**Optimized IncuCyte-Based Assays For Evaluating Nanoformulations in Colorectal Cancer**

**Srija Sur1**, Ghada Aboueid1, Moien Sadeghi1, Pegah Varamini1,2

1School of Pharmacy, Faculty of Medicine and Health, University of Sydney, Sydney, Australia.

2Sydney Nano Institute, University of Sydney, Sydney, Australia.

**Background and Aim.** Nanoformulations offer a promising alternative by improving drug targeting and reducing side effects. The IncuCyte Live-Cell Analysis System supports preclinical studies by tracking real-time cell behaviour. However, its use in evaluating nanoformulations is limited due to the absence of standardized protocols, highlighting the need for methodology development for nanomedicine advancement. This study aims to develop a robust method for evaluating the cellular uptake and *in vitro* anti-proliferative activity of targeted and non-targeted curcumin-loaded nanoformulations for Colorectal cancer (CRC). The targeted formulations include curcumin nanoparticles conjugated with luteinizing hormone-releasing hormone (LHRH) derivatives 1 (Cur-NP-LHRH-D1) and 2 (Cur-NP-LHRH-D2).

**Method.** Live-cell imaging using IncuCyte® ZOOM was employed to quantify nanoformulation uptake by CRC cells over 48 hours. Masking fluorescence imaging enabled selective quantification of curcumin, excluding background signals.

**Results.** Targeted nanoformulations showed greater curcumin uptake, validated by confocal immunohistochemistry imaging, with a higher fluorescence confluence (5500 GCU × μm², 100 μM curcumin) than non-targeted formulations (4500 GCU × μm²) over 48 hours. At 2 hours, Caco-2 cells (Figure 1) exhibited a higher fluorescence intensity than HCT-116 (Figure 2), demonstrating LHRH-R-mediated targeting efficiency. The MTT assay further confirmed greater cytotoxicity of targeted nanoformulations, showing a rapid cell viability decline at lower drug concentrations, reinforcing improved therapeutic efficacy through LHRH-targeted delivery.

 ****

**Figure 2.** Fluorescence confluence of HCC-116 cells.

**Figure 1.** Fluorescence confluence of Caco-2 cells.

**Conclusion:** LHRH-targeted nanoformulations demonstrated enhanced curcumin uptake and greater cytotoxicity in colorectal cancer cells, correlating with LHRH receptor expression levels. This validation supports IncuCyte as a reliable tool for real-time evaluation of targeted drug delivery in CRC treatment.