**MODULE NAME: INTRODUCTION TO RESEARCH**

**GRANT PROPOSAL**

Table of Contents

[NON-TECHNICAL SUMMARY: 3](#_Toc134545579)

[BACKGROUND TO RESEACH 3](#_Toc134545580)

[Research aims and objectives: 6](#_Toc134545581)

[PROGRAMME AND METHODOLOGY: 7](#_Toc134545582)

[RESOURCES NEEDED: 8](#_Toc134545583)

[IMPACT OF RESEARCH: 9](#_Toc134545584)

[TIMESCALE 10](#_Toc134545585)

[GANTT CHART: 12](#_Toc134545586)

[REFERENCES 12](#_Toc134545587)

**UNDERSTANDING THE FATE OF MESENCHYMAL STROMAL CELLS FOLLOWING INTRAVENEOUS ADMINISTRATION IN MICE.**

# NON-TECHNICAL SUMMARY:

The main aim of this study is to determine what happens to mesenchymal stromal cells (MSCs) when they are administered intravenously into the lungs of mice. Using immunofluorescence as well as hematoxylin and eosin staining, researchers are able to observe MSCs and other cell types in lung tissue. These additional cell types include endothelial cells, macrophages, and neutrophils. The present research aims to learn more about mesenchymal stromal cells (MSCs) and their potential use in treating kidney injury in the future. This will be achieved by locating these cells and determining how they interact with MSCs. This research will assist in enhancing cell therapies and determining how MSCs treat kidney injury. This research could improve therapies based on MSCs.

# BACKGROUND TO RESEACH

Mesenchymal stromal cells, also known as MSCs, are a type of stem cell that may be found in a wide range of tissues throughout the body. Some of these tissues include bone marrow, adipose tissue, and umbilical cord tissue, amongst others. In this proposal we will study the MSCS derived from the umbilical cord (Cona, 2023). Because of this, numerous studies have been conducted to investigate the distinctive traits that they possess. Umbilical cord-derived mesenchymal stem cells (UC-MSCs) are increasing in application as a source of MSCs for use in both research and therapeutic settings.

KEY CHARACTERISTICS OF MSC’S:

* Stromal cells from adipose tissue, fat, muscle, and bone can all be derived from MSCs.
* MSCs have the ability to divide and multiply, allowing them to grow and spread their culture. (Nagamura-Inoue T, 2014)
* The immune system is suppressed and inflammation is reduced by MSCs. The molecules they secrete have a positive effect on immunological cells such T cells, B cells, and natural killer cells, promoting tissue repair and regeneration. (Cona, 2023)
* MSCs are used in regenerative therapy because they travel to places that have been damaged or are inflamed.
* Tissue repair and immune response are aided by the secretome of mesenchymal stem cells (MSCs), which contains cytokines, growth hormones, and extracellular matrix proteins.
* It's non-invasive and simple to get: Donated cord blood and umbilical cord tissue after birth provide an easy and painless way to get MSCs (Marmotti, et al., 2012; Nagamura-Inoue T, 2014; Cona, 2023).

When MSCs are caught in the lungs, they can produce substances that can migrate to the kidneys and improve kidney function. MSCs, for example, can produce cytokines and growth factors that stimulate tissue repair and reduce inflammation. These substances can also increase the proliferation of kidney cells, such as tubular epithelial cells, which can aid in renal function restoration

(Wang, et al., 2022).

These microscopic particles have the potential to go to remote areas of the kidney injury and exert their therapeutic effects there. The injected EVs can engraft onto several cell types, including proximal and distal tubular cells, endothelial cells, and macrophages, just as injected MSCs (Eirin, et al., 2017).In contrast to EVs, MSCs may be able to extend and prolong their in vivo efficacy by replacing injured cells. This is because MSCs predominantly channel their protective effects via paracrine processes, such as the production of EVs, growth factors, and cytokines (Eirin, et al., 2017).

The techniques of immunofluorescence and immunostaining with hematoxylin and eosin, which is a stain that is used to differentiate between various types of tissues, are included in this study. Additionally, the results of this investigation will be discussed.

Mechanism of action of mesenchymal stromal cells

Paracrine and immuno-modulatory activities of MSCs have been receiving a lot of attention in recent years. It is becoming well accepted that mesenchymal stromal cells isolated from the umbilical cord (UC) have superior therapeutic potential to mesenchymal stromal cells isolated from other sources. In recent years, MSCs have received a lot of attention as a potential therapeutic for a variety of clinical applications, for the treatment of kidney injury. (Parekkadan & Milwid, 2010) (Saeedi, et al., 2019) (Payandeh, et al., 2019) (Norooznezhad, et al., 2022) (Ullah, et al., 2019). In addition, MSCs secrete soluble factors, Extracellular Vesicles (EVs) like exosomes, and micro vesicles (MVs), all of which can work in tandem to heal tissue and modify the tissue microenvironment via paracrine and autocrine mechanisms. (Joerger-Messerli, et al., 2018) (Eggenhofer, et al., 2012). Recent years have seen a surge of interest in the umbilical cord (UC), which is typically discarded as medical waste but may be collected without pain, effortlessly, and with minimal risk during delivery. MSCs have been reliably isolated in large numbers from umbilical cord (UC) tissues using the current isolation technique. In addition to a quicker doubling time (30-36 h), UC-MSCs exhibit a higher self-renewal capacity (300 cell divisions) (Koike, et al., 2014). UC-MSCs have been found to enhance blood vessel development and treat neuron, bone, heart, and kidney damage in clinical trials (Lv, et al., 2013) (Özmert & Arslan, 2020). The high proliferation and adaptability of UC-MSCs are the key advantages of these cells over MSCs from other sources (Pipino, et al., 2013) (Eggenhofer, et al., 2012).

