**Bioluminescent spheroids: new tools for high-throughput molecular imaging and biosensing**

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Bioluminescent (BL) cell-based assays based on two-dimensional (2D) monolayer cell cultures represent an invaluable tool for the early stages of the drug discovery process. 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. However, cells grown in 2D cultures do not often reflect the morphology and functionality of living organisms, thus limiting the predictive value of 2D cell-based assays. Conversely, 3D cell models have the capability to generate the extracellular matrix and restore cell-to-cell communications; thus, they are the most suitable model to mimic in vivo physiology. In this work, we first developed a nondestructive real-time BL imaging assay of spheroids for longitudinal studies on 3D cell models. To this end different luciferases have been explored including the small NanoLuc, a *P.pyralys* variant optimized for working in low ATP cellular environments (PLR3), and a thermostable green emitting *P. pyralis* variant (PGRTS). The feasibility of the assay was tested using the well-known transcriptional regulation of the nuclear factor k beta (NF-kB) response element in human embryonic kidney HEK293 cells. We obtained concentration-response curves and compared them with those obtained using conventional 2D cell cultures.

Encouraged by these results, we also evaluated the feasibility of implementing these 3D cell-based assays into portable formats. We fabricated a cell cartridge and smartphone adaptors 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. Moreover, multicolor bioluminescence was also implemented by exploiting two luciferases emitting at different wavelengths. Proof-of-principle applications are presented together with main limitations, such as those related to the limited shelf-life of cells.