**A Programmed Anti‐Inflammatory Nanoscaffold: Decoupling Brain Injury from Inflammation**

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**Introduction**

Astrocytes provide essential physiological functions within the brain that also included contributing to the inflammatory response post insult (Maclean et al., 2018). Whilst their role after brain injury or stroke is clear, their precise contribution to the inflammatory cascade is poorly understood, representing a significant opportunity for therapeutic targeting to promote repair and reconstruction of brain tissue. An *in vitro* model of the damaged brain where inflammation can be turned on and off, would allow the decoupling of progressive degeneration and inflammation, and ultimately the development of advanced regenerative therapies. Here, we demonstrate the dual functionalities of molecular hydrogels *in vitro*: as a delivery vehicle to enhance the anti-proliferative effects of polysaccharides on inflammatory astrocytes, and as a long-term 3D culture environment. As proof of concept we demonstrate how this programmable tool can be exploited for the *in vitro* investigation of the inflammatory mechanism of astrocytes.

**Methods**

Hydrogels were formed using a pH switch with sodium hydroxide and hydrochloric acid. Fucoidan was added to the peptide before hydrogel formation, resulting in the co-assembly of Fmoc-DIKVAV and fucoidan. FTIR and TEM confirmed assembly mechanism of the hydrogels. Cells were then seeded on top of the hydrogels the next day (400,000 cells/well for a 24 well plate) and maintained for 14 or 24 days. Immunohistological analysis, previously described (Nisbet et al., 2010), was performed to characterize the cell response to the hydrogels.

**Results and discussion**

Once the material properties were confirmed and match to that of brain tissue, we investigated the ability of the molecular hydrogels to induced cytoskeletal changes in the astrocytes. When astrocytes were grown on top of Fmoc-DIKVAV they significantly infiltrate the hydrogel and produced cytoskeletal network formations. Whilst we and others have shown extensive network formations (also termed cobblestone morphology) of non-inflammatory astrocytes in vivo, the formation of extensive astrocyte networking in vitro has not previously been reported. The observed networking was influenced by the Fmoc-DIKVAV hydrogel; maintaining astrocytes in a non-inflammatory phenotype. Astrocytes cultured our hydrogels were observed to have more ‘in vivo like’ cell-cell and cell-material interactions as compared to those growing underneath the hydrogels primarily in the XY plane. Additionally, our 3D culture system allowed us to significantly increase the cell seeding density from 20,000 – 400,000 cells/well, more representative of the cell organization and density within the brain. This was due to the 3D nature of the material system enabling the migration and physical support of a much greater extent to what could be obtained in 2D cultures.

**References**

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