CONFERENCE ABSTRACT BOOK OPEN ACCESS

2024-2025 SSGSA STEM Sustainability Case Competition: Regenerative Science

Massimo Maiuri, BSc Student [1]*, Sanya Sareen, BSc Student [2], Lauren Carscadden, BSc Student [2], Sukhjot Pooni, BSc Student [1], Rachel Nishizeki, BSc Student [2], Rameen Jafri, BSc Student [2], Mohammed Afridh, BSc Student [2], Youmna Asaad, BSc Student [2]

[1] College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

*Corresponding Author Details: ssgsa@uoguelph.ca

Abstract:
The SSGSA STEM Sustainability Case Competition is an annual research case competition hosted by undergraduate students from the STEM Students Guelph Support Association (SSGSA). The mission of this competition is to provide University of Guelph undergraduate students with an opportunity to develop their own research proposal while gaining valuable experience in innovative thinking and critical research analysis. Each year, students in teams of up to three are paired with an experienced mentor to develop and present a novel research proposal aligning with the competition's theme. During the competition, students are taught fundamental principles outlining three lab techniques that they could write about in their proposal. The theme of this year's competition was Regenerative Science, and competitors learned about Bioremediation, PCR, and CRISPR-Cas9. In the 2024-2025 SSGSA STEM Sustainability Case Competition, over 55 participants submitted abstracts for evaluation by faculty judges. We present to you the top 19 winning submissions in our competition abstract booklet. We hope you enjoy reading this year's best abstract submissions and encourage you to participate in the growing SSGSA community as we strive to encourage interest in novel scientific research fields surrounding STEM.

Keywords: STEM sustainability case competition; SSGSA; abstract submissions; regenerative science; bioremediation; 3D printing; phototonics engineering; undergraduate; STEM; University of Guelph

Table of Contents

SSGSA STEM Sustainability Case Competition Abstracts .... pg. A02-A10
The Targeting of Autoantigenic B Cells Using a Bi-Specific T Cell Engager as a Potential Therapeutic for Rheumatoid Arthritis .... pg. A02-A02
Treatment of Autosomal Dominant Retinitis Pigmentosa Using 3D-Bioprinted Scaffolds to Deliver CRISPR-Cas9 Gene-Edited Photoreceptors .... pg. A02-A03
From Medications to Microbes: Genetically Modified Escherichia coli Nissle 1917 as an Alternative to Traditional L-Dopa Therapy in Late Stage Parkinson's Disease .... pg. A03-A03
Investigating the Use of Glycyrrhizin as an Anti-Inflammatory Drug to Promote Neuroregeneration in Alzheimer's Disease Patients .... pg. A03-A04
Promoting Neuronal Proliferation of Cerebellar Granule Cells through Exogenous Brain Derived Neurotrophic Factor in Vitro .... pg. A04-A04
Enhancing Functional Innervation of 3D Printed Skeletal Muscle Using Neurotrophic Growth Factors ...... pg. A04-A04
Regenerative Nanobot-Tissue Interface for Dopaminergic Neuron Regeneration in Parkinson's Disease .... pg. A05-A05
Development of Bacterial Cellulose-Based Scaffolds for Diabetic Foot Ulcer Treatment: A Sustainable Approach .... pg. A05-A05
Application of CRISPR-Cas9 to Enhance OCT4 Expression in Menstrual-Derived Stem Cells: A Novel Approach to Treating Primary Ovarian Insufficiency as a Result of Chemotherapy .... pg. A05-A06
In-Vivo CRISPR-Cas9 Facilitated Gene Knockout to Eliminate EBV Antigen Attacks Associated with Multiple Sclerosis .... pg. A06-A06
Combining CRISPR-Cas9 Gene Therapy with IL-10 Administration in MPTP-Mouse Models of Parkinson's Disease .... pg. A06-A07

Integrating CRISPR-Cas9 Gene Editing, Stem Cell-Derived Photoreceptors, and Nanotechnology-Based Scaffolds for Vision Restoration in Retinitis Pigmentosa .... pg. A07-A07
Utilizing in Vivo CRISPR-Cas9 Gene Editing of APOE4 to APOE3 to Mitigate the Symptoms of Alzheimer's Disease .... pg. A07-A07
Novel Treatment for Renal Damage in Systemic Lupus Erythematosus .... pg. A08-A08
Innovative Bioprinting Solutions for Mitral Valve Regeneration: Addressing Stenosis, Regurgitation, and Prolapse .... pg. A08-A08
A Future Towards Safer Pain Relief: Evaluating the Combined Impact of Herbal Remedies on Menstrual Cycle-Related Discomfort .... pg. A08-A09
Investigating Patient-Specific Bioprinting to Address the Complications and Limitations of Split-Thickness Skin Grafting .... pg. A09-A09
In Utero Limb Regeneration Using 3D-Bioprinted Scaffolds and Genetically Modified Stem Cells .... pg. A09-A10
Integrating Fungal and Bacterial Metabolism for Methylmercury Bioremediation: Investigating Laccase Gene Transfer from Aspergillus flavus to Sphingomonas paucimobilis .... pg. A10-A10

Conference Abstracts

Note: These abstracts have been reproduced directly from the material supplied by the authors, without editorial alteration by the staff of the URNCST Journal. Insufficiencies of preparation, grammar, spelling, style, syntax, and usage are the authors.