Unknown facts about the fate of mesenchymal stromal cells administered intravenously in the lungs. Why do Mesenchymal stromal cells die in the lungs?

After being injected into the body, MSC quickly leave the lungs and move to other organs like the spleen and liver (Devine, et al., 2003) (Kraitchman, et al., 2005), with a strong preference for injury sites (Chapel, et al., 2003) (Assis, et al., 2010) (Jackson, et al., 2010) (Jin, et al., 2012). The results should be interpreted cautiously, though. The detection of living MSC is left out of studies that analyze MSC distribution following intravenous infusion and instead rely on PCR, immunofluorescence, or bioluminescence to detect MSC DNA, fluorescent label, or luciferase enzyme activity. The presence of label in either decomposing MSC or in macrophages that have consumed MSC is not out of the question (Eggenhofer, et al., 2012). Therefore, label detection does not shed light on where and how long MSCs have been present in the body. In several cases, researchers have used immuno-compromised recipient animals (Pereira, et al., 1995) (Liechty, et al., 2000) (Devine, et al., 2003) (Boulland, et al., 2012) to analyze MSC distribution. It is possible that the survival of MSC will be affected in human research by the fact that recipients will have a more robust immune system. Supporting the aims that MSC may not survive for long after administration is evidence showing that vast majority of MSC become apoptotic in response to administration (Liu, et al., 2012) (Eggenhofer, et al., 2012).

# Research aims and objectives:

Aims: this research is based on the following aims and experiments will be conducted on the basis of

* Endothelial cells and unfavorable circumstances in the lung microenvironment are hypothesized to be responsible for the death of MSCs in this study.
* Macrophage-derived suppressor cells (MSCs) are killed by the FAS ligand that is surface-expressed on macrophages.
* What role do mesenchymal stromal cells play in regulating the number of immune cells in the lungs?

Objectives:

The specific objectives are as follows:

* Learning the way around immunofluorescence staining protocols and microscopes.
* Improve staining methods for detecting injected MSCs in the lungs of mice.
* Enhancing methods of staining in order to recognize mouse lung host endothelial cells, neutrophils, and macrophages.
* Examine whether or whether or not the MSCs have any direct contact with the endothelium or immune cells of the host.
* To determine if MSCs have any effect on the lung immune cell populations.

# PROGRAMME AND METHODOLOGY:

The research plan and methodology comprise staining techniques like hematoxylin and eosin staining as well as immunofluorescence, with the hope that this will provide insight into the imaging and data visualization of MSCs in the lung tissues and their functions within the body.

Immunofluorescence (IF) is a biological technique that utilises a light microscope and fluorescently labeled antibodies to observe molecules. Successful immunofluorescence staining requires the use of an antibody capable of selectively detecting the antigen(s) within the target molecule (ONI, 2021). When studying individual cells or tissues, immunofluorescence is an effective approach used to conduct biological research. In order to see the target molecule under a microscope, this method involves antibodies that have been fluorescently labelled and then bind to it. Immunofluorescence is commonly used in the investigation of kidney injury in mouse models to detect and localize the cells involved in the injury process (Sonali & Dihua, 2017). Neutrophils are extremely short-lived cells that become senescent. That is, their population declines over time and endure apoptosis after only a few hours in circulation (Mauer, et al., 1960) (Basu, et al., 2002)During an infection or injury, tissue macrophages remove apoptotic neutrophils from the inflamed site, thereby resolving the inflammation (Cox, et al., 1995) (Peters, et al., 2005) to study more about this while conducting the experiment ,the cells can be identified using immunofluorescence. To determine the expression immune cells and endothelial cells expression we use the Fas-ligand, Fas-Ligand is a TNF (Tumour necrosis factor) family member that induces apoptosis by cross-linking its Fas receptor (Brunner, et al., 1995). FAS-ligand expression on immune and endothelial cells can be detected by immunofluorescence labelling. Tissue samples from the kidney or other organs can be removed and stained from mice models of kidney disease. Antibodies against FAS-ligand, as well as markers for immune cells (such as CD45) and endothelial cells (such as CD31), can be used to label these cells (Weng, et al., 2015). Once the samples have been labelled, they can be examined by fluorescence microscopy for FAS-ligand expression levels in various cell types. Co-staining techniques can also be used to assess the proximity of MSCs to immune cells and endothelial cells that express FAS-ligand (French, et al., 1996). In the context of kidney injury in mouse models, immunofluorescence labelling can be a useful technique for determining the expression levels of FAS-ligand in different cell types. The data presented here can aid in the comprehension of the probable role of FAS-ligand in the interaction between immune cells, endothelial cells, and MSCs in the damaged kidney (French, et al., 1996).

