

INDEX

A

- Aldehyde dehydrogenase (ALDH)
 activity 130, 134–135
- Alginate
 cell behavior
 histochemistry 170, 173–174
 immunostaining 169, 172–173
 metabolic activity assay 169, 171–172
 osteogenic differentiation 170, 175
 trypan blue dye exclusion assay 171
 viability assay 169
 cells and media 168–169
 internal gelation 168
 preparation 170–171
- Angiogenesis
 ECs (*see* Endothelial cells (ECs))
 MSCs 29
 and myogenesis 121
 neo-tubes branch 122
 tubular-like structures 36–37

B

- Bioengineered 3D matrix 30, 51–53
- Bioprinting 52
- Bone digestion 44, 49, 182, 188
- Bone homing 142
- Bone-marrow macrophages
 acquisition and analysis 189–191
 flow cytometry 182, 184
 immunostaining 183–184, 188–189
 isolation 183–185
 mice 182
 neonates 183, 187–188
 osteomacs 181, 183, 185–187
- Bone-marrow transplantation 142–145

C

- Cadherin 39–41, 47,
 102, 159
- Cancer cell inoculation 143–144
 intracardiac injection 146–147
 subcutaneous implant and resection 145–146
- Cancer stem cells (CSCs)
 cell culture selection 132, 135

- clonogenic assay 66–67
- culture techniques 61–62
- enzymatic activity 131, 134–135
- equipment and material list 62–63
- genetically modified mouse models 62
- Hoechst dye exclusion 131, 134
- markers 61
- mouse crypt isolation 63
- paraffin embedding 67–70
- reagents for paraffin embedding 63–64
- surface markers 131, 133
- Cardiovascular regeneration 52, 87, 88, 121
- Catenin 40, 41, 47, 159
- CD44 61, 130, 131, 133, 134
- CD166 181, 182, 184, 189–191
- Cell adhesion 51, 57, 102,
 103, 114, 181
- Cell aggregate
 appliances 89–90
 cell–surface and cell–cell interactions 88
 construction and optimization 92–93
 ECM 87
 isolation and culture 90–91
 morphological observation 93–94
 niche assessment 94–96
 reagents 88–89
- Chicken embryo 3, 5–7, 9, 41, 45,
 49, 153, 154
- Clonogenicity 62, 63, 66–67,
 69, 115
- Co-culture
 3-D and 2-D systems 104
 ECs 126
 HSPC/MSC 102
 UCB CD34⁺ 107, 112–114
 VCS 56
- Cranial neural crest 1–5, 8
- Crestospheres
 crest-associated genes 9
 cultures 5–6
 free-floating neuroepithelial 3D clusters 3
 immunostained 9
 molecular biology applications 2
 NC (*see* Neural crest (NC))
 validation and functional studies 6, 7

D

Disseminated tumor cells (DTCs) 142, 149

E

Embryo

crestospheres 9
 DMEM 5
 growth factor-rich chick 49
 hematopoietic niche (*see* Hematopoiesis)
 hierarchical clustering 153, 154
 migratory neural crest stream 6
 NC 3
 premigratory stage 3
 trunk-derived cultures 8
 in vitro manipulation 2

Endothelial cells (ECs)

aprotinin 122
 cell media 123
 co-cultures 126
 culture supports 123
 cytodex 3 beads 122
 embedding coated beads 124–125
 fibrinogen 122
 HUVEC coating 123–124
 multistep process 121
 preparing cells 123–124
 recording and analysis 125
 thrombin 123
 vascular stem cell niche 121

Explant 29–32, 37

Extracellular matrix (ECM)

alignment 122
 cell adhesion molecules 102
 cellular microenvironment 165
 cytokines 102
 gelatin 31
 integrin beta-1 14
 microenvironments 87
 niche assessment 95, 96
 NSCs 77
 secretion 176
 signaling molecules 165
 3D structure 77

Extracellular vesicles (EVs)

culture media 78
 exosome
 isolation 80
 labeling 78
 and transplantation 81
 intraluminal vesicles 75
 perinatal brains and SVZ 78–79
 primary cell culture and microdissection 77
 sedimentation 76

sucrose density gradient 77
 SVZ 76–77, 79–80
 transplantation 81
 viral nucleic acid sensing pathways 75

F

Fibrin gel 122, 124–125, 127
 Flow cytometry
 acquisition and analysis 184
 cancer stem cells 134
 fetal
 liver 22–23
 muscle 16, 24–25
 spleen 17, 25
 and immunohistochemistry 14
 peripheral blood 142
 physical properties 182
 placenta 15–16, 21–22
 p-Sp/AGM region 14–15, 21