SSGSA STEM Sustainability Case Competition Abstracts

The Targeting of Autoantigenic B Cells Using a Bi-Specific T Cell Engager as a Potential Therapeutic for Rheumatoid Arthritis

Christiana Lammers, BSc Student [1], Jamie Asselin, BSc Student [1], Erica D'Souza, BSc Student [2]
[1] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Rheumatoid arthritis (RA) is a chronic autoimmune disorder that primarily affects synovial joints, inflicting pain, inflammation, and progressive damage. A key molecular mechanism of pathogenesis is the citrullination of proteins such as fibrinogen, involving the conversion of arginine residues to citrulline. Anti-citrullinated protein antibodies (ACPAs) are autoantibodies that recognize the fib-α R84cit region of citrullinated fibrinogen (c-fib), contributing to immune complex formation, activation of inflammatory pathways, and subsequent tissue damage characteristic of RA. Currently, chimeric antigen receptor T-cell therapy has been shown to be effective in eliminating autoantigenic B-cells involved in rheumatoid diseases by targeting CD19. We hypothesize that using cytotoxic T-cells to eliminate ACPAs involved in RA may slow disease progression and limit the damaging effects of these autoantibodies. To test this hypothesis, we will design a bispecific T-cell engager (BiTE) with specificity for the CD3 region of cytotoxic T-cells and the fib-α R84cit region. A BiTE is an antibody-like protein consisting of two short-chain variable fragments (scFvs) and would be designed to have an affinity for cytotoxic T-cells and c-fib. This would bring the T-cells in proximity with the ACPA-producing B cells and destroy them. We will construct a plasmid vector that encodes both scFvs, clone our plasmid into *E. coli* for replication, and subsequently transfect murine cells using tissue culture to produce our BiTE therapeutic. The study will be conducted in HLA-DR4-IE transgenic mice; healthy controls will be injected with the BiTE while two treatment groups will be injected with c-fib to mimic the RA disease state. From these treatment groups, one will receive the BiTE injection and the other will not. Following the procedure, we expect to see decreased levels of ACPAs and hope to slow disease progression, particularly relating to joint tissue damage and inflammation levels within joint tissues.

Treatment of Autosomal Dominant Retinitis Pigmentosa Using 3D-Bioprinted Scaffolds to Deliver CRISPR-Cas9 Gene-Edited Photoreceptors

Aleena Gangji, BSc Student [1], Maina Patel, BSc Student [1]
[1] College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Autosomal dominant retinitis pigmentosa (ADRP) is caused by a mutation in the *RHO* gene, inducing the misfolding of rhodopsin protein and degeneration of rod photoreceptors. Aggregation interferes with essential cell functions, leading to rod apoptosis. Rods detect dim light, and their deterioration consequently causes night blindness and vision constriction over time.

Previous research demonstrates CRISPR-Cas9-mediated gene therapies to correct *RHO* mutations in ADRP animal models. However, its retinal administration and integration have not yet been optimized. This study will investigate a novel ADRP treatment using a tissue-engineered retinal scaffold containing photoreceptors that have been gene-edited through CRISPR-Cas9 to correct the RHO-P23H mutation. Somatic cells from $Rho^{P23H/P23H}$ transgenic mice will be extracted and reprogrammed into induced pluripotent stem cells (iPSCs) via adeno-associated viral vector delivery of Yamanaka transcription factors. CRISPR-Cas9 will correct the RHO-P23H mutation in the iPSCs by replacing histidine with proline at position 23 in rhodopsin. This is achieved by creating a double-stranded break, removing adenine at codon 68, and replacing it with cytosine to correct the mutation. Repaired iPSCs will then be differentiated into photoreceptors. Polylactic-co-glycolic acid (PLGA) will be 3D-bioprinted into biodegradable scaffolds, where photoreceptor cells will be seeded for optimal retinal integration. The photoreceptor-scaffold complex will be injected subretinally, between the retina and the retinal pigment epithelium layer. Within 2-3 weeks, we expect photoreceptors to form connections with surrounding cells, while scaffolds biodegrade over two months. The mice are anticipated to display corrected photoreceptors, reversing the effects of ADRP. This will be validated through operant maze training with light-based cues to evaluate visual function restoration. If successful in *RHO*-mutated transgenic mice, this approach may be applied to human ADRP patients. This breakthrough would represent a major advancement in regenerative science, offering hope for reversing retinal degeneration and restoring vision in patients.

From Medications to Microbes: Genetically Modified *Escherichia coli* Nissle 1917 as an Alternative to Traditional L-Dopa Therapy in Late Stage Parkinson's Disease

Ravleen Sandrah, BSc Student [1], Namita Seshadri, BSc Student [1]
[1] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Orally administered levodopa (L-Dopa) is the current primary treatment to address the dopaminergic deficiency characteristic of Parkinson's disease (PD). However, the clinical effectiveness of traditional L-Dopa therapy diminishes with continued use into late-stage PD, with additional challenges being created by pulsatile and non-continuous L-Dopa delivery. *Escherichia coli* Nissle 1917 (EcN) is a specific isolation of *E. coli* that has shown tremendous promise in both improving inflammatory and oxidative symptoms of gut dysbiosis, as well as in drug delivery. Therefore, we propose utilizing CRISPR-Cas9 to introduce the tyrosine hydroxylase (TH) gene into this bacteria, allowing for the continuous delivery of L-Dopa via the patient's own gastrointestinal (GI) synthesis. This bacteria has the potential combined effect of ameliorating PD-related gut dysbiosis and conferring continuous L-dopa delivery without the phasic dopaminergic signalling inherent to oral drug therapy. As for experimental procedure, the use of 6-OHDA-lesioned rats with consequent degeneration of dopaminergic neurons in the nigrostriatal tract constitutes a highly reliable animal model for PD. To establish $EcN_{L\text{-}Dopa}$, CRISPR will locate the PAM sequence at the lac operon, a well-characterized and reliably edited target site, allowing Cas-9 to cleave the DNA and insert the TH gene via a repair template. $EcN_{L\text{-}Dopa}$ synthesis rates can then be studied in vitro using HPLC-based quantification. Following this, 6-OHDA rats will be inoculated via oral gavage directly into the GI tract with either $EcN_{L\text{-}Dopa}$ or saline. After a habituation period, assays such as the cylinder test will be conducted to compare PD progression in the two groups. Findings should showcase improved sensorimotor skills in the group receiving therapeutic interventions. Overall, this proposal provides a basis for $EcN_{L\text{-}Dopa}$ inoculation as a novel intervention in the treatment of PD with greater potential therapeutic efficacy than any treatments currently available.

