

**UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2022/23**

***NB: This whole report will be posted on the Society’s website therefore authors should NOT include sensitive material or data that they do not want disclosed at this time.***

**Name of student:**

Louis Mulville

**Name of supervisor(s):**

Dr. Ian Woods

Dr. Adrian Dervan

**Project Title: (no more than 220 characters)**

Testing of a bioengineered drug-eluting synthetic dural patch for spinal cord injury applications

**Project aims: (no more than 700 words)**

Spinal cord injury (SCI) is a traumatic condition that often leads to lasting and debilitating changes in bodily functions, and currently, there are limited therapeutic options available. The complex pathophysiology of SCI causes widespread excitotoxic injury to injured neurons, which can lead to cell death (Park et al., 2004) and at the same time tearing of the protective overlying dural membranes leads to the leakage of cerebrospinal fluid (CSF). In a novel initiative, the Tissue Engineering Research Group (TERG) at RCSI in collaboration with the [Dziemidowicz research group at University College London](https://profiles.ucl.ac.uk/58083-karolina-dziemidowicz) seek to develop a sterile off the shelf synthetic drug-eluting dural patch. The approach involves utilizing a biodegradable electrospun polymer sheet capable of being surgically stitched into torn dural membranes, to seal off the injury site and at the same time, capable of delivering a preloaded candidate neuroprotective drug, to alleviate glutamate-induced excitotoxic neuronal injury (Xu et al., 2004). The overarching goal of this project was to assess several aspects of the neuroprotective dural patch through a series of well-defined objectives.

**Objective 1 (Weeks 1-4):** The first objective of the project is to verify that the concentration and release rate of the loaded candidate drug (hereafter called drug) does not induce adverse physiological effects on human spinal cord cells. Drug-loaded dural patches, with concentrations ranging from 0.01 uM to 100 uM, will first be incubated in culture media and the eluted drug collected at 24-hour intervals. Thereafter the drug-containing culture media will be applied to cultured human-derived neurons cultivated in 2D well plates to assess for cytotoxicity. Following a 7-day incubation period, assessments will include metabolic activity and cell stress/toxicity. The use of immunohistochemistry and fluorescent microscopy will enable a detailed analysis of the treated neurons.

**Objective 2 (Weeks 5-8):** Building upon the insights gained from the first objective, the second objective of the project will investigate the ability of patch-released drug to attenuate glutamate-induced neuronal injury within a 2D and later 3D in vitro cell culture environment. TERG's expertise in the production of neuron -optimized 3D scaffolds will be instrumental in assessing the neuroprotective potential of the dural patch. Neurons will be seeded, grown, and exposed to chronic (24 hour) excitotoxic injury (55mM glutamate). Collected media containing patch-eluted drug (to simulate release onto the cord) will be applied to the 3D-growing neurons over a 7-day period. The subsequent analysis will involve the processing and evaluation of the well plates and scaffolds, mirroring the methodologies employed in the first objective.

**Objective 3 (Weeks 8-10):** The third objective will evaluate the mechanical properties of the dural patch, specifically focusing on two candidate fibre alignments in the form of randomly aligned fibre patches. Uniaxial tensile testing will be conducted to measure the tensile strength of the patch types and also assess the impact of drug loading on the structural integrity and mechanical strength of the patches. This work will provide a comprehensive understanding of the dural patch's functionality, incorporating both its biological and mechanical aspects, crucial for a thorough evaluation in clinical applications.

The data generated from these experiments will contribute to a future publication on the potential efficacy and safety of this innovative therapeutic approach for SCI repair. The collaborative nature of the project, involving experts from two research groups and institutions, underscores the interdisciplinary effort required to tackle the challenges associated with spinal cord injuries.

