer chains, in hydrogels, 215. *See also* Synthetic polymers
Polymer-chondrocyte preparation, with photoinitiated hydrogels, 221
Polymer networks
in hydrogels, 215
photopolymerization in, 216
Polymer scaffolds. *See also* Polyglycolic acid scaffolds; Poly(lactic acid-co-glycolic acid) (PLGA) scaffolds; Polylactic acid scaffolds; Poly-L-lactic acid (PLLA) scaffolds
assembly of, 305–306
three-dimensional cultures on, 72
Polymethylmethacrylate bone fixation, in ACL tissue engineering, 195
Polystyrene, as culture substrate, 9
Pore size, in hydrogels, 215
Porous scaffolds, 139, 144–146
gel-cell seeding of, 144–146
perfusion of, 145–146
Powell, Courtney, 239
Precursor cells
from bone marrow, 137
isolated endothelial, 75–77
Predigestion medium, for human articular cartilage, 85
Primary cultures, 11
of bovine cartilage, 119–120
cross-contamination of, 16
of human bone marrow stromal cells, 199
of mesenchymal stem cells, 34–35
microbial contamination of, 16–17
Primary explant, 4, 5
Primary explant culture, of human ligament, 200. *See also* Explant culture
Primary explantation, 4
Primary hepatocytes, in vitro stabilization of, 420–422. *See also* Hepatocytes
Primary rat hepatocytes
isolation of, 425–428
purification of, 427–428
Progenitor cells, 98–100
in vitro chondrogenesis of, 100–104
in stromal system, 25–26
in tissue engineering, 25
Proliferating monolayer, 171–172
Proliferation, cell, 4–7
Pronase digestion, 170
Pronase digestion solution, preparation for articular cartilage cultures, 161
Propagation, of mesenchymal stem cells, 35–37
Prostheses
ACL replacement with, 193, 194–195
artificial vascular, 297
cardiovascular, 294
Proteinase K buffer, preparation for articular cartilage cultures, 166
Proteinase K digestion, of alginate beads, 182–183
Proteinase K solution, preparation for articular cartilage cultures, 166–167
Protein formation, extracellular matrix, 204
Proteins. *See also* Therapeutic proteins
in engineered blood vessels, 300
in silk, 327, 328
Proteoglycan assays, for articular cartilage cultures, 163–164
Proteoglycan content, of chondrocyte cultures, 176–178
Proteoglycan content assays, 176–178
Proteoglycan dilution buffer, preparation for articular cartilage cultures, 163
Proteoglycans (PGs), 122
analysis of, 221–222
calculation of amount in samples, 178
Proteoglycan standard stock, preparation for articular cartilage cultures, 163
Prototyping tools, in liver tissue engineering, 460
Pulsatile perfusion system, 307, 308, 310
Purification
of plasmid DNA, 116
of silk, 328–329
Purification schemes, for chondroprogenitor cells, 99

Qualitative cytochemical alkaline phosphatase assay, solutions for, 30–31
Quantitative biochemical alkaline phosphatase assay, solutions for, 30
Quarantine, of cell cultures, 17

Rat hearts, implantation of engineered heart tissues onto, 264. *See also* Neonatal rat cardiac myocytes