Investigating the Use of Glycyrrhizin as an Anti-Inflammatory Drug to Promote Neuroregeneration in Alzheimer's Disease Patients

Ben A. Smith, BSc Student [1], Colin J. Carscadden, BSc Student [1]
[1] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Alzheimer's Disease (AD) is a neurodegenerative disorder with no effective treatment or cure. AD impairs cognitive function and it is characterized by various complex mechanisms. Some of these mechanisms include the formation of amyloid-β (Aβ) plaques and neurofibrillary tangles (NFT). The hippocampus, crucial for memory and learning, is markedly damaged in an AD affected brain. This hippocampal damage is driven by prolonged inflammation which exacerbates Aβ and NFT formation. A key contributor to inflammation is the high mobility group box 1 (HMGB1) protein. HMGB1 is a damage-associated molecular pattern (DAMP) released by necrotic brain cells that binds to receptors responsible for initiating a cascade, ultimately resulting in the release of proinflammatory factors. To test whether glycyrrhizin (GL) acts as an anti-inflammatory agent in the hippocampus via inhibition of HMGB1 pathways, mice will be given GL, and cognition tests will be conducted. Fully matured 5xFAD (AD-BXD) mice will be modified with CRISPR-Cas9 via a *MAPT* gene knock-in so that they best mimic the human AD microenvironment. GL will be injected into the brain encapsulated in a synthetic

DOI Link: https://doi.org/10.26685/urncst.796

biomaterial, allowing for controlled release of the drug. Attentional set-shifting tasks and the Morris Water Maze assessment will be conducted before and after the GL injection to determine changes in cognitive function. Tissue analysis using the Western Blot technique will be carried out to evaluate the levels of free HMGB1. It is expected that mice not treated with GL will display a higher concentration of free HMGB1 and poorer performance on the tests as compared to their GL-treated counterparts. This would indicate a decline in neuroinflammation and provide evidence for GL's role in neuroregeneration. The anticipated findings would provide foundations for the further investigation of GL injections as an efficacious treatment in human AD patients.

Promoting Neuronal Proliferation of Cerebellar Granule Cells through Exogenous Brain Derived Neurotrophic Factor in Vitro

Fatima Askary, BSc Student [1], Maryam Khan, BSc Student [2], Andra Popa, BSc Student [2]
[1] College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Cerebellar granule neurons (CGNs), the most abundant neuronal cell type in the brain, are crucial for supplying contextual information necessary for fine motor movements in the cerebellum. Abnormal neuronal apoptosis of CGNs contributes to the progression of neurodegenerative diseases and their underlying symptoms such as Parkinson's disease, multiple sclerosis and Huntington's disease. Brain-Derived Neurotrophic Factor (BDNF), a key neurotrophic factor, is significantly depleted in these diseases. Previous research has shown the role of BDNF in counteracting neurodegeneration in CGNs. However, the effect of BDNF in hypoxic cerebellar conditions has yet to be elucidated. Here we aim to promote neuronal proliferation by exogenously adding BDNF to cerebellar hypoxic mice brain slices in vitro using an organotypic slice culture (OTC) model, which allows for the 3D organization of the CNS to be maintained. We hypothesize that BDNF will lead to increased proliferation of CGNs. OTC dissection protocol will be done on mice cerebellum. Two groups of OTCs will be kept in culture for 21 days. Hypoxia will then be induced in both groups to promote CGN apoptosis. Following this, exogenous BDNF will be administered to one of the groups, while the other will remain untreated as a control. Both groups will be kept in culture for an additional 21 days. Immunohistochemistry will be performed using calretinin, a CGN-specific marker, to assess proliferation in CGNs, comparing cell counts between both groups. As a result, CGN proliferation is expected to increase following addition of BDNF compared to the non-treated hypoxic group. Understanding the role of BDNF in the cerebellum following neurodegeneration will contribute to developing crucial therapies to treat neurodegenerative diseases and identify key prevention strategies.

Enhancing Functional Innervation of 3D Printed Skeletal Muscle Using Neurotrophic Growth Factors

Palina Dubavets, BSc Student [1], Yi Zhen Bao, BSc Student [2]
[1] Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Non-restorable skeletal muscle damage caused by severe trauma, surgical removal, genetic disorders, and atrophy, severely impairs motor ability and quality of life. Although muscle tissue has innate post-injury regeneration capacity, volumetric muscle loss exceeding 20% requires surgical intervention to restore native function. 3D bioprinted grafts are effective at replicating the functional and structural properties of host muscle, while minimizing the risks of adverse immune responses. However, current bioprinting approaches face challenges in facilitating sufficient innervation to develop working neuromuscular junctions (NMJs) throughout the muscle. Neurotrophic factors, derived from ciliary and glial cells, are biomolecules that have been shown to promote the migration of neuron projections in vivo. This study aims to investigate the role of neurotrophic factors in enhancing NMJ outgrowth in bioprinted skeletal muscle constructs to achieve functional innervation. Human muscle progenitor cells (hMPCs) will be cultured to induce proliferation and myotube formation. Two hMPC bioprinting mediums will be prepared: a control and a treatment group containing neurotrophic factors introduced via microspheres. Muscles will be constructed using the 3D Integrated Tissue-Organ Printing (ITOP) system, and subsequently cultured in differentiation media. Dorsal root ganglia of chicken embryos will be co-cultured with the constructs as an origin of neuronal growth. Compound muscle action potential (CMAP) amplitude will be measured at multiple time points using electromyography following electrical stimulation. Additionally, the distribution and density of NMJs along neurite projections will be evaluated through immunostaining with antibodies targeting NMJs, followed by micrograph analysis. Muscle tissues grown in the presence of neurotrophic factors are expected to have a greater amplitude of CMAP due to wider distribution and increased density of NMJs along neurite projections. This approach aims to advance strategies for enhancing functional innervation within bioprinted muscle implants to accelerate rehabilitation for patients experiencing significant muscle loss.