For microscopic analysis of fixed, processed, embedding, and sectioned tissues, hematoxylin and eosin (H&E) stain is the recognised standard technique. Either a human or an automated system can carry it out. Mouse tissues were manually H&E stained after being fixed, paraffin embedded, processed, and sectioned. In H&E-stained tissues, nucleic acids appear dark blue, while proteins stain a variety of colours from red to pink to orange (Cardiff, et al., 2014).

# RESOURCES NEEDED:

|  |  |  |  |
| --- | --- | --- | --- |
| Sr. no. | Resources | Rationale | Cost estimate |
| 1 | PECAM-1 (platelet endothelial cell adhesion molecule-1) or CD31 | Antibody to detect mouse endothelial cells | £ 305 |
| 2 | F4/80 | Antibody to detect mouse macrophages | £ 295 |
| 3 | Neutrophils elastase | Antibody to detect mouse neutrophils | £ 250 |
| 4 | FAS- ligand | To study the mechanisms of MSCS and to induce apoptosis in kidney cells | £ 325 |
| 5 | TST- monoclonal | Antibody to detect human specific nuclear antigen | N/A  (Already present in the lab) |
| 6 | Kidney patients |  | N/A |
| 7 |  | **Total cost estimated** | **£ 1,174** |

# IMPACT OF RESEARCH:

This research is thoroughly based on understanding the fate of MSCs following intravenous administration in mice has significant potential for future benefits. This research sheds light on how MSCs operate in the body and how can they be applied in the field of medical treatments to treat the human patients who suffer with kidney injury also there can be a vast development done to improve the safety measures and efficacy and further to improve the methods to carry forward this research to deliver the MSCs to the target organs. Furthermore, this research has the ability to share new and improved techniques to track the stromal cells in vivo and to gain knowledge about imaging due to various staining procedures this will help widely in the phase of clinical trials and in the MSC based therapies in the patients.

# TIMESCALE

The proposed investigation will take place over the course of three months.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Task no. | Task of description | Start date month | End date month | Milestone (M) | Deliverables (D) |
| 1 | Become competent in the application of immunofluorescence staining techniques and microscopy. | 0.0 | 0.5 | M1.1 investigating into the previous research | D1.1 Developed a staining and microscopy protocol for immunofluorescence |
| 2 | Optimise staining protocols to identify administered MSCs in the mouse lung | 0.5 | 1.0 | M2.1 Samples of lung tissue were successfully stained to see MSCs. | D2.1 Staining Procedure for optimal outcomes |
| 3 | Optimise staining protocols to identify host endothelial cells, neutrophils and macrophages in the mouse lung | 1.0 | 1.5 | M3.1 Lung tissue samples were effectively stained, allowing host cells to be seen. | D3.1 Staining Procedure for optimal outcomes |
| 4 | Investigate if there is any direct interaction between the MSCs and host endothelial or immune cells | 1.5 | 2.0 | M4.1 Use immunofluorescence microscopy to locate MSCs in close contact to host cells. | D4.1 Insights into the interactions between MSCs and host cells |
| 5 | Investigate if the immune cell populations in the lung are affected by the presence of the MSCs. | 2.0 | 3.0 | M5.1 Investigated successfully and experiment completed | D5.1 Immune cell population data analysis for lung tissue samples and report submitted |

Table: (Tabular representation of the experiment and the proposed investigation)

# GANTT CHART:

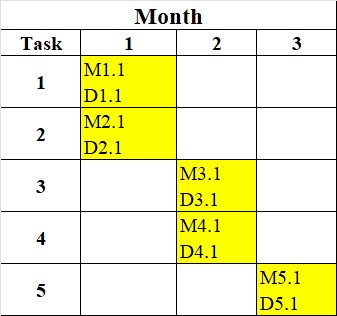


Fig:(Gantt chart representing the investigation and timescale, milestone and deliverables for the course of three months)

# REFERENCES

Assis, A. C. et al., 2010. Time-dependent migration of systemically delivered bone marrow mesenchymal stem cells to the infarcted heart.. *Cell Transplant.,* Volume 19, p. 219–230.

Basu, S., Hodgson, G., Katz, M. & Dunn, A., 2002. Evaluation of role of G-CSF in the production, survival, and release of neutrophils from bone marrow into circulation.. *Blood.,* 100(3), p. 854–861.

Boulland, J. L. et al., 2012. Evaluation of intracellular labeling with micron-sized particles of iron oxide (MPIOs) as a general tool for in vitro and in vivo tracking of human stem and progenitor cells.. *Cell Transplant.*

Brunner, T. et al., 1995. Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas.. *Nature ,* Volume 373, p. 441–444.

Cardiff, R., Miller, C. & R.J., M., 2014. