UTHealth
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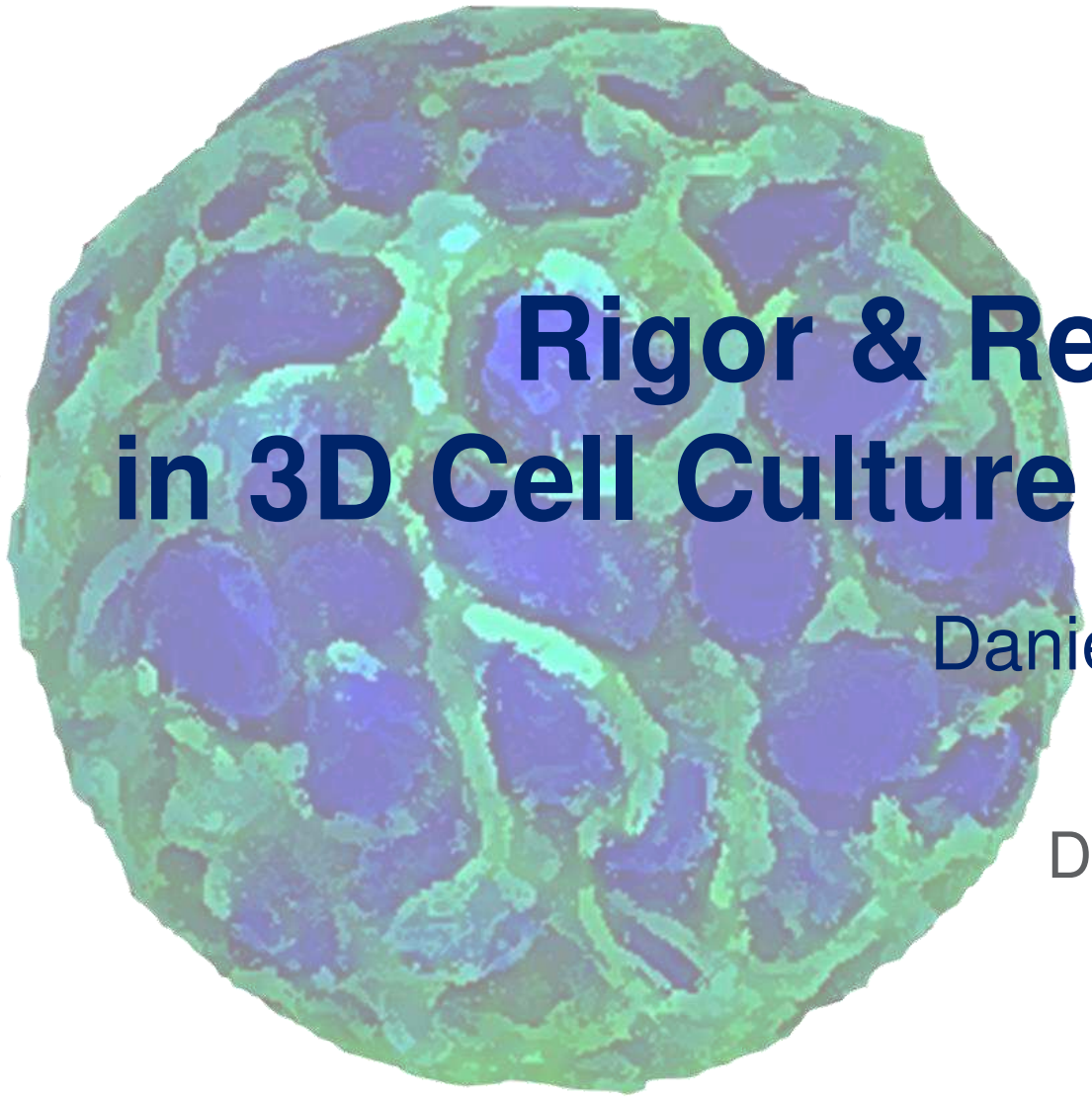