Regenerative Nanobot-Tissue Interface for Dopaminergic Neuron Regeneration in Parkinson's Disease
Oranoos Beissed, BSc Student [1], Zahraa Suhail Shaikh, BSc Student [1]
[1] College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, resulting in motor dysfunction and diminished quality of life. Current symptomatic treatments, such as dopamine replacement therapy and deep brain stimulation, offer no regenerative solutions to address the underlying neuronal degeneration. This research aims to develop a Regenerative Nanobot-Tissue Interface (RNTI) system integrating 3D bioprinting, CRISPR-Cas9 gene editing, and nanobot biomonitoring to regenerate dopaminergic neurons and restore motor function. The RNTI system uses patient-derived induced pluripotent stem cells (iPSCs) that are genetically edited using CRISPR-Cas9 to upregulate dopamine synthesis enzymes, like *TH* (tyrosine hydroxylase) and *AADC* (aromatic L-amino acid decarboxylase). The edited cells are embedded in bioprinted scaffolds engineered to mimic the extracellular matrix of the substantia nigra. Embedded nanobots with biosensors will monitor dopamine levels, synaptic activity, and tissue oxygenation to ensure functional integration and feedback-driven optimization. This 12-week study will test the RNTI system in preclinical murine models of MPTP-induced PD. Experimental outcome evaluation includes weekly dopamine quantification using high-performance liquid chromatography (HPLC), motor tests (rotarod and open field) every two weeks, and histological analysis at the end of 12-weeks to assess neuronal survival and synaptic connectivity. We hypothesize that the RNTI system will enhance dopaminergic neuron differentiation, restore dopamine in the substantia nigra closer to normal, and improve motor function in treated models. This improvement will be measured through reduced latency in rotarod performance and increased activity in open field tests. Real-time biomonitoring will validate the neuronal integration and functional recovery of the regenerated neurons, establishing a replicable and scalable protocol for future clinical applications in PD. By addressing a critical gap in regenerative therapies for PD, this research offers a transformative approach to regenerative medicine and personalized healthcare.

Development of Bacterial Cellulose-Based Scaffolds for Diabetic Foot Ulcer Treatment: A Sustainable Approach
Matthew R.M. Arai, BSc Student [1], Swara Bangali, BSc Student [1]
[1] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Diabetic foot ulcers (DFUs) impact millions worldwide, with approximately 25% of patients facing amputation due to poor wound healing driven by infection, poor vascularization, and elevated matrix metalloproteinase-9 (MPP-9) activity. Addressing this challenge requires innovative materials to support tissue regeneration and improve prognosis. Bacterial cellulose (BC), derived from purified symbiotic cultures of bacteria and yeast (SCOBY), holds mechanical properties that could be used for DFU wound patches, including increased tensile strength and a highly adjustable filamentous matrix. Our study explores the development of inoculated BC-based scaffolds as a cost-effective, bioactive solution for treating DFUs. By incorporating (R)-ND-336, a MPP-9 inhibitor, these scaffolds aim to reduce inflammation, enhance angiogenesis, and promote tissue healing. Tissue culture will be used to culture and inoculate fibroblast cells onto the BC scaffolds to utilize their ability to support cell adhesion, proliferation, granulation tissue formation, and tissue integration. These cells are expected to enhance angiogenesis through paracrine vascular endothelial growth factor (VEGF), further supporting tissue regeneration. Functionalized BC scaffolds are expected to mitigate elevated MPP-9 activity, reduce inflammation, and enhance granulation tissue formation, leading to improved angiogenesis and more efficient wound healing. This could significantly lower the risks of amputation, osteomyelitis, and death for patients with DFUs. Furthermore, the low-cost production and adaptability of SCOBY-derived BC make these scaffolds particularly suitable for resource-limited settings. This study explores the potential of BC-based scaffolds as a sustainable and accessible treatment for DFUs. Future work will focus on optimizing bacterial strains, improving scaffold bioactivity, and conducting clinical trials to test effectiveness.

Application of CRISPR-Cas9 to Enhance OCT4 Expression in Menstrual-Derived Stem Cells: A Novel Approach to Treating Primary Ovarian Insufficiency as a Result of Chemotherapy
Grace Davis, BSc Student [1], Lydia Lawrence, BSc Student [1]
[1] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Chemotherapy induces primary ovarian insufficiency (POI) by causing DNA breaks and apoptosis of follicles, depleting the ovarian reserve, and resulting in irregular menstrual cycles. Assisted reproductive technologies for women post-puberty, including oocyte cryopreservation, can foster ovarian cancer resurgence. Menstrual-derived stem cell (MenSC) therapy is a sustainable alternative in mitigating resurgence while addressing the needs of prepubescent individuals. The presence of

DOI Link: https://doi.org/10.26685/urncst.796

octamer-binding transcription factor, OCT4, in MenSC enhances differentiation in mural granulosa and primordial germ cells; however, levels of OCT4 within these cells are inconsistent. MenSC lacking OCT4 have decreased proliferative and differentiating ability, suggesting the need to enhance this function. Strict OCT4 regulation is essential given the suspected correlation of overexpression to tumorigenesis. Through CRISPR-Cas9 in a CD1 female mouse model, we explore performing a gene knock-in, increasing proliferative capability of MenSC into functional primordial follicles and ultimately replenishing the ovarian reserve, as confirmed by restoration of typical hormonal fluctuations. Characterized by high gonadotropin (Gn) levels, through reintroducing viable primordial follicles in POI that may regulate synthesis of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), we expect typical LH and FSH production and restoration of the menstrual cycle. Mice in control and test groups will be administered cyclophosphamide, a chemotherapeutic agent inducing POI by damaging the ovarian reserve. MenSC will be harvested from the endometrium of donor CD1 female mice. MenSC designated to the test group will receive CRISPR-Cas9 intervention, increasing OCT4 expression as confirmed by western blot analysis. Controls will receive MenSC without genetic intervention. Intervention efficacy will be confirmed by measuring serum estrogen levels over three estrous cycles. Return of typical estrogen fluctuation is expected, indicating successful restoration of the menstrual cycle. Given the high-cost nature of stem cell therapy, using CRISPR-Cas9 to enhance MenSC differentiation may provide a minimally invasive, sustainable option in replenishing the ovarian reserve.