H

Heart microenvironment

bioengineering 52
 VCS (*see* Vascularized cardiac spheroids (VCSs))

Hematopoiesis

embryogenesis 13–14
 endothelial cells 14
 erythroid progenitor cells 13
 flow cytometry 14–17, 21–26
 immunohistochemistry 17–21, 26

Hematopoietic niche (HSC niche) 102, 141, 142

bone matrix 181
 mouse embryo (*see* Mouse)
 3D co-culture 101–118
See also Hematopoietic stem cells (HSC)

Hematopoietic stem cells (HSC)

BM MSC 102
 bone microenvironment 142
 CD34⁺-enrichment 107
 engraftment analysis 144, 147–148
 feeder layer 106
 hematopoietic lineage 101
 HSPC 103
 human

BM-derived MSC 103–105
 UCB CD34⁺-enriched cells 107

immunophenotypic and functional characterization

adhesion assay 108–109
 CAFC 108
 cell counting and viability 108
 CFUs 108
 migration assay 109
 UCB CD34⁺-enriched cells 108

microwells	110
MS-5 murine stromal cells	105
niche competition assay workflow	142
peripheral blood	141
preparation of microwells	105–106
spheroids	103, 106
thawing and expansion	
human BM-derived MSC	109–110
MS-5 murine stromal cells	110
3D cellular interactions	102
UCB MNC	102, 106–107
Hematopoietic stem/progenitor cells (HSPC)	
blood-borne viruses	101
CD34 ⁺ 417 ⁺ enrichment	112
cellular components	181
co-culture	103
MSC co-culture microenvironment	102
populations	16
UCB	107
in vitro	102
Human ES cell-derived neural crest	2, 5
Hybridization chain reaction (HCR)	152, 156
antibody staining	159–160
crestosphere validation	7, 9
and immunostaining	157–160
signal amplification	152
Hydrogel	
alginate (<i>see</i> Alginate)	
ECM	166
gelation	168
internal gelation	168
MSC-laden RGD-alginate	173
polymeric 3D networks	166
I	
Immunohistochemistry	26
ECM niche assessment	94, 96
fetal	
liver	18–19
muscle	19–20
spleen	20–21
placenta	18
p-Sp/AGM region	17–18
Induced pluripotent stem cells (iPSC)	51, 52, 57
Integrin	14, 40, 41, 89, 95, 96, 166
Invasion	29, 31, 35
In vivo single-cell analysis	6, 21, 22, 62, 135, 151, 152, 159, 182
Isolation	
cell and culture	90–91
CS formation	53
flow cytometer	21
functional characterization	30

mesenchymal precursors	31–32
mouse	
crypt reagents	3
EDL muscle	42–44
proximal and distal small intestine	64–66
myofibers	40–41, 44–45
neural tubes	3–5, 8
VCSs (<i>see</i> Vascularized cardiac spheroids (VCSs))	

M

Mesenchymal stem/stromal cells (MSC)	
adult tissue niches	29
alginate hydrogels	168–169
alginate crosslinking	167
BM-derived	103–105
CaCO ₃	168
ECM	165
feeder layer	106
and HSC	102
HSPC	103
human	
BM-derived	109–110
UCB CD34 ⁺	107, 112–114
hydrogels	166
self-renewal capacity	29
2D models	165
UCB CD34 ⁺ -enriched cells	108–109
<i>See also</i> Hematopoietic stem cells (HSC)	
Microenvironment	
CSCs	61
HSC niche	142
morphogenesis and regeneration	87
SCs	39
VCSs (<i>see</i> Vascularized cardiac spheroids (VCSs))	
Microglia	76, 79, 81
Microvesicle	75, 76, 82
Microwells	103, 105–106, 110, 117
Migration	
assay	31, 109, 116
cell adhesion	103
hematopoietic stem cells	13
mesenchymal precursor	34
regenerative properties	29
transwell assay	34–35
in vitro scratch assay	33–34
Mouse	
BMMSCs	91
crypt culture medium	64
EDL muscle	42–44
hematopoietic niche (<i>see</i> Hematopoiesis)	
human HSCs	148
isoflurane inhalation	145
isolation (<i>see</i> Isolation)	
model	62