Rigor & Reproducibility in 3D Cell Culture Experiments

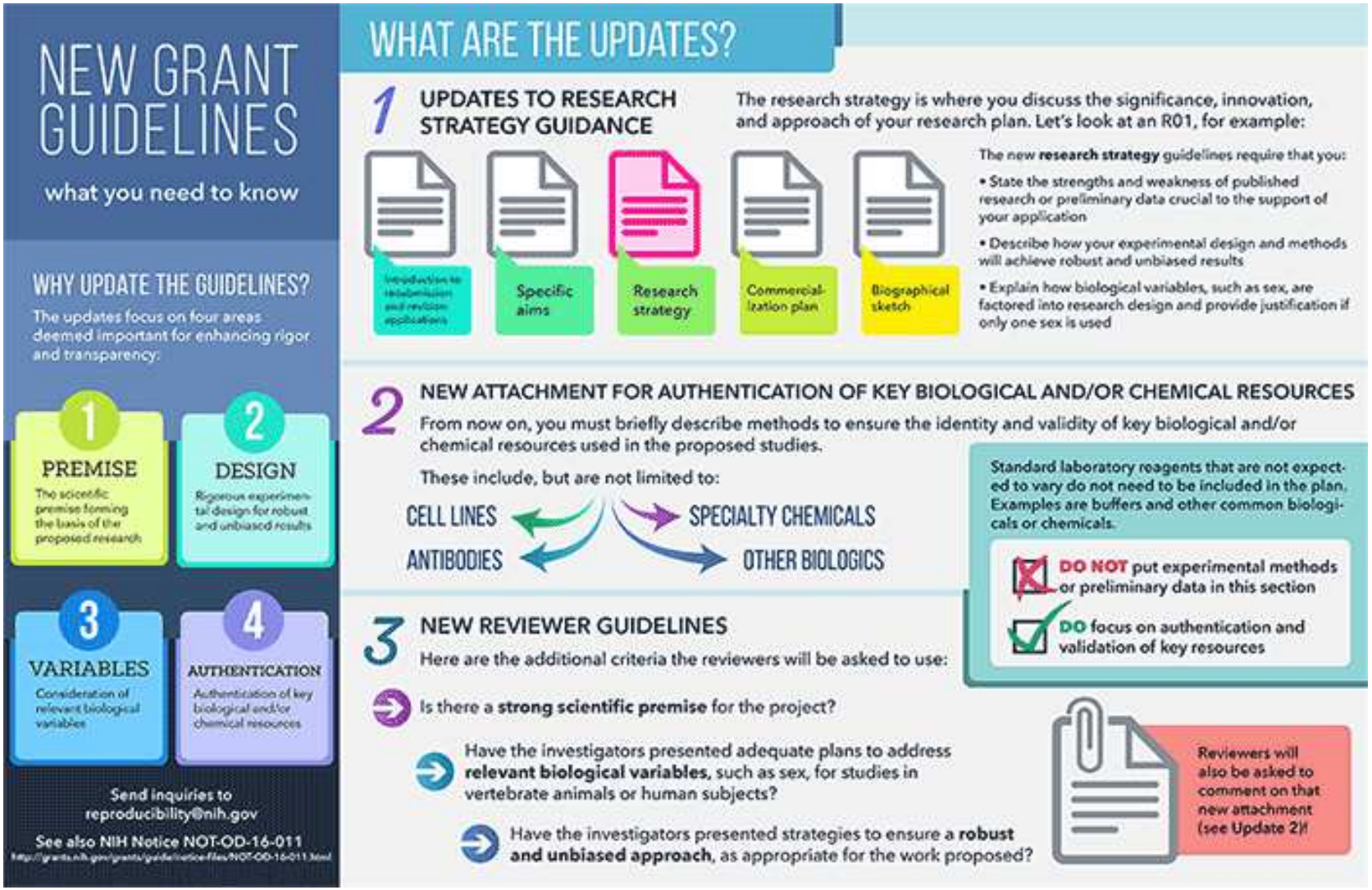
Daniel A. Harrington, PhD

Assistant Professor
Department of Diagnostic
& Biomedical Sciences

How to Define 3D Cell Culture?

- Broadly described: Cell culture methods that advance beyond 2D cell growth on tissue culture plates
- Multiple incarnations:
  - Spheroids (hanging drop, low-adhesion plates)
  - Culture in/on porous scaffolds
    - Various materials: polymers, hydrogels (including biologics), ceramics, porous metal structures
    - Cells may be fully encapsulated (within gels) or seeded throughout a highly porous material
- Common applications:
  - Tissue engineering, regenerative medicine
  - Cancer biology, drug screening

Authentication of Key Resources



NIH/NIDCR

From my own grant!

Authentication of Key Biological and/or Chemical Resources

Cell lines
Human cell lines in the proposal were obtained as described below:

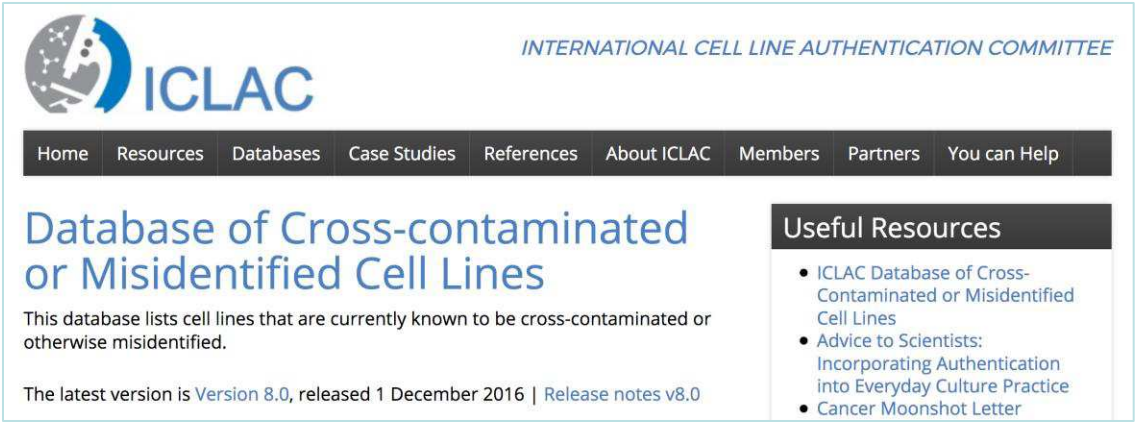
Cell line	Source	Date acquired	Lot #	Culture medium
UM-SCC-11-fib	Carey/Brenner – UM	7/2016	N/A	DMEM-HG w supplements
UM-SCC-11A	Carey/Brenner – UM	7/2016	N/A	DMEM-HG w supplements
UM-SCC-11B	Carey/Brenner – UM	7/2016	N/A	DMEM-HG w supplements
UM-SCC-26-fib	Carey/Brenner – UM	7/2016	N/A	DMEM-HG w supplements
UM-SCC-26	Carey/Brenner – UM	7/2016	N/A	DMEM-HG w supplements
UM-SCC-42-fib	Carey/Brenner – UM	7/2016	N/A	DMEM-HG w supplements
UM-SCC-42	Carey/Brenner – UM	7/2016	N/A	DMEM-HG w supplements
HN31	Frederick – UCHSC	11/2008	N/A	DMEM-HG w supplements
UM-SCC-47	Carey - UM	7/2008	N/A	DMEM-HG w supplements
MOC1	Uppaluri - WUSTL	10/2015	N/A	IMDM MOC
MOC2	Uppaluri - WUSTL	10/2015	N/A	IMDM MOC
mEERL	Lee – U-Iowa	2015	N/A	DMEM/F12

Vials of cells were frozen in growth medium supplemented with 5% DMSO and stored in liquid nitrogen. In our laboratory, each cell line undergoes follow-up STR verification upon creation of each new frozen batch. The STR profile is generated by our Characterized Cell Line Core (CCLC) supported by the Cancer Center Support Grant CA016672 by using the Promega Power PLex 16HS Kit. Following analysis, CCLC staff will compare the STR profile against the inclusive commercial database generated by this core, and our laboratory staff will compare it to our own database of STR profiles for HNSCC lines.

Antibodies. We use commercially available antibodies recognizing multiple proteins including FAK, PAK4, ROCK2 to conduct our cell biomarker analyses. These antibodies are typically sourced from the following vendors: Biolegend, abcam, Santa Cruz Biotechnology Inc. and Sigma Aldrich. (At the time of this submission, our laboratories have identified the few antibodies in use that are generated by Santa Cruz in USDA-regulated species, and we have transition plans to shift these to other vendors. None of our analyses will be affected by this transition.)

What Cells are Those?

