Multicolor bioluminescent 3D cell biosensors for effect-directed smartphone-based analysis

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Introduction

Bioluminescent (BL) cell-based assays represent bioanalytical tools for drug discovery process providing simple, fast and cost-effective assays avoiding the use of animals. Thanks to their easy adaptability to high-throughput and high-content screenings, cell models can identify bioactive molecules interacting with molecular targets well in advance of preclinical studies. In the last years, three-dimensional (3D) cell-culture models have gained great attention due to their capability to faithfully replicate intrinsic physiological conditions and in vivo cellular responses to external stimuli. Firstly, a transcriptional biosensor system relying on BL 3D spheroids has been developed for high-throughput tumor necrosis factor α (TNFα) detection in a 96-well micro-patterned microplate.[1] For implementing these 3D cell-based assays into portable formats we developed a smartphone-based platform for effect-based analysis relying on multicolor bioluminescent 3D cell biosensors.

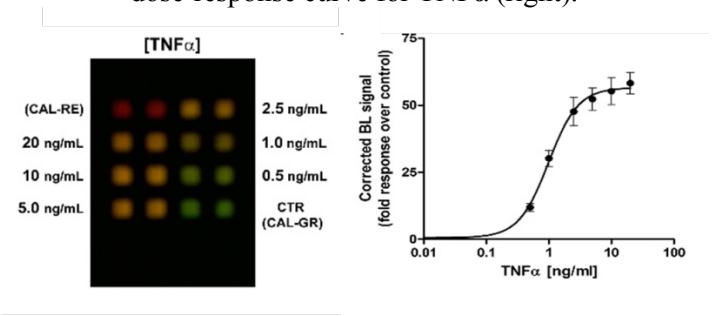
Materials and Methods

Two powerful *P. Pyralis* variants, PpyGR-TS and PpyRE-TS luciferases emitting at different wavelengths have been selected respectively as viability control and inflammation reporter. Spheroids were incubated with different concentrations of TNFα (concentration range 0.5-20 ng/mL) for 5 h. We fabricated a cell cartridge and smartphone adaptor using a desktop 3D printer to provide a mini-darkbox and an aligned optical interface between the smartphone camera and the cell cartridge for BL signals acquisition.

Results and Discussion

We report the development of a dual-color BL spheroid-biosensor in which a red-emitting luciferase is induced by the presence of pro-inflammatory molecules and a green-emitting reporter is constitutively expressed and used as viability control allows to obtain a color-coding visual information in which the green emission is associated to "safe" while the red correspond to "harmful" samples. Smartphone-based bioassay provides a limit of detection (LOD) of 0.15 ± 0.05 ng/mL and an EC50 of 1.0 ± 0.1 ng/mL TNFα.

Figure 1: Image acquired with the smartphone obtained by incubating the spheroid-biosensor with increasing concentration of TNFα (left), corrected dose-response curve for TNFα (right).



Conclusion

To the best of our knowledge this is the first implementation of a multicolor bioluminescent spheroid biosensor in a smartphone-based platform. The proposed biosensing platform could become a useful tool for an initial on-site screening of potentially toxic substances, prioritizing samples for a more accurate chemical analysis.

Bibliography

[1] L. Cevenini et all., Bioluminescence Imaging of Spheroids for High-throughput Longitudinal Studies on 3D Cell Culture Models Photochem. Photobiol., 2017, 93(2), 531-535.