BT5130 Tissue Engineering

Neural Tissue Engineering: Designing constructs for better emulation

The proposed solution - *Submitted by Sahana Gangadharan (BE17B038)*

Context of the problem:

This project aims to find a better neural tissue engineering approach in the context of a traumatic brain injury (TBI), especially **Diffuse Axonal Injury** (DAI) that is caused due to sudden, external and physical assault to the brain. DAI results in shearing of axons at the junction of grey-white matter in the prefrontal lobe, upper brainstem and corpus callosum, while also causing the axons to swell and disconnect. This project will mainly focus on proposing a solution to DAI in the prefrontal lobe amongst other TBIs while ensuring a minimally invasive technique for implantation of the scaffold and regeneration of the neurons.

Choice of biomaterial for the scaffold, cells and the cell seeding technique, with reasoning:

A commonly observed side effect due to *in vivo* differentiation of implanted scaffolds is the context of NTE is allodynia (1), which causes increased pain to a stimulus that is otherwise noxious. Hence, **electrical stimulation** is used to induce differentiation. Invasive surgery for the implantation of scaffolds causes glial scars, which affects axonal regeneration by producing inhibitory molecules such as CSPGs, tenascin and ephrin-B2 (2). To ensure reduced glial scars, **Laminin-Hyaluronic acid (HA)-based composite injectable hydrogel** is employed. This combination of materials for the hydrogel is very similar to what is found in the brain, hence the biocompatibility issues are also sorted. In general, hydrogels are favourable for their porous structures, biodegradability, non-antigenicity and chelating properties. Various methods to assess the **rheological** properties of the hydrogel is explained by Chen et al (3). In general, the scaffold should display a soft and viscoelastic structure to be able to protect the neurons during the injection. *In situ* crosslinking of the hydrogel can be accomplished by attaching small moieties such as tetrazine and TCO to the HA hydrogel and use them as a heterogel to form a

click-crosslinked hydrogel *in vivo* (4). It has also been reported that the recombinant spider protein (**4RepCT**) is known to act as a biocompatible substrate upon which, stem cells gets differentiated into neuronal cells that include astrocytes, neurons and glia (5). The 4RepCT and other spider silk proteins have relatively high isoelectric points (around 9), which means that at physiological pH, they carry a net positive charge. Neurons are known to have higher adhesion to positively charged surfaces. (6) (7). To support electrical conduction by the 4RepCT matrix, we allow **carbon nanotube** to uniformly adhere on the silk fibre surface to produce flexible and electrically conducting fibres. The experimental methodology is explained here (8). The **hUMSCs** are easily accessible and are more immature than MSCs and they can differentiate into a varied set of cells belonging to all three germ layers. This unique ability of hUMSCs allows it to be cultured effectively and straightforwardly. The differentiation can be induced by specific factors such as Edaravone (9) and Resveratrol (10).

Justification for the novelty of the project:

Diffuse axonal injury, cannot be examined through MRI and is one of the most common forms of traumatic brain injury. This is also one of the neural injuries where not much progress has been reported in terms of tissue engineering. Hence, the proposed method is a novel strategy to help in the regeneration process of DAI. The current experiments used to treat TBI is invasive as it requires a surgery which might cause secondary cell death and other damages. This solution employs injectible hydrogel so that not much damage is done to the system. This solution brings together two of the brightest techniques that were developed in recent terms. The use of hUMSCs for differentiating into neurons are not extensively studied, despite its advantages and the use of spider silk protein for scaffolds is just upcoming. The idea of using hUMSCs and conductive-spidroin together is novel. The factors that induce differentiation, are taken from the literature, and they are known to not elicit any immunological responses, either. Overall, the system is set to mimic the ECM better and is in all sense biodegradable and biocompatible.

References:

1. Matsumoto M, Imura T, Fukazawa T, Sun Y, Takeda M, Kajiume T, et al. Electrical stimulation enhances neurogenin2 expression through β-catenin signaling pathway of mouse bone marrow stromal cells and intensifies the effect of cell transplantation on brain injury. Neurosci Lett. 2013 Jan 15;533:71–6.
2. Madhusudanan P, Raju G, Shankarappa S. Hydrogel systems and their role in neural tissue engineering. J R Soc Interface. 2020 Jan 29;17(162):20190505.
3. Chen MH, Wang LL, Chung JJ, Kim Y-H, Atluri P, Burdick JA. Methods To Assess Shear-Thinning Hydrogels for Application As Injectable Biomaterials. ACS Biomater Sci Eng. 2017 Dec 11;3(12):3146–60.
4. Wei Z, Zhao J, Chen YM, Zhang P, Zhang Q. Self-healing polysaccharide-based hydrogels as injectable carriers for neural stem cells. Sci Rep. 2016 Nov 29;6(1):37841.
5. Lewicka M, Hermanson O, Rising AU. Recombinant spider silk matrices for neural stem cell cultures. Biomaterials. 2012 Nov 1;33(31):7712–7.
6. An B, Tang-Schomer M, Huang W, He J, Jones J, Lewis RV, et al. Physical and Biological Regulation of Neuron Regenerative Growth and Network Formation on Recombinant Dragline Silks. Biomaterials. 2015 Apr;48:137–46.
7. Allmeling C, Jokuszies A, Reimers K, Kall S, Choi CY, Brandes G, et al. Spider silk fibres in artificial nerve constructs promote peripheral nerve regeneration. Cell Prolif. 2008 Apr 2;41(3):408–20.
8. Steven E, Saleh WR, Lebedev V, Acquah SFA, Laukhin V, Alamo RG, et al. Carbon nanotubes on a spider silk scaffold. Nat Commun. 2013 Sep 10;4(1):2435.
9. Shi Y, Nan C, Yan Z, Liu L, Zhou J, Zhao Z, et al. Synaptic Plasticity of Human Umbilical Cord Mesenchymal Stem Cell Differentiating into Neuron-like Cells In Vitro Induced by Edaravone. Stem Cells Int [Internet]. 2018 Oct 28 [cited 2020 Oct 4];2018. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6230402/
10. Guo L, Wang L, Wang L, Yun-peng S, Zhou J, Zhao Z, et al. Resveratrol Induces Differentiation of Human Umbilical Cord Mesenchymal Stem Cells into Neuron-Like Cells. Stem Cells Int [Internet]. 2017 [cited 2020 Oct 4];2017. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5415670/