UCSF LARC



Misidentified Cell Line	Claimed Species	Claimed Cell Type	Misidentified Cell Line, Cellosaurus AC	Contaminating Cell Line	Actual Species	Actual Cell Type	Contaminating Cell Line, Cellosaurus AC	Misidentification Reported By	Reference PubMed ID
REH-6	Human	Leukemia, acute lymphoblastic, B cell precursor	CVCL_L803	Unknown	Mouse	Unknown	None	Drexler et al, 2003	125923
REPC	Human	Kidney, normal renal cells	CVCL_W815	Hep 3B	Human	Liver, hepatocellular carcinoma	CVCL_0326	Frede [retrac	2 06725
RERF-LC-MA	Human	Lung carcinoma, small cell	CVCL_3153	SK-MES-1	Human	Lung, squamous cell	CVCL_0630	Cape Da et al, 201	2313 38
RERF-LC-OK	Human	Lung carcinoma	CVCL_3154	Marcus	Human	Astrocytoma	CVCL_3019	JC 3 w	No PMID
RGC-5	Rat	Retinal ganglion	CVCL_4059	661W	Mouse	Retina, photoreceptor cells	CVCL_6240	Van Bergen al, 2009; Krishnamoorthy et al, 2013	19 13730 3975727
RM-10	Human	Leukemia, chronic myeloid, blast crisis	CVCL_8463	K-562	Human	Leukemia, chronic myeloid, blast crisis	CVCL_0004	Drexler al, 2 3	1259 42
RMUG-L	Human	Ovarian carcinoma	CVCL_3157	SNG-II	Human	Endometrial carcinoma	CVCL_3170	JCRB w site	No
RO-D81-1	Human	Thyroid, medullary carcinoma	CVCL_M779	HT-29	Human	Colon carcinoma	CVCL_0320	Dadon et a 2013	23472229
RO-H85-1	Human	Thyroid, medullary carcinoma	CVCL_A666	647-V	Human	Bladder carcinoma	CVCL_1049	et al, 2013	23472229
RPMI-4788	Human	Colon carcinoma	CVCL_0U46	HeLa	Human	Cervical adenocarcinoma	CVCL_0030	Capes-Davis et al, 2013	23136038
RPMI-6666	Human	Lymphoma, Hodgkin	CVCL_1665	Correct name, incorrect cell type	Human	EBV+ B-lymphoblastoid cell line	None	Drexler et al, 2003	12592342
RPTC-1	Human	Thyroid, papillary carcinoma	CVCL_V277	TPC-1	Human	Thyroid, papillary carcinoma	CVCL_6298	Zhao et al, 2011	21868764
RS-1	Human	Leukemia, acute myeloid, M7	CVCL_8423	K-562	Human	Leukemia, chronic myeloid, blast crisis	CVCL_0004	Drexler et al, 2003	12592342
RTSG	Human	Ovarian carcinoma	CVCL_1671	SNG-II	Human	Endometrial carcinoma	CVCL_3170	JCRB website	No PMID
								Harris et al, 1981;	

488 LINES

Real Example: Paper Review

- Reviewing a submission to a journal with an Impact Factor ~ 6.
- Some parts of the paper felt rushed or sketchy, but one particularly caught my eye: Use of an epithelial cell line that I had never heard of!
  - At first I was worried that we were completely behind in the literature, having missed these human “hEG” * cells!
  - And then I searched for more information on them…
- “hEG” cells are actually on the ICLAC list – indistinguishable from HeLa cells!
- The paper was rejected
- And later published anyway in another journal, IF < 2, describing the use of “epithelial cells”

* (not the real name)

Target: Descriptions that Enable Experimental Reproducibility

- Goal: Without your notebook, could you reproduce the experiments in your paper?
- Why write this way?
  - Your own reproducibility
  - Junior lab members will be using your paper as a trail guide
  - Other researchers will be validating your work
  - Journals are starting to require it
- Nearly every journal has Supporting Information addenda where you can add all of these details

Example: Antibody Listing from Our Own Paper / SI

Target Protein	Vendor	Item #	Host Species	Dilution
SMMHC	Abcam	ab53219	rabbit	1:50
Cytokeratin 14	Abcam	ab49747	mouse	1:100
α-SMA	Abcam	ab2413	rabbit	1:50
Perlecan	Farach-Carson Lab	*	Polyclonal - rabbit	1:50
Collagen IV	AbCam	ab6586	rabbit	1:100
Laminin	Pierce	PA-36119	rabbit	1:50
M3 muscarinic receptor	Santa Cruz Biotech	Sc-9108	rabbit	1:50
B2-adrenergic receptor	Abcam	ab13989	chicken	1:250
Ki67	BD Pharmingen	550609	mouse	1:50
Cytokeratin 19	Abcam	Ab9221	mouse	1:100
AQP-5 (H-200)	Santa Cruz	Sc-28628	Polyclonal-rabbit	1:20

Key Elements: Source Materials

- Cells: validation of cell identity & applicability
  - Immortalized (from where?)
  - Primary (derivation method? characterization?)
  - STR confirmation
  - SABV
- Matrix (if any): beyond basic composition
  - If purchased: What item #? Details about material?
  - If synthesized: Describe details about composition, purity, molecular weight (if applicable)
- Media: often multiple variants of the same name
  - “DMEM”: glucose content? Sodium bicarbonate?
  - “FBS/FCS”: from where? purity/grade?

Key Elements: Culture & Analyses

- Culture Conditions:
  - Incubator (CO_2 level? Matched to media? Calibrated recently?)
  - Media exchanges
  - Any flow/perfusion/active motion vs. static cultures
- Analyses:
  - Immunostaining: specific antibody vendors, clones, concentrations, protocols, images of secondary controls
  - Imaging: which instrument? which detectors?
  - Protein/RNA extraction: precise methods & kits

“Reviewer #2”

- Reviewers will often ask for one extra experiment, or one additional validation
- But increasingly, journals will also require documented validation of cells & materials
- Data sharing plans often require:
  - that you retain “data” for 4-5 years
  - that you provide “data” upon request to other researchers
- Always save an aliquot of your cells, materials, or a small portion of your 3D cell/scaffold for a post-submission analysis!