Modelling the Blood-Brain Barrier with Hyaluronic Acid-Based Hydrogels

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Introduction:

The blood-brain barrier (BBB) is implicated in many diseases, including Altzheimer's disease, Autism, and stroke. It is also an obstacle for drug delivery to the brain due to its impermeability to many molecules. Currently, researchers use both *in vivo* and 2D *in vitro* models to examine diseases and screen for drugs. However, the results of *in vivo* rat and mouse models cannot always be directly correlated to humans. Also, *in vitro* cell culture models may use human cells, but are short-lasting and do not fully capture the 3D environment of the actual BBB.

Hyaluronic acid (HA)-based hydrogels provide a unique tool to examine the complex structure of the BBB. HA is found in the natural extracellular matrix of the brain, and is able to be remodelled by neuronal cells. HA is also readily chemically modified so that it may be chemically crosslinked to form hydrogels, which are solvent-swollen networks of crosslinked polymers. This experiment aims to evaluate the efficacy of these HA hydrogels as matrices to study neural stem/progenitor cells (NSPCs) and endothelial cells, the two major cell types found in the BBB. We examined different combinations of crosslinkers, including polyethylene glycol (PEG) and peptide crosslinkers, in combination with an adhesive peptide sequence. Our hypothesis is that NSPCs will adhere to HA hydrogels containing RGD sequences, but will not adhere to purely PEG-crosslinked hydrogels.

Materials and Methods

Hyaluronic acid was modified with furan groups at 47% functionalization as determined by H-NMR. Solid-phase peptide synthesis was used to synthesize various RGD-containing peptides that contain maleimides that can selectively and effectively bind to the furan groups of the HA. Gels were prepared containing PEG crosslinker and various concentrations of peptides. The resulting gels were then seeded with cells.

Mouse neural stem/progenitor cells (NSPCs) were derived from adult mice and plated at concentrations of 10 000 cells per well, and cultured in serum-free media for 2-5 days. Immunocytochemistry was used to characterize cells.

Results

Gels crosslinked with PEG alone were relatively nonadhesive, while the gels crosslinked with RGD peptides were moderately adhesive. Gels crosslinked with PEG and a hydrophobic RGD sequence showed the greatest amount of cell attachment and spreading on the hydrogel surface.

Discussion

This experiment shows that HA hydrogels are supportive of NSPC growth for periods of at least 5 days. This study supports previous work that shows the benefit of the fibronectin-derived adhesive RGD sequence. Interestingly, the incorporation of a hydrophobic spacer seems to promote adhesion. This hydrogel may provide a good basis for 3D co-culture of mouse NSPCs with endothelial cells with potential for use as an *in vitro* model for a variety of BBB-related diseases or for drug screening.