Rat heart tissue. *See also* Engineered rat heart tissue
preparation of, 268, 269–271
preparing casts for and culturing, 271–272
Rat hepatocyte isolation, surgical procedure for, 426–427
Rat peritoneum, implantation of engineered heart tissues in, 263
Rats, adrenal medullary chromaffin cells from, 377–378
Rat Sertoli cells, isolation of, 396–397. *See also* Sertoli cells
Reagent preparation
for articular cartilage cultures, 160–168
for bone engineering, 332–335
for cartilage tissue engineering, 85–89
for cellular photoencapsulation, 217
for dorsal root ganglia cultures, 402–403
for engineered blood vessels, 302–303
for engineered heart tissues, 265–266
for human embryonic stem cells, 64–65
for ligament tissue engineering, 197–199
for lipid-mediated gene transfer, 116–118
for liver tissue engineering, 424–425
for neuroendocrine/neuronal cell culture, 379–380, 395–396
for skeletal muscle tissue engineering, 241–242
Real-time reverse transcriptase-polymerase chain reaction (RT-PCR), 225, 347, 360–362. *See also* Reverse transcriptase-polymerase chain reaction (RT-PCR)
of cartilage hydrogels, 222–223
in ligament tissue engineering, 204
Recombinant DNA technology, 114
Recombination, 7, 8, 9
Reconstituted rat heart tissue, preparation of, 268, 269–271. *See also* Engineered rat heart tissue; Rat hearts; Rat heart tissue
Record keeping, 11
Redifferentiation culture models, 95, 98
Refrigeration, tissue, 10, 134
Regeneration, 214
of cartilage, 84–85
liver, 460–461
of liver cells, 419
Regenerative cells, 5
Regenerative medicine, embryonic stem cells in, 63
Remodeling of tissue, 134
Repair capacity, of articular cartilage, 159
Repair of tissue, 134
Replacement, of defective cardiac tissue, 286–288
Reporter gene expression, in transfected cartilage, 122–123
Reverse transcriptase-polymerase chain reaction (RT-PCR), 225, 230, 232. *See also* Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)
of cartilage hydrogels, 222–223
RGD motif, coupling to silk, 329–331. *See also* Arginine-glycine-aspartic acid (RGD) surface modification
Richmond, John, 191
Ring-shaped engineered heart tissues. *See* Circular engineered heart tissues
RNA. *See* mRNA assays
RNA extraction, 230
from bone constructs, 357–360
RNA isolation, in ligament tissue engineering, 204
RNase contamination, preventing, 358
RNeasy Mini Kit, 225, 230
Rolled cell sheets, in engineered blood vessels, 301
Rotating vessels, 140, 141. *See also* Rotating wall vessel (RWV) bioreactors; Spinner flasks
Rotating wall vessel (RWV) bioreactors, 379, 392, 394. *See also* High Aspect Ratio Vessels (HARVs); Slow Turning Lateral Vessels (STLVs)
dynamic formation of PC12 organoids in, 383, 384–385
Rotatory Cell Culture Systems (RCCS), 383
Ruffner, Melanie, 213

Safranin-O staining, of sections of cartilage constructs, 365
Sah, Robert L., 157
Saline, sterile, 87
Saline wash, preparation for articular cartilage cultures, 160
Samples, calculation of DNA in, 183
Saphenous vein grafts, 294
Saporta, Samuel, 375, 377, 390
Sarcomeric structures, of engineered heart tissues, 281
Sarcomeric tropomyosin, 249, 250
Saturation density, 13
Scaffold biomaterials, comparative mechanical properties of, 326
Scaffold-cultured cells, DNA assay of, 348–350
Scaffold-free alginate-recovered chondrocyte (ARC) method, 173–176
Scaffolds, 9, 325. *See also* Bone tissue scaffolds; Polyglycolic acid scaffolds; Polylactic acid scaffolds; Polymer scaffolds; Silk scaffolds; Tubular PGA scaffold
biomaterial, 138–139
in bone tissue engineering, 366–368
cell seeding in, 122
in engineered blood vessels, 301
for engineered heart tissues, 286–287
fibrous, 139, 143–144
hydrogel, 215, 219–221
for liver tissue engineering, 423–424
osteoconductive, 325
porous, 139, 144–146
seeding differentiated cells onto, 78
in tissue engineering, 214–215

Scaffold seeding, 141, 142–146
Scanning electron microscopy (SEM)
buffers for, 198
of engineered vessels, 313
in morphology analysis, 204
Scanning electron microscopy solutions, for blood vessel engineering reagents, 303
SCID mice, 64, 77
implantation of ceramic cubes into, 41–43
Screening, of cell cultures, 17
SDS lysis buffer, preparation for liver tissue engineering, 425
SDS-PAGE, 182
Seeding. *See also* Cell seeding; Luminal seeding; Scaffold seeding
dynamic, 142–144
gel-cell, 144–146
smooth muscle cells into bioreactor, 308–310
Seeding chamber, 201
Selectable markers, genetically engineered, 74
Senescence, 12
Sensory neurons, 400
Serial subculture, 14, 15
Sericin-extracted silk, in ACL tissue engineering, 195, 206
Sericins, in silk, 327
Sertoli cells (SCs), 391, 392. *See also* Rat Sertoli cells
effect on NT2 cell dopamine production, 394
preparation of, 396–397
Sertoli-NT2N-aggregated-cell (SNAC) tissue constructs, 392, 393, 394
preparation of, 399–400
Sertoli-NT2N tissue constructs, to treat neurodegenerative disease, 390–400
Serum
in articular chondrocyte cultures, 94–95
for neuroendocrine/neuronal cell culture, 379–380
Serum dependence, of engineered heart tissues, 263
Serum-free hepatocyte culture medium, preparation for liver tissue engineering, 425
SH2 antibodies, 335
Shansky, Janet, 239
Sialoprotein, silk matrix design and, 207
Silicone rubber casting molds, 249
formation of, 250–251
Silk, 336
in ACL tissue engineering, 195, 200–201, 201–202
biodegradation of, 327, 328
as a biomaterial, 325–328
in bone tissue engineering, 366–368
purification of, 328–329
Silk fibroin, as scaffold for ACL tissue engineering, 205–207
Silk matrices, 200–201
Silk matrix cords, seeding with bone marrow stromal cell culture medium, 201–202
Silk matrix design
in ligament tissue engineering, 205–207
study of, 201
Silk matrix surface modification, in ligament tissue engineering, 207–208
Silk-RGD, preparation of, 329–331. *See also* Arginine-glycine-aspartic acid (RGD) surface modification
Silk-RGD scaffolds, culture of hMSCs on, 344–347
Silk scaffolds. *See also* Silk-RGD scaffolds
in bone tissue engineering, 366–368
preparation of, 331–332, 345
3D, 325–332
Silk surface modification, study of, 202
Silkworms, 367
Silkworm silk, 326
Skeletal muscle growth medium (SKGM), 241
Skeletal muscle needle biopsy, 242–244
Skeletal muscle tissue engineering. *See also* Skeletal muscle needle biopsy
applications of, 254–255
for clinical applications, 239–257
myoblast characterization in, 246–249
myoblast isolation/culture in, 244–246
reagent and media preparation for, 241–242
sources of materials for, 255–256
Slow Turning Lateral Vessels (STLVs), 141, 383, 385. *See also* Bioreactors; Rotating vessels
generation of PC12 organoids with beads in, 387–390
SMC markers, immunostaining for, 311–312. *See also* Smooth muscle cells (SMCs)
Smooth muscle cell culture medium, for blood vessel engineering reagents, 303
Smooth muscle cell isolation/culture, for vascular tissue engineering, 305
Smooth muscle cells (SMCs), 301, 303, 304. *See also* SMC markers; Umbilical smooth muscle cells
seeding into vascular cell bioreactor, 308–310
Sodium cacodylate buffer, for scanning electron microscopy, 198
Spider silk, as biomaterial, 326. Sources of Materials, *See* Materials sources
Spinal cord injury, 400
Spinner flasks, 140–141
in bone tissue engineering, 343–344
dynamic seeding in, 142–144
preparation of, 344–347
Split ratio, 14
Spontaneous transformation, 18
Staining. *See also* Coomassie Blue staining solution; Immunostaining; MTT staining
for cultured mesenchymal stem cell mineralization, 48–49
for microbial contamination, 17
Mallory–Heidenhain, 43, 44
Nile Red, 51, 52–53
Safranin-O, 365

Staining. *See also* Coomassie Blue staining solution; Immunostaining; MTT staining (*Continued*)
Toluidine Blue, 43
von Kossa, 48–49, 364–365
Starter dishes, 140
Static culture systems, hepatocytes in, 421
Static flasks, 140
Stationary phase, 12–13
Statistical analysis, in ligament tissue engineering, 205
Steady-state oxygen gradients, formation of, 447–449
Steady-state stirred suspension reactors, 77
Stem cell isolation/expansion, bone marrow- derived, 218–219
Stem cells, 5, 6. *See also* Human mesenchymal stem cells (hMSCs); Mesenchymal stem cells (MSCs)
in bone marrow, 335
in tissue engineering, 25
Stereolithography, in liver tissue engineering, 460. *See also* Photopatterning
Sterile saline, 87
Stewart, Matthew, 83
Strain
in cardiac myocyte-populated matrix, 261–262
in engineered blood vessels, 300
Strength
of engineered blood vessels, 299–302
of silk, 327
Streptomycin, 85
Stroma, 340. *See also* Bone marrow stromal cells (BMSCs); Human bone marrow stromal cells (hBMSCs)
Stromal system, progenitor cells in, 25–26
Student-Newman-Keuls test, in ligament tissue engineering, 205
Subculture, 11–14
of human mesenchymal stem cells, 35–36, 37
serial, 14, 15
Substrate-based culture models, for articular chondrocytes, 95
Substrate buffer, 30
Substrates, 9
microfabrication of, 438–441
Substratum coating, for dorsal root ganglia cultures, 403
Superficial zone protein (SZP), tissue formation and, 183–184
Supplemented tris-buffered saline (STBS), preparing for engineered heart tissues, 266
Suppliers list, 473–481
Surface antigen analysis, authentication of, 340, 341–342
Surface modifications. *See also* Silk-RGD entries
photolithographic patterning of, 438–439
of silk fibers with RGD, 200–201, 207–208
Surgeon, in culture projects, 10
Suspension, of hepatocytes, 421, 422
Sympathoadrenal (SA) cell lineage, 377–378
Synovial tissue, chondroprogenitor cells from, 99–100
Synoviocytes, 99–100
Synthesis, liver, 428
Synthetic polymers. *See also* Polymer entries
ACL replacement with, 193
in ACL tissue engineering, 195
Synthetic vascular grafts, 294–295