In-Vivo CRISPR-Cas9 Facilitated Gene Knockout to Eliminate EBV Antigen Attacks Associated with Multiple Sclerosis

Sehara D. Perera, BSc Student [1], Parth Kumar, BSc Student [1], Amelia Luong, BSc Student [1]
[1] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Multiple Sclerosis (MS) is an autoimmune disease that targets the brain. Recent research has proposed that Epstein-Barr Virus (EBV) can trigger MS through molecular mimicry. EBV antigens that have undergone somatic hypermutations can bind and attack GlialCAM (a protein residing in the myelin sheath). This mimicry was epidemiologically and pathologically linked to the progression of MS through humanized mice. Our study will investigate the effectiveness of CRISPR-Cas9 gene knockout in eliminating or slowing the progression of MS triggered by EBV. MS progression by EBV will be mimicked in mice through the insertion of the EBNA1 gene section that codes for anti-EBNA1$_{386–405}$, a peptide sequence linked to the progression of MS. PCR and appropriate primers will be used to amplify amino acids 386 to 405. The DNA will be integrated into mouse-egg nuclei producing transgenic mice that mimic progression of MS triggered by EBV. This DNA will also be injected into Experimental Autoimmune Encephalomyelitis (EAE) mice (animal models for MS) to provide further insights. Following the development of MS, half of the EAE models and EBV-MS models will be injected with mRNA that encodes CRISPR-Cas9 and small guide RNA (sgRNA). Once the EBNA1 gene is located, Cas9 will create a double-strand break to induce mutations, knocking out the gene. The knockout will end ongoing attacks by EBV antigen to GlialCAM and thus the myelin sheath. Magnetization transfer techniques will be used to analyze the progression of demyelination in MS. Following CRISPR treatment, magnetic ratios are expected to increase in EBV-MS models indicating an elimination of MS progression; EAE models should mimic this trend to some extent. This study provides a framework for evaluating the effectiveness of eliminating EBV antigens as a treatment for EBV-driven MS.

Combining CRISPR-Cas9 Gene Therapy with IL-10 Administration in MPTP-Mouse Models of Parkinson's Disease

Ava Quinn, BSc Student [1], Aleeshah Sohail, BSc Student [2]
[1] Department of Biomedical Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] Department of Psychology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Parkinson's disease (PD) is a complex neurodegenerative disorder that is characterized by the degeneration of dopaminergic neurons (DAN) and neuroinflammation in the substantia nigra (SN). Previous studies have shown that Pitx3-deficient mice demonstrate DAN loss in the SN and accurately model both non-motor and motor symptoms of PD. Administration of anti-inflammatory cytokines, such as IL-10, has been shown to reduce inflammation and is proposed as a promising treatment for PD. CRISPR-based gene therapy is commonly used for the delivery of genes that play a role in neurodegenerative diseases but has not been used to alter the expression of Pitx3. Although both CRISPR-based and anti-inflammatory cytokine treatments have been studied independently, the potential benefits of combining the two treatments remain unexplored. We propose using CRISPR-Cas-9 gene therapy to stimulate Pitx3 expression in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model — a common mouse model used to study PD. We will explore whether combining this therapy with the delivery of IL-10 can mitigate both neuroinflammation and neurodegeneration. Preliminary motor and cognitive tests will be conducted on a control and an experimental group of MPTP-treated mice. In the treatment group, IL-

10 will be administered intranasally and CRISPR-Cas9 will be used to enhance Pitx3 expression. Following treatment, the motor and cognitive tests will be repeated in both groups, and immunohistochemical analyses and Western blotting will be used to assess the efficacy of this treatment. We expect that the combined therapy will enhance DAN survival, reduce inflammation, and improve motor and cognitive function in the treated MPTP mice. This research will provide insight into whether the combination of gene and cytokine-based treatments is effective at treating neurodegenerative diseases such as PD.

Integrating CRISPR-Cas9 Gene Editing, Stem Cell-Derived Photoreceptors, and Nanotechnology-Based Scaffolds for Vision Restoration in Retinitis Pigmentosa

Tina Aella, BSc Student [1], Ayesha Asif, BSc Student [2]
[1] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Retinitis Pigmentosa (RP) is an inherited retinal degenerative disorder affecting 1 in 4,000 individuals worldwide, making it a leading cause of inherited blindness. RP is characterized by progressive photoreceptor loss due to mutations in genes like RPE65 and RPGR, which disrupt the retinoid cycle and photoreceptor cilia function. Current treatments slow disease progression but do not restore lost vision, highlighting the need for regenerative strategies. The purpose of this study is to restore vision in RP patients by integrating gene therapy, stem cell-derived photoreceptors, and electrospun nanofiber scaffolds. CRISPR-Cas9 will be used to correct RPE65 and RPGR mutations in patient-derived iPSCs differentiated into light-sensitive photoreceptors. These will be seeded onto electrospun nanofiber scaffolds made of PCL and collagen, functionalized with BDNF to mimic the retinal extracellular matrix. In vitro studies using retinal organoids and postmortem tissue will validate photoreceptor integration through confocal microscopy and multi-electrode arrays. In vivo, rodent models of RP will assess vision restoration through behavioral tests. Retinal activity will be monitored using ERG, and postmortem analysis will evaluate photoreceptor survival and synaptic formation. Photoreceptor transplantation has shown potential to restore retinal function, but challenges persist. BDNF-functionalized scaffolds improve cell survival, and CRISPR-Cas9 effectively corrects mutations, but current methods fail to restore functional vision fully. This research combines CRISPR-corrected iPSC-derived photoreceptors with scaffolds to address these gaps. We expect improved photoreceptor growth and light responsiveness in vitro and restored vision in RP rodent models. If successful, this approach offers a regenerative solution for RP, advancing retinal therapies. RP affects thousands worldwide, causing vision loss. Combining CRISPR-Cas9, stem cell-derived photoreceptors, and BDNF-functionalized scaffolds could provide a scalable regenerative solution, transforming treatment for RP and similar diseases.