Mouse (<i>cont.</i>)	
Periostin antibody	89
proximal and distal small intestine	64–66
reagents	63
samples	30
xenografts	130–132
Multipotency	
lineage-restricted progenitors	103
NC stem cells (<i>see</i> Neural crest (NC))	
Muscle	
and bone marrow	13
and connective tissues	90
fetal	16, 19–20, 24–26
liver hematopoietic progenitor cells	14
regeneration	121
<i>See also</i> Muscle stem cells	
Muscle stem cells	
fixing and staining reagents	41–42
imaging and analyzing junctional	
components	42, 47–48
initial preparation	42
mouse EDL muscle	42–44
myofiber isolation reagents	40–41, 44–45
SCs	39
in vivo approaches	40
Myofibers	
blocking	46
fixation	45
mouse EDL muscle	44–45
permeabilization	45
staining	46
Myogenesis	121
N	
Neural crest (NC)	
cell culture models	1–2
crestospheres	2–3
culture	3–4
embryos	3
isolation of neural tubes	3–5
stem cell niche	152, 155
transient stem cell population	1
Neural stem cell (NSCs)	
EVs (<i>see</i> Extracellular vesicles (EVs))	
hematopoietic stem cells	121
heterogenous clusters	77
SOX2	7
SVZ (<i>see</i> subventricular zone (SVZ))	
Niche	
angiogenic assays	31
cell	
aggregate (<i>see</i> Cell aggregate)	
culture	30–31
invasion	35
migration assessment	34–35
competition	142
ECs (<i>see</i> Endothelial cells (ECs))	
explant technique	30
hematopoietic (<i>see</i> Hematopoiesis)	
imaging and analyzing junctional	
components	47–48
invasion assay	31
mesenchymal precursor isolation	31–32
migration	
assay	31
measurement	33–34
mouse samples	30
MSCs	29
muscle stem cells (<i>see</i> Muscle stem cells)	
tubular-like structures	36–37
vertebrate body	2
in vitro techniques	30
O	
Oral cancer	
cancer cell lines	131–132
cell-cycle/G2-M	130
CSCs	129–130
mouse xenografts	133–135
primary tumor/mouse xenografts	130–133
xenograft formation assay	136
Organoids	
IHC	67–70
Lgr5+ stem cells	62
mouse colon	62
paraffin embedding	63, 64
phase contrast images	67
Orosphere	132, 135
P	
Peptide-modified alginate	175
Pluripotency	155
Q	
Quiescence	39, 121, 142
R	
Regeneration	30, 52, 77, 87, 88, 121
S	
Satellite cell (SCs)	39, 40, 45, 47–49, 121
Self-renewal	
cell–cell interactions	14
HSC differentiation	141
lymphoid and myeloid lineages	101

and multipotency (<i>see</i> Multipotency)	
neural crest-associated genes	9
tumorigenic potential	130
Side population	130, 131, 134
Single-molecule fluorescent in situ hybridization (smFISH)	151
Spatial genomic analysis (SGA)	
buffers	153–155
cover glasses	155–156
data analysis	161
HCR signal amplification	152
hybridization (<i>see</i> Hybridization chain reaction (HCR))	160–161
imaging	156
probe design	157
sample preparation and cryosectioning	152
signal amplification	151
single-cell RNA sequencing-based methods	110–111
Spheroids	
BM MSC	113
CD34 ⁺ Cells	103
culture plates	106
formation	103
heterogeneous sizes	102
mimic stem cell niche interactions	102
VCSs (<i>see</i> Vascularized cardiac spheroids (VCSs))	
Stem cells	
aggregate engineering (<i>see</i> Cell aggregate)	
HSC niche (<i>see</i> Hematopoietic niche (HSC niche))	
human heart microenvironment (<i>see</i> Vascularized cardiac spheroids (VCSs))	
isolation (<i>see</i> Isolation)	

MSCs (<i>see</i> Mesenchymal stem cells (MSCs))	
muscle (<i>see</i> Muscle)	
NC (<i>see</i> Neural crest (NC))	
SVZ (<i>see</i> Subventricular zone (SVZ))	
Subventricular zone (SVZ)	76–81

T

3D cultures	51, 56, 62
<i>See also</i> Endothelial cells (ECs)	
Tissue bioengineering	52
Tissue regeneration	88
Trunk neural crest	1, 3

V

Vascularized cardiac spheroid (VCSs)	
building blocks	52
cell	
culture	52–53
preparation	54–55
CS formation and isolation	53
fixation and immunolabeling	53–57
formation and maintenance	55
hanging drop cultures	51–52
isolation from hanging drop culture	55

X

Xenograft	
formation assay	132, 136
mouse strain	138
primary tumor	130–133