Telomerase, 12
Tensile strength, of silk, 327. *See also* Mechanical strength; Strength
Thawing, of human mesenchymal stem cell culture, 339. *See also* Cryopreservation
Therapeutic proteins, from bioartifical muscles, 254
Thombin, 297
3-D culture models, for liver tissue engineering, 423–424. *See also* Three-dimensional cultures
Three-dimensional (3D) cardiac tissue constructs, 261–263, 284–288
Three-dimensional cultures
of articular chondrocytes, 95
on polymer scaffolds, 72
Three-dimensional hydrogels, photopatterned, 451–457
Three-dimensional neuroendocrine/neuronal differentiation, of PC12 pheochromocytoma cells, 377–390
Three-dimensional polymer scaffolds, in bioreactors, 146
Three-dimensional scaffolds, cell seeding in, 142
3-D PC12 organoids, generation of, 383
3D silk scaffolds, 325–332
Thrombomodulin, 317
Thrombosis, 316–317
in engineered blood vessels, 297–299
Tissue(s)
collection and transportation of, 9–10
as stem cell repositories, 25
Tissue analyses, outcome of, 183–184
Tissue constructs
Sertoli-NT2N-aggregated-cell (SNAC), 392, 393, 394, 399–400
transplanted into the brain, 393–394
Tissue culture equipment, for lipid-mediated gene transfer, 117–118
Tissue culture suppliers, 20
Tissue digestion, trypsin solution for, 395
Tissue disaggregation, 4, 11
Tissue-engineered anterior cruciate ligament, criteria for, 194
Tissue-engineered blood vessels, 293–322. *See also* Engineered blood vessels
genetic manipulation in, 316–318
sources of materials for, 318–319

Tissue-engineered vascular grafts, 316
culture of, 303–311
requirements for, 296–297
Tissue engineering, 131–155. *See also* Anterior cruciate ligament tissue engineering; Bone tissue engineering; Cardiac tissue engineering; Embryonic stem (ES) cells; Engineered entries; Ligament tissue engineering; Liver tissue engineering; Skeletal muscle tissue engineering; Mesenchymal stem cells (MSCs); Vascular tissue engineering
of articular cartilage, 157–189
biomaterial scaffolds in, 138–139
bioreactor cultivation in, 146–149
bioreactors in, 139–141
cells in, 135–137
functional, 133
goals and strategies of, 214–215
history of, 25–27
human embryonic stem cell culture for, 61–82
of human skeletal muscle, 239–257
in vitro cultivation of engineered tissues, 132–135
media used in, 137–138
model system of, 133–135
neuroendocrine and neuronal cell culturing for, 375–415
perfusion of porous scaffolds for, 145–146
preparation of media and reagents for, 29–31
protocols for using embryonic stem cells in, 78
for replacement of defective cardiac tissue, 286–288
scaffold seeding in, 141, 142–146
scale-up of embryonic stem cells in, 77–78
sources of materials related to, 150
Tissue harvesting
for cellular photoencapsulation, 217–219
in ligament tissue engineering, 199–200
Tissue regeneration, 134
Tissue remodeling, 134
Tissue repair, 134
Tissue stem cells, 6
Toluidine Blue metachromatic matrix, 102
Toluidine Blue staining, 43
Total joint arthroplasty (TJA), chondrocyte removal via, 89
Totipotent stem cells, 6
Trabecular networks, in bone tissue engineering, 366–368
Transcript levels, in ligament tissue engineering, 204
Transfected cartilage, reporter gene expression in, 122–123
Transfection, 317. *See also* Adenoviral gene transfer;Gene transfer
Transfection reagent, 117
Transforming growth factor-β-1 (TGF-β_1), 234
in bioreactor cultivation, 146–147
preparation of, 335
stock, 88
Transmission electron microscopy (TEM)
of cardiac myocytes, 280–282, 285
of engineered heart tissue, 275–276
of engineered vessels, 312–313
Transmission electron microscopy solutions, for blood vessel engineering reagents, 303
Transplantation
engineered tissue constructs for, 391
liver, 419
for Parkinson disease, 390
of tissue constructs into brain, 393–394
Tris-base, for collagen ELISA buffers, 198
Tris buffer, preparation for neuroendocrine/neuronal cell culture, 395
Tris-buffered saline, supplemented, 266
Tris-EDTA (TE) buffer, 118
Tris-HCl, for collagen ELISA buffers, 198
TRIzol RNA extraction, from bone constructs, 357–360
Trypsin, passaging human embryonic stem cells with, 68
Trypsin-EDTA, for ligament tissue engineering, 197
Trypsin-EDTA solution, preparation for neuroendocrine/neuronal cell culture, 395
Trypsin solution
for dorsal root ganglia cultures, 403
for tissue digestion, 395
Trypsin stock, 242
Trypsin stock solution, preparing for engineered heart tissues, 266
T-tubule-SR junctions, of EHT cardiac myocytes, 285
Tube formation, in vitro, 76
Tubular PGA scaffold. *See also* Polyglycolic acid (PGA) scaffolds
assembly/treatment for vascular tissue engineering, 306
for vascular cell bioreactor, 307–308, 309
2-D culture, of PC12 cells, 381
2-D culture models, for liver tissue engineering, 423
Two-dimensional (2D) confluent cultures, differentiation of embryonic stem cells in, 71
Type I collagen, 232, 444
in ACL tissue engineering, 194–195
in articular cartilage repair, 159
assays of, 347
biodegradation of, 326
in bone, 325–326
for engineered heart tissue cultures, 266–267, 271
protein content of, 204
Type II collagen, 84, 122, 134, 226, 232
analysis of, 221–222
in articular cartilage repair, 159, 160
silk matrix design and, 207
Type II collagenase, 86

Tyrode's solution, preparing for engineered heart tissues, 266
Tyrosine hydroxylase (TH), 378, 392–393

Ultimate tensile strength (UTS), in silk matrix design, 206
Ultrafoam™, 139
Ultrastructure, of engineered heart tissues, 279–282, 285
Ultraviolet (UV) light, 451, 456
Umbilical smooth muscle cells, in arterial grafts, 295–296
Underhill, Gregory H., 417
Undifferentiated embryonic stem cells, seeding for in vivo differentiation, 78
Undifferentiated PC12 cells, 380–382
  culturing and passaging, 382–383
Undifferentiated precursor cells, 6
Unsworth, Brian R., 375, 377
Urea synthesis, liver, 428
Urea synthesis assay, in cultured hepatocytes, 433

Validation, cell culture, 16–20
Vandenburgh, Herman H., 239
Vascular cell adhesion molecule-I (VCAM-I), 316
Vascular cell bioreactor. *See also* Bioreactors
  luminal seeding of endothelial cells into, 310–311
  seeding smooth muscle cells into, 308–310
Vascular constructs, immunostaining for SMC and EC markers in, 311–312
Vascular disease, atherosclerotic, 294
Vascular grafts, 294–295, 296–297, 300–301. *See also* Tissue-engineered vascular grafts
  culture of, 303–311
Vascularization, of implanted engineered heart tissues, 263, 264
Vascular tissue engineering
  assembly and treatment of tubular PGA scaffold for, 306
  efforts in, 295–296
  isolation and culture of endothelial cells for, 304–305
  isolation and culture of smooth muscle cells for, 305
Vasculogenesis, 76–77
Vasoconstriction tests, 313, 314
Vasodilation tests, 313, 314
Venous grafts, 294, 301
Vessel culture, 306–311
  enhanced culture medium for, 303
Vessel formation, in vivo, 76–77. *See also* Blood vessels
Viruses. *See* Adenoviral gene transfer
Volloch, Vladimir, 191
Volunteers, human skeletal myoblast biopsies on, 244
von Kossa staining, 48–49
  of sections of bone constructs, 364–365
Vunjak-Novakovic, Gordana, 131, 191, 323

Washing buffer, preparation for liver tissue engineering, 425
Water-soluble polymer chains, in hydrogels, 215
Williams, Christopher, 213
WST-1 analysis, of photoinitiator toxicity, 219, 220–221

X-Gal cleavage, by β-galactosidase, 122–124
X-Gal staining fixative, 118
X-Gal stock solutions, 118
XYZ gyrator, 140

Yeast infections, of cell cultures, 17
Yoo, Jung, 83

Zimmermann, W. H., 259
Zonated CYP2B expression, resulting from bioreactor culture, 449–450. *See also* Cytochrome P450 B2 (CYPB2)
Zonated features, 445
  assessment of, 449–451
Zonated gene expression, in hepatocytes, 447
Zonation, liver, 444, 445, 447, 449–451
Zone-specific biosynthetic activity, of hydrogel-encapsulated cartilage, 226–228
Zyderm working solution, 252, 253