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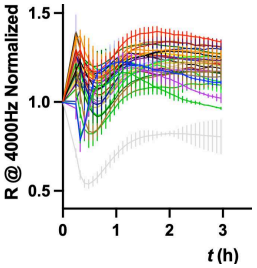
ECIS Data Analysis for Stimulation of Human Pulmonary Microvascular Endothelial Cells (HPMECs) with Human Serum V.3



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Michael Bokoch[1]

[1]University of California, San Francisco

UCSF Transplant Anesth...

Michael Bokoch
University of California, San Francisco

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We use this protocol and it's working

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Abstract

Sera from patients in our UCSF Liver Transplant Biobank are used to stimulate human pulmonary microvascular endothelial cells (HPMECs) grown to confluency on ECIS platform. This protocol prospectively defines the plan for data analysis from these experiments.

Troubleshooting

ECIS Data Analysis for HPMEC stimulation by Liver Biobank Sera

1 **Determine Difference in Area-under-the-Curve (Normalized Resistance at 4000 Hz)**
To be calculated after recording full ECIS curve after stimulation with human serum from liver transplant (LT) patients.

Goal: To calculate the difference in AUC between the normalized ECIS curves generated by (*a*) Liver transplant serum and (*b*) Healthy pooled serum.

$$\Delta AUC = AUC_{LT\ serum} - AUC_{Healthy\ pooled\ serum}$$

Sign conventions:
- Baseline resistance is normalized to 1
- AUC of curve that drops below 1 = Negative AUC ⟶ interpreted as an increase in EC permeability
- AUC of curve that rises above 1 = Positive AUC

e.g., for a typical Liver serum sample that induces permeability:
$AUC_{LT\ serum}$ = Negative number
$AUC_{Healthy\ pooled\ serum}$ = Positive number
ΔAUC = Negative number - Positive number = Large negative number

e.g., for a typical Liver serum sample that does not induce much permeability:
$AUC_{LT\ serum}$ = Smaller positive number
$AUC_{Healthy\ pooled\ serum}$ = Larger positive number
ΔAUC = Smaller positive number - Larger positive number = Small negative number

Final AUC analysis will use data from 0-3 hrs.

Prepare ECIS data for analysis

1.1 Using ECIS software, normalize the resistance. This is done by dividing the resistance at each time point by the baseline resistance (the plateau resistance reached just prior to stimulation with serum). This will set the baseline resistance = 1.

1.2 Export "Graph Data" and import into GraphPad prism.

Name data table "*raw normalized_mm-dd-yy_cell line*"

Align t=0

1.3 Duplicate X-Y tables with data from 0-6 hrs → name duplicate "*_adjustedt_0-3h*"

1.4 Align time = 0

- Inspect normalized curves for a given experiment (day and cell line)
- Determine time point (t_{stim}) where curves begin to diverge from baseline resistance = 1
- The time point prior to that time (t_{stim-1}) will be set = 0 hrs
- Calculate time shift in Excel using the follwing formula:

$$t_{adjusted} = t_{original} - t_{stim-1}$$

- Use $t_{adjusted}$ for determination of AUC 0-3 hrs.

Determine AUC from 0-3 hrs

1.5 Delete data points with $t_{adjusted}$ > 3 hrs

1.6 Analyze → XY analyses → Area under curve

1. **Baseline** Y = 1
2. **Minimum Peak Height** = ignore peaks < 5%
3. **Minimum Peak Width** = leave blank
4. **Peak direction** = "Also consider 'peaks' that go below the baseline."

1.7 From AUC Results → Copy "**Net Area**" and "**Std Error**" to Excel.

Note: It is not necessary to copy/paste transpose each result individually. Copy the "**Net Area**" rows from the whole table and then use "**=transpose()**" function in Excel.

Calculate ΔAUC, error, and store data

2 Calculate ΔAUC

$$\Delta AUC = AUC_{LT\ serum} - AUC_{Healthy\ pooled\ serum}$$

Notes:

- Steps 2 and 2.1 can be performed in a single Excel sheet after running the "transpose" function.
- Both AUC and ΔAUC can be tabulated in a single Prism worksheet (Grouped)
- Store results in a new "**Grouped**" table in Prism (Mean ± SEM) named "AUC" with column labels:

AUC, 0-3h

$\Delta AUC_{LT - Healthy}$, 0-3h

2.1 Standard error calculation for ΔAUC

$$\text{Std Error}_{\Delta AUC} = sqrt(dx^2 + dy^2)$$

Where:
dx = $\text{Std Error}_{AUC,\ LT\ serum}$
dy = $\text{Std Error}_{AUC,\ Healthy\ pooled\ serum}$

Standard errors are obtained from the "**Net Area**" section of the Results tab in Prism after an AUC analysis.

2.2 Plot average curves with error bars from 0-6 h:

- Healthy serum with positive controls {LPS, TNF, IL-1b} and negative control {Media} - *one for each day of experiment.*
- Healthy serum with Liver serum {S1, S2, S3, S4, S5} - *one for each patient.*
- Generally there are 3 replicates, occasionally 2.

2.3 Upload $\Delta AUC_{0\ to\ 3\ h}$ in REDCap for each patient, timepoint, and cell line.

REDCap variables for Cell Line #1 are named:
auc_mean_ln1_s1
auc_se_ln1_s1
auc_mean_ln1_s2
auc_se_ln1_s2
...
auc_mean_ln1_s5
auc_se_ln1_s5

... similarly for Cell Line #2:
auc_mean_ln2_s1
auc_se_ln2_s1

Predefined comparisons for analysis

2.4 Perform one-way ANOVA with the following pairwise comparisons:

Note: Control Column should be S1, Start of Surgery for all

1. S1 vs. Healthy Serum - *Q: Is liver serum at the start of surgery different from Healthy controls?*
2. S1 vs. S2 - *Q: What is the effect of surgery (dissection phase) alone with no liver reperfusion?*
3. S1 vs. S3 - *Q: What is the effect of liver reperfusion?*
4. S1 vs. {S4 or S5} - *Q: If there is an effect of liver reperfusion, does it get worse with more time from reperfusion?*

Note: If both S4 and S5 are present, keep whichever is "less" (i.e., keep the more negative, or less positive AUC)

- S1 = Start of Surgery
- S2 = End of Dissection (before liver removal)
- S3 = 15 min after Portal Vein reperfusion
- S4 = 60 min after Portal Vein reperfusion
- S5 = 120 min after Portal Vein reperfusion (or End of Surgery, whichever comes first)

Supplementary details for each experiment

3

- Cell line #
- Passage #
- Old plate (yes/no)
- Seeding density (cells/well) - *should be 40,000*
- Plateau capacitance at 64,000 Hz, average and st. dev - *prior to stimulation*
- Plateau resistance at 4,000 Hz, average and st. dev - *prior to stimulation*
- Time of stimulation - *elapsed from seeding ECIS plate*