

## Cell budding at a narrow concentration span upon exposure to Growth Factor on Cline Nano Gradients

*Cline Nano Gradients are here used to study how a change in concentration of a surface-bound growth factor affect iPS cells that are induced to develop into limb structures. Study performed by Stina Simonsson, PhD, Gothenburg University Sweden (2016).*

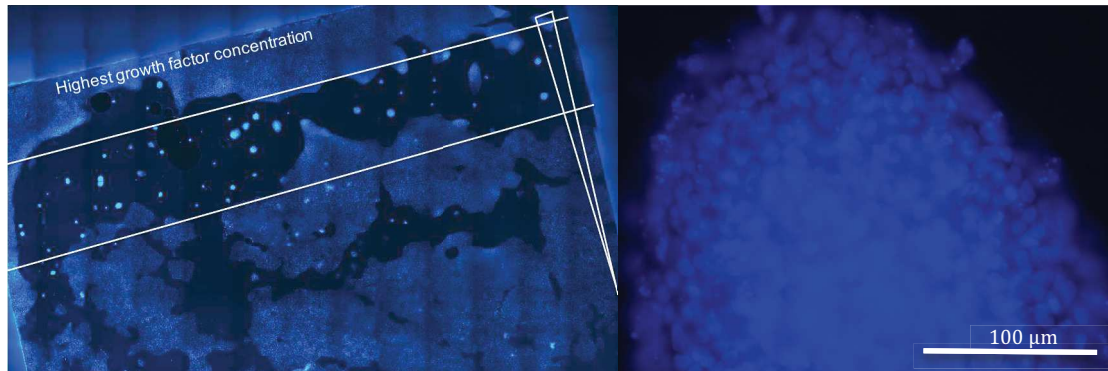


Figure 1. A) Differentiation of iPS cells on a Cline Nano Gradient, from low concentration of growth factor (bottom) to high (top). A specific concentration of growth factor generated budding cell clusters (two white lines indicate the "budding" area). Nuclei are stained with DAPI-blue. B) Magnification of one cell cluster from the "budding area". Nuclei stained with DAPI-blue.

### Cell budding zone found using Cline Nano Gradients

Limbs are formed due to orchestration of growth factors that function as morphogenes. Thus, depending on the gradual change in concentration, different signals are given to the cells. In this experiment cells were grown on a surface with laminin and a gradient of growth factor, resulting in clustered cells at a specific surface composition. This "budding zone" is marked by two white lines in Figure 1. The concentration interval in the "budding zone" also seem to correspond to the apoptotic zone, an event important for joint development. The particular concentration of growth factor and laminin was determined and further used in new experiments with a Cline Nano Surface of that selected uniform composition.

### Unique concentration of growth factor on a Cline Nano Surface

Cline Nano Surfaces were prepared with two different uniform concentrations of laminin and growth factor. Interestingly, high density cell budding clusters were detected on the whole surface with the high concentration of growth factor (Figure 2A) corresponding to the selected "budding zone" on the gradient surface. For comparison, a low concentration Cline Nano Surface was also produced showing no budding, as expected (Figure 2B).

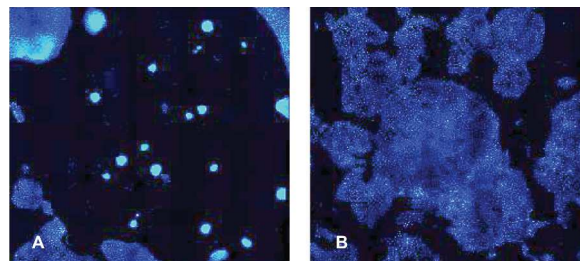


Figure 2. A) Dense cell clusters appeared all over the surface at high concentration of growth factor compared to B) that has a low concentration of growth factor. Nuclei are stained with DAPI-blue. Both images are from Cline Nano Surfaces with two different uniform surface concentrations.

This may be an *in vitro* model for limb bud formation from iPS cells.