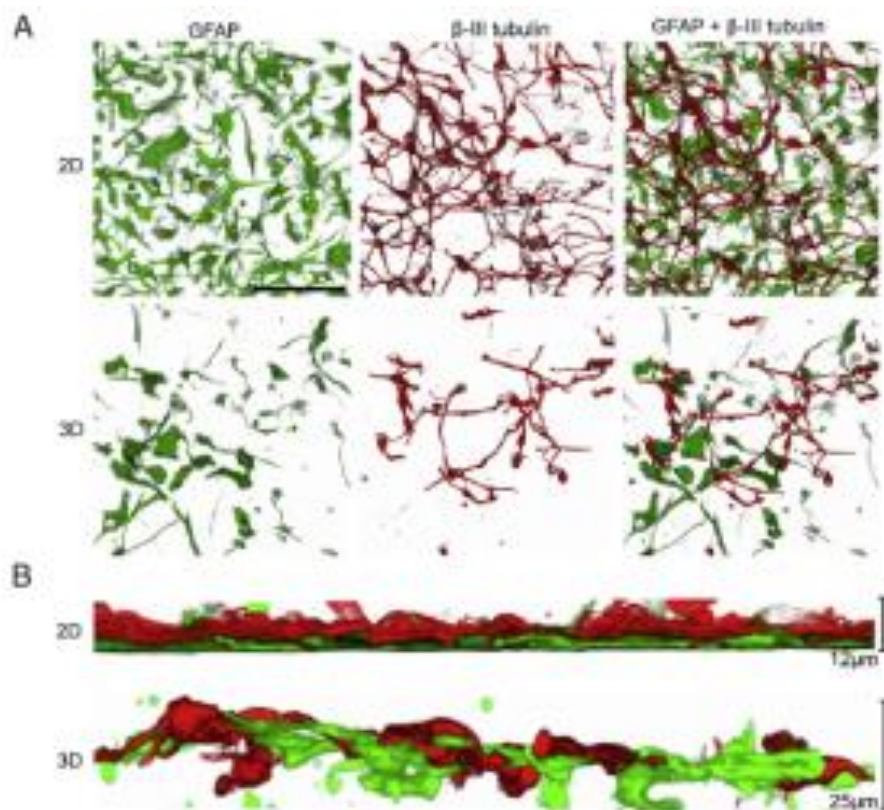


Proof of Concept: Co-Culture

Jakobsson, A. et al. Three-dimensional functional human neuronal networks in uncompressed low-density electrospun fiber scaffolds. *Nanomedicine: Nanotechnology, Biology and Medicine* 13, 1563–1573 (2017) doi: 10.1016/j.nano.2016.12.023.

A remarkably different distribution pattern of astrocytes and neuronal cells had formed depending on culture substrate. At 2D surfaces astrocytes were found as a dense, flat monolayer attached to the culture surface, while β -tubulin III-positive neurons were located predominantly on top of the glial cell monolayer (Figure 3, B). Occasional neurites were found growing on top of adjacent neurons. In contrast, at 3D scaffolds the glial and neuronal cells were always found intermingled, and never found to have formed a layered structure (Figure 3, B).



Neuronal cell integration and neurite extension in the 3D scaffold. (A) Confocal images of HNPC cultured for 20 days in 2D and 3D substrates. Both β -III tubulin-positive (red) and GFAP-positive (green) cells were found with similar morphologies in the two cultures. (B) Notably, in 2D culture a side view of the same images revealed a confluent layer of glial cells beneath a layer of neurons. The 2D culture measured about 14 μ m in thickness. In contrast, in 3D scaffold culture, the glial and neuronal cells were found intermingled, in networks spanning ≥ 25 μ m in depth. Scale bar in A: 100 μ m. (Jakobsson 2017)

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