

## **Cytotoxicity test of doxorubicin-loaded fibrinogen microspheres with U-2 OS using Juli™ FL**

The effect of anticancer drug Doxorubicin-loaded fibrinogen (Dox-Fbg) microspheres were investigated on GFP-expressed human osteosarcoma U-2-OS cancer cells. The cytotoxicity of Dox-Fbg microsphere was measured by time-lapse images video of cell proliferation with Juli™ FL (NanoEntek Inc., Seoul, South Korea), which could provide continuous monitoring of live cell imaging. The GFP-expressed U-2-OS cells were pre-cultured for 36 hours in 12 well plates until the confluence was reached at 45%. Dox-Fbg and Dox-free (bared) microspheres were added to the GFP-expressed U-2-OS cells, and cells were incubated for 48 hours in 37°C CO<sub>2</sub> incubator. The cell images were taken at every 10 minutes by Juli™ FL and composed into movie. After 48hours, confluence data were compared. Initial confluences were 45.77% for Dox-Fbg microspheres and 45.15% for Bared-Fbg microsphere, respectively. Final confluences for Dox-Fbg Microspheres and Bared-Fbg microsphere were 22.92% and 69.88%, respectively. As a result, significant decrease of confluences (22.85 %) was observed for DOX-linker-Fbg microsphere treated U-2-OS cancer cells, revealing continuous cell death by the function of doxorubicin. On the other hand, an increased confluence (24.73%) was detected in a bare-Fbg microsphere treated cells, demonstrating the biocompatibility of Fbg. Through this experiment, we concluded that DOX-linker-Fbg microspheres could be applied for cancer drug delivery, and also the Fbg drug carrier system possessed the biocompatibility. Since GFP-expressed human osteosarcoma U-2-OS cancer cells were commercially available, ethics were not considered.