**Project Outcomes and Experience Gained by the Student (no more than 700 words)**

**Project Outcome**

The study revolved around the evaluation of a synthetic drug-eluting dural implant, specifically employing the candidate drug as a potent blocker of glutamate-induced excitotoxic neuronal injury. To assess the toxicity of the drug in 2D culture, cells from the SH-SY5Y neuronal cell line were incubated with varying concentrations of the drug ranging from 0.01 uM to 100 uM. Analysis of cell viability (Presto blue assay) demonstrated that exposed cells tolerated all tested drug concentrations save for 100 uM which significantly reduced viability to 5% after 72 hours of culture. Next, in order to induce glutamate-induced excitotoxic injury, SHSY-5Y cells were exposed to concentrations of glutamate ranging between, 0.1 mM and 100 mM for 24 hours. Our findings showed significant reduction in viability at concentrations over 55 mM glutamate in cells treated for 72 hours of culture. Based on this data the 55mM and 100mM concentrations of glutamate were selected for future experiments and align with the levels of the neurotransmitter measured in the injured cord from literature (e.g. Xu et al. (1998) Neuroscience, 1011-21)

Next, the ability of different concentrations of drug to rescue neurons exposed to 24 hr glutamate exposure was assessed. To do so growing SHSY-5Y cells were first incubated with either 55 mM or 100 mM glutamate for 24 hours, which was then followed by post-administration with varying concentrations of the drug to assess the effect of concentration to rescue injured cells. Notably, a rescue effect was observed for the 55 mM Glutamate injury with 0.1 µM and 10 µM drug concentrations, while the 100 mM glutamate injury proved too toxic for effective rescue, indicating widespread cell death. Next, the temporal ability of the patches, loaded with 0.125%, 0.25, 0.5, 1, 2 and 4% drug to elute neuroprotective concentrations over a 72-hour period was assessed. To collect eluted drug, circular (8mm) patches containing the drug were first placed in new 24 well plates, covered in culture media and the eluted drug collected daily. Thereafter it was transferred to the glutamate-exposed cells for 72 hours. Results demonstrated a significant rescue effect from the 55 mM glutamate Injury with the drug collected from 0.25% and 0.5% loaded patches, while media collected from >1% drug loaded patches exhibited significant toxicity. The 100 mM Glutamate injury, consistent with previous findings, remained too toxic for rescue in 2D culture.

Expanding our investigation to a 3D culture setting, hyaluronic acid scaffolds, functionalised with collagen IV and fibronectin, were seeded with SHSY-5Y cells that were subsequently differentiated to neurons by changing foetal bovine serum containing culture media to a media containing a serum substitute (B-27) supplemented with 5 mM retinoic acid. Neurons grown in the 3D scaffolds and exposed to the glutamate-induced injury paradigm and subsequently treated with patch eluted 1% and 2% drug concentrations, demonstrated improved survival and contrasts with the results 2D culture (c.f. 0.25% & 0.5%). This discrepancy underscores the importance of considering the microenvironment and culture dimensionality in evaluating drug efficacy.

Next, to assess the tensile strength of the patches, mechanical testing was carried out using the Zwick/Roell materials testing machine (model No. Z005) to assess the tensile properties of patches constructed from randomly aligned electrospun fibres. The objective was to assess the mechanical characteristics and to evaluate them against the tensile properties of the human dura mater. Dog-bone-shaped patches were cut and prepared for analysis, and measurements of length, thickness, and width were taken prior to conducting tensile tests. The focus of the analysis was on Young's Modulus and ultimate tensile strength, which provide valuable information on the ability of the patches to withstand deformation-based stresses.

The results from testing several replicates of each type indicated that random aligned drug-loaded patches exhibited an elastic Young’s modulus (a measure of tensile stiffness and elasticity) of between 5-12MPa compared to non-drug loaded patches (2.5 MPa). This implies greater resistance to deformation within the elastic range and an overall higher stiffness in drug-loaded patches, suggesting that drug loading may influence patch stiffness. However, it is noteworthy that the mechanical properties of the drug-loaded patches remained considerably below those of the human dura mater which has a Young’s modulus of 70±44MPa (Zwirner et al.(2019) Sci Rep, 9:16655). This highlights the need for further refinement and enhancement of the mechanical properties of the drug-loaded patches to approach or surpass the biomechanical characteristics of the human dura mater.

