Real-Time Biomonitoring of Cytotoxicity in 3D Autobioluminescent Human Tissue Culture Models

Haylie Lam
Chemical Engineering and Biochemistry and Cellular and Molecular Biology

Center for Environmental Biotechnology
A University of Tennessee Research Center of Excellence

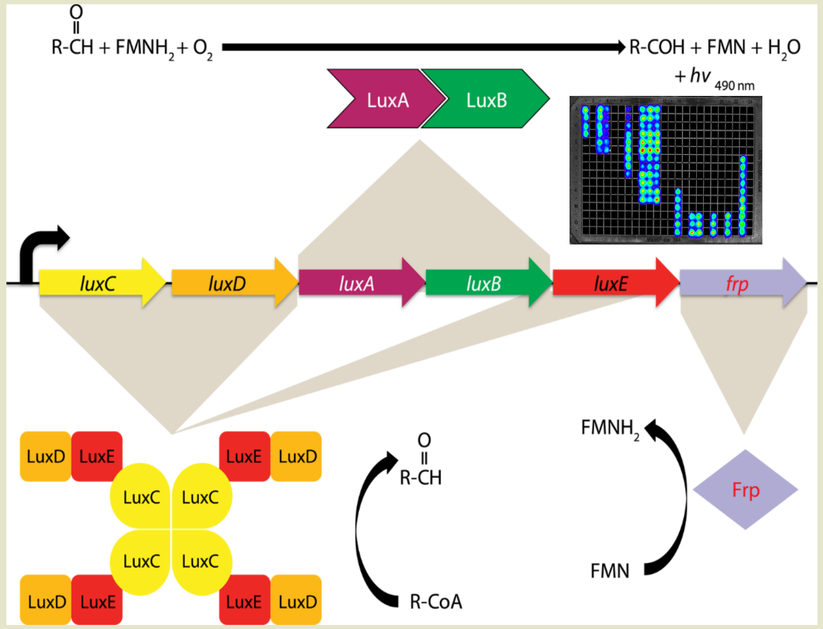
Introduction

- Current optimal imaging technologies (e.g., fluorescence and firefly luciferase-based bioluminescence) require an external stimulation be applied prior to signal generation.
- To overcome this limitation, a 'humanized' bacterial luciferase reporter operon (*lux*) has been developed for expression in eukaryotic organisms, resulting in human cell lines capable of self-producing bioluminescent output without external stimulation.
- These cells can be programmed to continuously produce an autobioluminescent signal with declining intensity correlating with exposure to toxicants detrimental to cellular health or to auto-initiate bioluminescent production only in response to the detection of specific target agents.
- This study compares the 'lights-off' and 'lights-on' reporter functions of these cells when deployed in collagen-encapsulated 3D matrix mimicking typical organs-on-a-chip platform technologies relative to their function under traditional 2D growth conditions.

Background

Synthetic six gene (*luxCDABEfrp*) bioluminescent reporter system

- Based on the bacterial luciferase gene cassette
- Allows for endogenous synthesis and regeneration of the substrates required for signal production
- Eliminates the need for exogenous substrate addition
- Permits continuous and/or repetitive monitoring of the same sample



Experimental Design

'Lights-off' bioreporter for detecting cytotoxicity

Constitutively autobioluminescent HEK293 cells (CMV promoter)

Encapsulated in collagen hydrogel in 96-well plates (3D)

Plated as monolayers in 96-well plates (2D)

Treated with various concentrations of Zeocin (0 to 1000 µg/ml)

Bioluminescence imaged using the IVIS Lumina imaging system

'Lights-on' bioreporter for detecting target compounds

Tet-On inducible autobioluminescent HEK293 cells (doxycycline inducible Tet-On promoter)

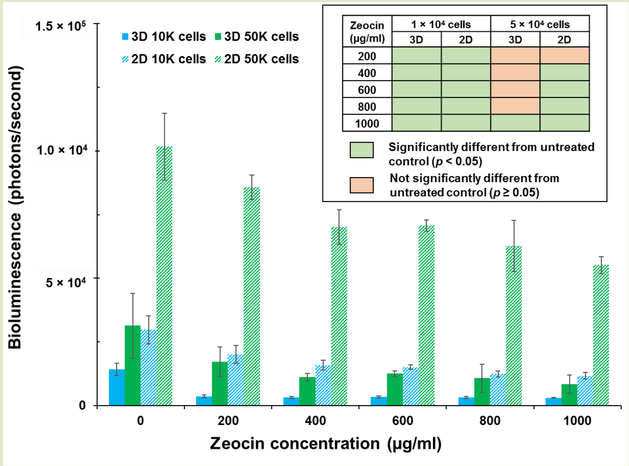
Encapsulated in collagen hydrogel in 96-well plates (3D)

Plated as monolayers in 96-well plates (2D)

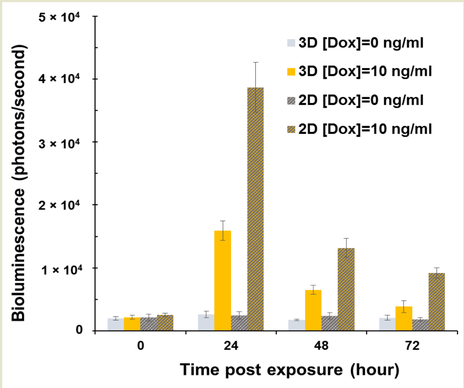
Exposed to doxycycline for 3 days

Bioluminescence imaged using the IVIS Lumina imaging system every 24 hours

Results



- Zeocin-triggered bioluminescent response in collagen encapsulated (3D) and monolayer (2D) autobioluminescent HEK293 cells was measured in the IVIS imaging system
- Significant decreases in bioluminescence ('lights-off') were observed after 48 hours of treatment with 1000 µg/ml and 400 µg/ml Zeocin concentrations in 3D and 2D conditions, respectively.



- Peak bioluminescence occurred 24 hour post-treatment in both 2D and 3D conditions
- A higher fold of induction was observed in 2D conditions (16-fold) than that of 3D conditions (6-fold).

Conclusions

- These results demonstrate the utility of a reagent-free autobioluminescent cellular system for continuous, real-time toxicity monitoring in 3D tissue culture models.
- Collagen encapsulated cells are more resistant to toxicants than cells grown as monolayers, possibly due to their increased cell-to-cell interactions and the extracellular scaffold support facilitated by the collagen matrix.

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