Assignment 7: Cell Adhesion and Migration

EN 585.729 Cell and Tissue Engineering

Problems

1. Name that molecule or complex (adhesion/junction):

   a) Thin alpha helix fibrils, found in intervertebral disks **microfibril**
   b) Motors composed of this protein are used to contract the cell during migration **myosin**
   c) Cell-cell adhesion that links to intermediate filaments **desmosome**
   d) A dimer that contains a heparin-binding domain which facilitates binding to other ECM molecules as well as growth factors resulting in haptotactic gradients **fibronectin**
   e) A monomer that participates in homotypic bonds during the leukocyte adhesion cascade **selectin**
   f) Comes in many lengths and (with one exception) covalently attach to proteins increasing their sugar content **glycoprotein**
   g) An adhesion that utilizes integrins and connects to the actin cytoskeleton **focal adhesion**
   h) Three chains joined together in a cross or "t" shaped **laminin**

2. (1 page or less) Provide a critical response to the assigned reading article "Directed Migration in Neural Tissue Engineering" by Wrobel and Sundararaghavan. First, concisely summarize the goals of this review paper (why was it written?). Second, respond to the paper by thinking critically about what the authors have told you → In the response please consider the different methods of directed migration and comment on which methods are the most advanced, have been the most successful and are good candidates for combination with other directed migration methods.

The article starts describing the challenges faced by neural tissue engineering and the limitations of autograft tissue. Using directed cellular migration, the authors believe that future nerve growth conduits (NGCs) will eventually address these challenges and will replace autografting. The authors review in details the existing major studies to create a variety of gradients to initiate guided cell migration: chemotaxis (chemical cues), hapoptaxis (adhesive substrate), durotaxis (substrate mechanics, stiffness factor), topographical cues, electric field (EF) stimulation, and contact guidance-mediated growth. In conclusion, the article mentions combination strategies as the most promising strategies for tissue engineering. For in vitro studies, the authors also

recommend coculturing as a more physiologically relevant method to determine the effects of gradient on cells.

First, we can observe that for both chemotaxis or haptotaxis studies, most of the results are not reported or available (**Table 1 and Table 2**), and there is a wide range of values for the threshold slope required for neurite orientation (from 0.017μg/mL/mm to 60 μg/mL/mm0. The lowest threshold of 0.017 μg/mL/mm was obtained by Dodla and Bellamkonda with an agarose scaffold and Laminin-1 isoform (LN-1) as gradient molecule. At this threshold, cells grew at an average of 27.8 μm/h. The same team replicated their success by promoting in vivo, for 4 months, neural regeneration in a 20 mm nerve sciatic gap of a rat model.
In chemotaxis research, Kithapalli et al., have created a novel structure in the shape of a "T" with netrin-1 (chemoattractant) on one side of the T-junction and slit-2 (chemorepellent) on the other side, directing a large proportion of hippocampal neurites and DRG neurites down the gradient. Similarly, Webber et al. have prevented neurite growth in the wrong direction. Pinato et al, had an interesting approach in encapsulating netrin-1 solution into liposomes, achieving a remarkable average neurite growth of 10μm in 5 min. This technic allows a fine control of molecules needed to cause chemotaxis. In hapoptaxis scaffolding, Masand et. Al, showed that PSA-, HNK-, and PSA/HNK grafted gels promoted significantly not only neurite growth but Schwann cell proliferation and migration.

Several research studies have been conducted to measure the impact of an increase in substrate rigidity on different cell types. Many confirmed a decrease of neurite branching, longest process and an increase of cell density, spread area and proliferation (**Table 3**). Sundararaghavan et al. used a novel "H-shaped" microfluidic device to direct neurites with a gradual stiffness gradient (75±3 Pa untreated collagen, 797±50 Pa for 10mM genipin). Experiment from Engler et al. showed that Durotaxis can play a role in cell differentiation. Combining scaffold mechanic properties with stem cell cultures or chemotactic cues is a promising future research work for inducing specific cell differentiation. Topographical gradients can change neurite orientation and growth. Lee et al. concluded that a zigzag pattern was the most effective for cell adhesion and Schmalenberg and Uhrich showed that on patterned surface laminin stripes with in-between graduated PMMA intervals, cells aligned within laminin stripes.

Several studies have established that EFs can have an effect on neurite growth and cell migration (**Table 4**). This is particularly important as electrotaxis is easier to modulate and maintain compared to chemical gradient. They could be combined for a synergistic effect; for example, NT-3 can initiate cathodal attraction. More studies are required to quantify and understand the combinatorial effects.
One important factor the article mentions is related to co-culturing and preseeding which can improve significantly neuron migration and growth; Mattotti et al., obtained a 3-fold improvement in average speed and overall migration distance by placing neurons on RGLC-covered PMMA lines.