Utilizing in Vivo CRISPR-Cas9 Gene Editing of APOE4 to APOE3 to Mitigate the Symptoms of Alzheimer's Disease

Chau V.P. Ngo, BSc Student [1], Fatima Farhan, BSc Student [1]
[1] Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Alzheimer's disease (AD) is a progressive neurodegenerative disease, which is characterized by memory loss and cognitive impairment. Different types of AD are categorized on the age-onset and familial AD. Late-onset AD is associated with the mutation of the Apolipoprotein E (APOE), specifically isoform 4 (APOE4) on chromosome 19. APOE4 is known to increase AD risk and decrease the age of onset through its effect on the accumulation of amyloid-β (Aβ) plaque in the brain due to its role in cholesterol transport. This study aims to investigate the use of in vivo CRISPR-Cas9 gene editing to mitigate the symptoms of Alzheimer's disease by converting APOE4 to APOE3, the neutral isoform of APOE. Aβ peptide plaques will be administered in the brain using adult Wistar mice. They will be divided into two groups: a control group and a genetically modified group. The plaque deposition in mice will be evaluated using Amyloid PET scans. In the genetically modified group, adeno-associated virus (AAV) will be used as a vector carrying Cas9, a single guide RNA (sgRNA) targeting APOE4, and the APOE3 replacement sequence. This will be delivered into the brain via intracerebroventricular (ICV). The sgRNA will guide the Cas9 protein in recognizing and cleaving the APOE4 sequence, resulting in the insertion of APOE3 at the APOE4 location. It is expected that the accumulation of Aβ plaque in the genetically altered group will decrease compared to the control group due to the presence of APOE4. This will be observed through the Amyloid PET scans. This study demonstrates the utility of using CRISPR-Cas9 gene editing to reduce the symptoms of AD. Further research is needed to refine this approach, evaluate its long-term effects, and test its translational potential in animal models.

Novel Treatment for Renal Damage in Systemic Lupus Erythematosus
Lauren Bruce, BSc Student [1], Tess Nuttall, BSc Student [2]
[1] Department of Human Health and Nutritional Science, University of Guelph, Guelph, Ontario, Canada N1G2W1
[2] Department of Biological Science, University of Guelph, Guelph Ontario, Canada N1G2W1

The prevalence of renal damage in systemic lupus erythematosus (SLE) is one of the primary contributors to lupus-related mortality, making it one of the most severe complications of the disease. The purpose of this study is to minimize lupus-induced renal damage. It has been found that the G1 and G2 alleles of the APOL-1 gene can be responsible for the progression of lupus nephritis to end-stage renal disease (ESRD), decreasing the time from diagnosis of SLE to diagnosis of ESRD by 2 years. Furthermore, approximately 35% of the African-American population with SLE was found to express the APOL-1 risk variants. Gene editing via CRISPR/Cas9 involves the incorporation of foreign DNA into CRISPR to create a genetic record of the disease. Future infection of the same disease is recognized by CRISPR, and cleaved off by the Cas9 protein. CRISPR/Cas9 would be applied to the APOL-1 gene, cleaving off and destroying risk variants to prevent their expression. Furthermore, 3D bioprinting is an emerging medical technique that has the potential to become a treatment for individuals with kidney disease. In vitro studies have shown that kidney replacement utilizing 3D bioprinting techniques leads to improved kidney function, through the removal of damaged renal tissue, and increased organogenesis/organoid maturation. Current challenges faced with kidney 3D bioprinting include selecting appropriate cell types, biomaterials, and other biological factors. Once this research develops, it is proposed that 3D bioprinted kidney transplant should be used preceding CRISPR/Cas9 treatment to ensure effective removal of necrotic tissue and prevention of further damage. The desired outcome of the modification of the APOL-1 risk genotypes in combination with the replacement of the diseased kidney would be improved renal functioning and decreased risk of lupus nephritis.

Innovative Bioprinting Solutions for Mitral Valve Regeneration: Addressing Stenosis, Regurgitation, and Prolapse
Madelyn E. Budweth, BSc Student [1], Isabel M. Louwers, BSc Student [1], Caroline É.M. York, BSc Student [1]
[1] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Mitral valve disease, including prolapse, regurgitation, and stenosis, disrupts systemic blood flow and heart function, often leading to complications such as right ventricular failure and pulmonary congestion. Current treatments, such as mechanical and bioprosthetic valves, have major drawbacks, including thrombosis, limited durability, and lack of growth potential. These challenges emphasize the need for innovative alternatives. This research explores the use of extrusion-based 3D bioprinting to create regenerative, patient-specific mitral valve scaffolds that replicate the biomechanical and structural properties of natural heart valves. Collagen, fibrin, and alginate bio-inks are used to create mitral valve scaffolds, mimicking the natural extracellular matrix, while human mesenchymal stem cells encourage tissue remodelling and endothelialization. The resultant scaffolds have the potential for growth and durability since they are made to conform to the patient's anatomy. Additionally, these scaffolds are designed to replicate the anisotropic structure and biomechanical properties of the native valve, ensuring proper leaflet coaptation under hemodynamic conditions. In vitro testing evaluates mechanical properties like tensile strength, flexibility, and durability, while bioreactors stimulate cardiac conditions to assess functionality. This ensures the scaffold meets both structural and performance requirements for clinical application. Preliminary findings indicate that the bioprinted supports exhibit favourable cellular viability, structural stability, and mechanical strength. However, despite their potential, 3D-bioprinted valves have drawbacks, such as the requirement for additional preclinical testing and the usage of immunosuppressants following implantation. This revolutionary approach may be able to overcome the limitations of existing treatments, providing a durable, biocompatible solution for young individuals with mitral valve dysfunction. Preclinical studies optimizing scaffold makeup and improving cellular integration will be the main areas of ongoing research that aim to improve clinical viability.

