

# Is NanoShuttle™ Biocompatible?

**n3D**  
Biosciences, Inc.

## Yes!

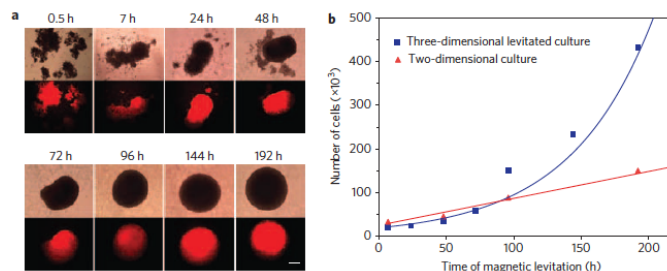
We get asked this question all the time, and the answer is always **yes**. NanoShuttle™ is a nanoparticle assembly (~50 nm) consisting of gold, iron oxide, and poly-L-lysine (PLL)<sup>1</sup> that attaches to the plasma membrane electrostatically (50 pg/cell).

NanoShuttle™:

- consists of biocompatible components: iron oxide and PLL are recognized as safe by the FDA<sup>2,3</sup>; and gold nanoparticles are in clinical trials for therapeutic use, with no indications for systemic toxicity<sup>4</sup>
- does not bind any specific receptors, works with all cell types
- will release off the cell over 7-8 days into the surrounding extracellular matrix, as shown by transmission electron microscopy (TEM)
- requires magnetic forces (30 pN) only strong enough to aggregate but not harm cells
- will not effect proliferation<sup>5,6</sup>, viability<sup>6</sup>, metabolism<sup>5,7</sup>, inflammatory<sup>5</sup> or oxidative stress<sup>8</sup>, phenotype<sup>5,7,8</sup>, and other macro cell functions
- does not cause any chromosomal abnormalities in cells, as shown by comparative genomic hybridization (CGH)

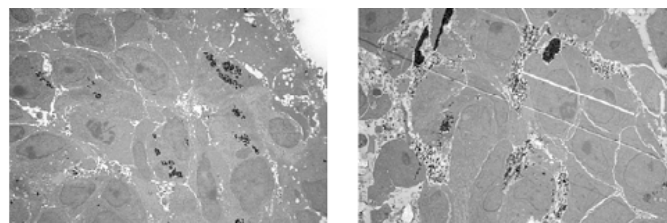
**Overall, NanoShuttle™ is biocompatible and facilitates rapid 3D culture formation.**

### Spheroid Growth



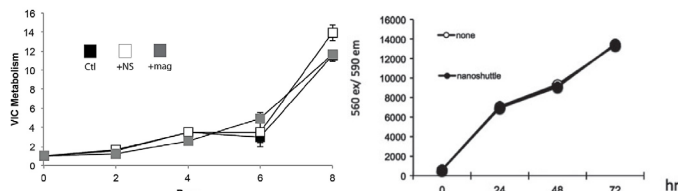
Over 8 d, mCherry-tagged glioblastoma grow faster in 3D v. 2D<sup>1</sup>

### Transmission Electron Microscopy



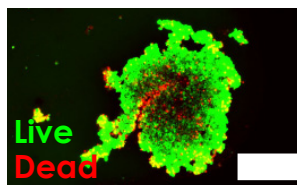
After 24 h (left), NanoShuttle™ is localized with the cells, but by 8 d (right) move out of the cell and into the extracellular space<sup>1</sup>

### Proliferation, Metabolism



Neither NanoShuttle™ nor magnetic forces have any effect on the proliferation of valvular interstitial cells (VIC, left)<sup>7</sup> and 3T3 fibroblasts<sup>6</sup> (right)

### Viability



NanoShuttle™ has no effect on viability, as demonstrated by live/dead staining (live = green, red = dead) on magnetically 3D bioprinted spheroids of 10,000 HepG2 hepatocellular carcinoma cells in a 384-well plate. Scale bar = 500 μm

### References

1. Souza et al. *Nat Nanotech.* (2010)
2. GRN No. 135, FDA. (2004)
3. CFR, 21.I.A §73.200. (1994)
4. ClinicalTrials.gov #NCT00848042. (2014)
5. Tseng et al. *Tissue Eng C.* (2013)
6. Daquinag et al. *Tissue Eng C.* (2013)
7. Tseng et al. *Acta Biomater.* (2014)
8. Haisler et al. *Nat Protoc.* (2013)

## 3D in a 2D Workflow™

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