Acetylcholine-based biomaterial enhances neuronal differentiation

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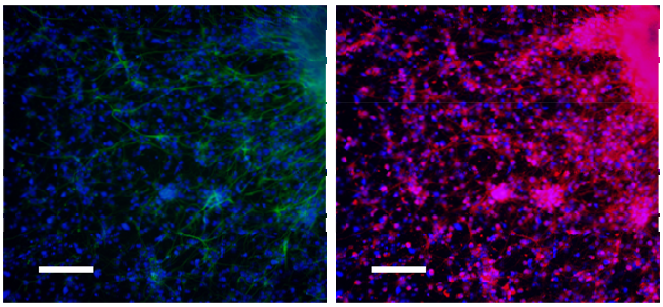
Statement of Purpose: Neural progenitor cell (NPC) is an important cell source with extensive potential in regenerative medicine because of its abilities to self-renew and to differentiate into neurons and glia. It is known that the proliferation and differentiation of NPCs are affected by extracellular signals from culture substrates, medium components, and cell-cell interactions. Several methods have been described for NPCs differentiation and the most common culturing substrate is laminin. One challenge of natural substrates such as laminin is batch variation. Typically most NPCs will differentiate into glia *in vitro*, and the efficiency of neuronal differentiation is also desired. We thus set out to explore the possibilities of using a bioactive material to differentiate NPCs, because neurotransmitters play an important role in neurogenesis, we postulate that biomaterial with neurotransmitter functionalities can provide a niche that would increase neuronal differentiation.

Methods: The acetylcholine based polymer ($PSA_{70}L_{30}$) was synthesized according to the method listed in reference. Embryonic neural progenitor cells (NPC) were isolated from of Sprague Dawley rat subventricular zone (embryo day 14), and cultured in a serum-free DMEM/F12 medium containing B-27 supplement, epidermal growth factor and fibroblast growth factor. The polymer's capability at promoting NPC differentiation *in vitro* was evaluated by culturing NPC neurospheres and dissociated NPC cells on laminin-1 and $PSA_{70}L_{30}$ surfaces. Immunocytochemistry was used to examine the *in vitro* differentiation of NPCs on both laminin and $PSA_{70}L_{30}$ surfaces under an identical experimental condition. Neurofilament and glial fibrillary acidic protein (GFAP) were used as identification markers for neurons and astrocytes, respectively.

Results: The purpose of this study was to explore the potential of $PSA_{70}L_{30}$ in facilitating the neuronal differentiation of embryonic neural progenitor cells isolated from rat subventricular zone. We found that neurospheres and dissociated NPCs can attach on the $PSA_{70}L_{30}$ substrates within an hour after seeding. The neurospheres broke down gradually on $PSA_{70}L_{30}$ surfaces and cells migrated out from the aggregates over time. The migrated cells differentiated around original aggregates, and formed networks. Immunocytochemistry results indicated that there were significantly more NPCs on $PSA_{70}L_{30}$ surfaces differentiated into neurofilament-positive cells than that on laminin-1 surfaces under identical experimental condition. There were little GFAP-positive cells on $PSA_{70}L_{30}$ surfaces (Figure 1). In contrast, there were many GFAP-positive cells on laminin-1 surfaces.



A B

Figure 1. Rat embryonic NPC cells differentiated into more neurofilament-positive cells and less GFAP-positive cells on $PSA_{70}L_{30}$ surfaces. (A) DAPI (blue) and GFAP stain (green); (B) DAPI (blue) and Neurofilament stain (red). Scale bar: 100μm

Conclusions: We have designed and synthesized a bioactive and biodegradable biomaterial that derive its biological activity from acetylcholine. The results showed that significantly more NPCs on $PSA_{70}L_{30}$ surfaces differentiated into neurofilament-positive cells than on laminin-1 surfaces. This suggests that $PSA_{70}L_{30}$ can induce more NPCS to differentiate into neurons. This research may lead to new pathways to control the differentiation of stem cells.

References: Gumera, C. *et. al*. Modulating neuronal responses by controlled integration of acetylcholine-like functionalities in biomimetic polymers. Advanced Materials, 19, 24, 4404-4409, 2007.