A Future Towards Safer Pain Relief: Evaluating the Combined Impact of Herbal Remedies on Menstrual Cycle-Related Discomfort
Disany Thayalan, BSc Student [1], Harsharan Bharj, BSc Student [2]
[1] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Most females of reproductive age experience menstrual cycles and can experience dysmenorrhea or menstrual pain that can last several days and significantly impact their daily lives. Females can experience debilitating cramps, heavy bleeding, and mood swings, which can span the entirety of their menopausal period, averaging over 40 years. Currently, 30% to 70% of

DOI Link: https://doi.org/10.26685/urncst.796

females of reproductive age experience some form of menstrual cycle-associated pain or discomfort. The overuse of pharmaceutical pain relievers, like opioids, may pose long-term health risks, including hormonal dysregulation, infertility, and delayed or absent menstrual cycles. Progesterone is a primary factor in the menstrual cycle as it mediates acute inflammation. Estrogen works with progesterone when a hormone imbalance is present, inducing inflammation and cell proliferation. Despite this, current pain management options primarily rely on pharmaceuticals, requiring a need for safer alternatives such as herbal remedies. Ginger, chamomile, and cinnamon have been used for centuries to alleviate menstrual discomfort; however, these herbs have not yet been combined into an all-in-one pain management product. The purpose of this study is to explore this single-product combination of multiple herbal remedies for managing menstrual cramps and heavy bleeding using an experimental and a control group of human endometrial cells to measure and assess the impact of herbal supplements on menstrual cycle-related pain and discomfort. Using tissue culture, human endometrial cells will be cultured and exposed to herbal extracts. Researchers will monitor how the herbal extracts affect the inflammation, myometrium, and progesterone and estrogen levels during the menstrual cycle. The herbal extracts are expected to reduce inflammation, decrease smooth muscle activity, and help regulate progesterone and estrogen levels. This research could result in an over-the-counter herbal product that provides a safer way for females to manage their discomfort compared to long-term pharmaceutical use, improving the quality of life for millions worldwide.

Investigating Patient-Specific Bioprinting to Address the Complications and Limitations of Split-Thickness Skin Grafting

Humna Chaudhry, BSc Student [1], Sapphire Aberdeen, BSc Student [2], Hashir Ahmed, BSc Student [3]
[1] College of Engineering and Physical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[3] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Split-thickness skin grafting (STSG) is the current gold standard for treating severe burns, trauma, or chronic wounds. However, complications such as graft rejection/necrosis, scarring, unwanted hair growth, hypo/hyperpigmentation, and reduced elasticity cause significant psychological distress in patients, many of whom seek further treatment due to dissatisfaction. STSG is limited by its reliance on healthy patient skin, which may be insufficient or unavailable in cases of extensive burns. Additionally, STSG lacks the full dermal layer including its intrinsic blood supply, making revascularization failure a common reason for STSG rejection. Emerging technologies like 3D bioprinting offer a promising regenerative solution by creating patient-specific multilayered and vascularized skin constructs. These constructs eliminate the need for extensive graft harvesting while providing stable grafts with improved aesthetic and functional outcomes. This methodology involving the induction of fibroblasts, melanocytes, and keratinocytes has been previously established. In this study, we aim to build upon this approach by adopting a similar process with modifications to enhance vascularization and skin maturation. The study will investigate individuals with STSGs, comparing their outcomes to 3D-bioprinted skin grafts through in vitro and clinical analyses. Induced pluripotent embryonic stem cells (iPSCs), fibroblasts, melanocytes, and keratinocytes will be cultured and mixed with biomaterials to form a bioink. Imaging of wounds will be processed using computer-aided design (CAD) software to create precise 3D models for printing. Histological analysis of printed tissue will include Hematoxylin and Eosin (H&E) and immunohistochemistry staining to visualize structural integrity, vascularization, and biomarkers (CK14, CK10, and filaggrin) indicative of skin maturation. Dermal-epidermal junction formation, vascular network development, and extracellular matrix composition will be monitored. We expect superior vascularization and improved epidermal differentiation with the biomarkers in the 3D model. The successful application of bioprinted skin grafts has the potential to transform wound care by addressing current limitations and patient outcomes.

In Utero Limb Regeneration Using 3D-Bioprinted Scaffolds and Genetically Modified Stem Cells

Jasmine Gill, BSc Student [1], Presley Vinet-Cecha, BSc Student [2]
[1] College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Childhood limb malformations are primarily congenital, resulting from abnormal development of a portion or an entire limb during pregnancy. In-utero therapies aim to address fetal issues early on, using the restorative ability of developing tissues to promote limb regeneration, ultimately improving health outcomes prior to birth. Current treatments for congenital limb anomalies focus on postnatal management, including surgical correction and the use of prosthetic devices. However, these approaches may not fully meet the child’s developmental needs and often delay early functional progress. In contrast, the fetal environment offers a more conducive setting for treatment, supporting regenerative processes and reducing immune

DOI Link: https://doi.org/10.26685/urncst.796

responses. This study proposes using 3D bioprinting to create a scaffold populated with genetically modified placenta-derived stromal cells to facilitate in-utero limb regeneration within the fetus. Placenta-derived stromal cells will be reprogrammed into pluripotent cells through the delivery of transcription factors, such as OCT4, SOX2, KLF4, and MYC5, and non-integrative techniques, including RNA and episomal vectors. The genetic defects in induced pluripotent stem cells (iPSCs) will be corrected using CRISPR/Cas96. These corrected iPSCs will then be differentiated into the specific cell types needed in the 3D bioink. 3D bioprinting will ensure the precise placement of cells into scaffolds, sustaining homogeneous cell distribution while supporting vascularization via endothelial cells to mimic blood vessel networks. To create scaffolds with both structural strength and bioactivity, combinations of materials such as tricalcium phosphate (TCP) and polycaprolactone (PCL) will be used, supporting effective cell development and integration with the natural tissues of the fetus. The 3D-printed scaffold will be introduced and inserted through fetoscopic surgery. Past research has shown that adult human fibroblasts can be reprogrammed to generate iPSCs. This study hopes to replicate their success, however further trials are needed to ensure viability.