In summary, the findings from my fellowship provide insights into the ability of the candidate drug to attenuate glutamate-induced injury seen in the injured spinal cord. The 3D culture model, with cells grown in protein functionalised Hyaluronic acid scaffolds, offers a promising avenue for further exploration of neuronal responses to the drug, revealing distinct responses compared to the 2D neuronal culture experiments.. These discoveries contribute to the ongoing pursuit of effective therapeutic strategies for SCI.

**Experience Gained**

During this project, I developed proficiency in a diverse range of laboratory techniques under the supervision of Dr Ian Woods. Beginning with instruction in laboratory health and safety, I progressed to mastering aseptic and other techniques necessary for cell culture through comprehensive training from Dr Woods and other TERG researchers. This equipped me not only with the ability to apply these techniques but also strengthened my confidence and allowed me to conduct my research work independently after 3 weeks.

My training also extended to various quantitative and qualitative techniques, including metabolic and cytotoxic assays, and immunofluorescent staining. I mastered biomimetic freeze-dried scaffold production to produce neuron-functionalised scaffolds and I was fully trained in cryo-sectioning, which was necessary for more detailed examination of scaffold cell distribution and morphology.

Beyond the lab, I received training in light and confocal microscopy and sharpened my scientific writing and presentation skills. I actively engaged daily with colleagues in the spinal cord injury team and the wider Tissue Engineering Research Group community (> 50 active members) and I attended weekly meetings, presented updates, and engaged in scientific discussions. Online meetings were held with our partner lab at University College London, and they provided insightful feedback on my progress. Attending the mid-summer spinal cord injury public-patient involvement advisory panel meeting offered unique insights into a different aspect of SCI research, understanding the views and needs of individuals with long-term disabilities. This experience added great depth to my research journey.

**Please state which Society Winter or Summer Meeting the student is intending to present his/her poster at:**

*Anatomical Society Summer Meeting July 2024*

**Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)**

Development of a bioengineered drug-eluting synthetic dural patch for spinal cord injury applications

**Brief Resume of your Project’s outcomes**: **(no more than 200-250 words)**.

*The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.*

**Development of a bioengineered drug-eluting synthetic dural patch for spinal cord injury applications.**

Spinal cord injury (SCI) is a devastating paralysing event that results in physical damage to the spinal cord. At the same time, the protective dural sheath overlying the cord is often torn, exposing the cord and allowing cerebrospinal fluid to leak and requires surgical intervention to prevent further injury. At present, there is an unmet need for an appropriate synthetic dural patch capable of sealing the torn dura while also simultaneously delivering much needed therapeutics to the injured cord. As part of a collaboration between the Tissue Engineering Research Group (TERG) at the Royal College of Surgeons in Ireland and the Department of Pharmaceutics at University College London, this project aimed to test the mechanical properties of a biodegradable electrospun polymer patch loaded with a candidate neuroprotective drug and assess its ability to attenuate glutamate-induced neuronal death.

Using a combination of 2D and 3D *in vitro* cell culture models it was found that dural patches loaded with low concentrations of the candidate drug were capable of significantly rescuing neurons exposed to concentrations of glutamate found in the injured cord (55 mM). Mechanical testing of the drug-loaded patches demonstrated a Young’s modulus of 5-12MPa showing they have a high tensile strength but retain necessary flexibility. These data provide significant insight into the mechanical properties of drug-loaded synthetic electrospun dural patches and their ability to rescue injured neurons from cell death.



**References**

1. Park E, Velumian AA, Fehlings MG. The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J Neurotrauma. 2004, 21: 754-74.
2. Xu GY, Hughes MG, Ye Z, Hulsebosch CE, McAdoo DJ. Concentrations of glutamate released following spinal cord injury kill oligodendrocytes in the spinal cord. Exp Neurol. 2004, 187: 329-36.
3. Zwirner J, Scholze M, Waddell JN, Ondruschka B, Hammer N. Mechanical Properties of Human Dura Mater in Tension - An Analysis at an Age Range of 2 to 94 Years Sci Rep 2019, 9: 16655.

**Other comments: (no more than 300 words)**

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*Signature of student............Louis Mulville................................Date…13/11/2023…….*

*Signature of supervisor………Ian Woods…………….............. Date…14/11/2023……….…*

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