Integrating Fungal and Bacterial Metabolism for Methylmercury Bioremediation: Investigating Laccase Gene Transfer from *Aspergillus flavus* to *Sphingomonas paucimobilis*

Olga Loktaev, BSc Student [1], Matteo Girardo, BSc Student [1], Melody Nikseresht, BSc Student [2]
[1] Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[2] Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Heavy metal accumulation in soils from increased anthropogenic activity is linked to high rates of biotoxicity in humans and wildlife, and decreased soil microbial diversity. Since heavy metals do not undergo biological degradation, they remain in the soil for millennia after contamination. The neurotoxin methylmercury (MeHg), in particular, is the only heavy metal that undergoes bioaccumulation in food chains, comprising most of the total mercury in top predators. Fungi can form mycorrhizal networks and penetrate deep into the soil to reach toxins inaccessible to bacteria. The efficiency of mycoremediation has been studied, with positive results. However, many bacteria abundant in mercury-contaminated soils lack the fungal genes that drive bioremediation. This study explores the transfer of laccase genes (lac1, lac2, and lac3) from the *Aspergillus flavus* (*A. flavus*) KRP1 strain to the *Sphingomonas paucimobilis* (*S. paucimobilis*) bacterium as a means of maximizing bacterial MeHg bioremediation in soils with reduced fungal diversity. *A. flavus* KRP1 spores will be grown in Potato Dextrose Broth and incubated at 27 ℃ for 5 days. Using qPCR, lac1, lac2, and lac3 will be amplified and transformed into *S. paucimobilis* through the heat shock method. *S. paucimobilis* will be cultured on a guaiacol substrate and incubated at 37℃ for 24hrs. Upon transformation, laccase-positive colonies will be isolated and incubated at 37 ℃ for 24hrs. To determine the efficacy of the transformed bacterium, *S. paucimobilis* will be added to 3 samples of 50 g soil containing 5 mL of 1 M MeHg: a control group with no inoculants, a sample inoculated with 4 Log *A. flavus*, and a sample inoculated with 4 Log transformed *S. paucimobilis*. We hypothesize that the transformed *S. paucimobilis* will effectively remediate the soil regardless of the presence of *A. flavus*, providing valuable insight into the advancement of enzymatic mercury bioremediation.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors' Contributions
MM: Head Author of Authorship Committee, drafted the SSGSA Regenerative Science competition case package, peer-reviewed the abstract submissions and ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.
SS: Co-President of the SSGSA, member of Authorship Committee, drafted the SSGSA Regenerative Science competition case package, peer-reviewed the abstract submissions and ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.
LC: Co-President of the SSGSA, member of Authorship Committee, drafted the SSGSA Regenerative Science competition case package, peer-reviewed the abstract submissions and ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.
SP: Drafted the SSGSA Regenerative Science competition case package, peer-reviewed the abstract submissions, ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.
RN: Drafted the SSGSA Regenerative Science competition case package, peer-reviewed the abstract submissions, ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.

RJ: Drafted the SSGSA Regenerative Science competition case package, peer-reviewed the abstract submissions, ensured that they adhered to correct formatting standards and gave final approval of the version to be published.
MA: Drafted the SSGSA Regenerative Science competition case package, peer-reviewed the abstract submissions, ensured that they adhered to correct formatting standard and gave final approval of the version to be published.
YA: Drafted the SSGSA Regenerative Science competition case package, peer-reviewed the abstract submissions, ensured that they adhered to correct formatting standards and gave final approval of the version to be published.

Acknowledgements
We would like to acknowledge the SSGSA STEM Sustainability Case Competition Organizational Committee for their hard work and contributions to ensure that this competition was a complete success. The members of this dedicated team include; Tithi Jain as the Case Competition Coordinator; Avery Bendell and Alisha Gandhi as the Case Competition Events Coordinators; Victoria Scallen and Rachel Hershoran as the Case Competition Outreach Coordinators; and Alice Nielissen as the Case Competition Ambassador Coordinator. Additionally, we would like to thank the professors who judged both rounds of the competition, the graduate students who dedicated their time as mentors throughout the competition, the ambassadors who helped advertise and run the annual Poster Conference, and the 50+ University of Guelph undergraduate students who competed in our fourth annual competition. Without all of those mentioned, this competition would not be made possible. We look forward to building on this year's success in the upcoming years of the SSGSA STEM Sustainability Case Competition.

Funding
We would like to thank BeMo Academic Consulting, and the University of Guelph's own Student Life Enhancement Fund, College of Biological Science Student Council, College of Engineering and Physical Sciences Student Council, Interhall Council and Central Student Association. This funding provided monetary support for the execution of the 2024/2025 SSGSA STEM Sustainability Case Competition, including but not limited to conference platform, advertising, and resource development.

Article Information
Managing Editor: Jeremy Y. Ng
Article Dates: Received Jan 31 25; Published Feb 11 25

Citation
Please cite this article as follows:
Maiuri M, Sareen S, Carscadden L, Pooni S, Nishizeki R, Jafri R, Afridh M, Asaad Y. 2024-2025 SSGSA STEM Sustainability Case Competition: Regenerative Science. URNCST Journal. 2025 Feb 11: 9(2). https://urncst.com/index.php/urncst/article/view/796
DOI Link: https://doi.org/10.26685/urncst.796

Copyright
© Massimo Maiuri, Sanya Sareen, Lauren Carscadden, Sukhjot Pooni, Rachel Nishizeki, Rameen Jafri, Mohammed Afridh, Youmna Asaad. (2025). Published first in the Undergraduate Research in Natural and Clinical Science and Technology (URNCST) Journal. This is an open access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in the Undergraduate Research in Natural and Clinical Science and Technology (URNCST) Journal, is properly cited. The complete bibliographic information, a link to the original publication on http://www.urncst.com, as well as this copyright and license information must be included.

Do you research in earnest? Submit your next undergraduate research article to the URNCST Journal!
| Open Access | Peer-Reviewed | Rapid Turnaround Time | International |
| Broad and Multidisciplinary | Indexed | Innovative | Social Media Promoted |
Pre-submission inquiries? Send us an email at info@urncst.com | Facebook, X and LinkedIn: @URNCST
Submit YOUR manuscript today at https://www.urncst.com!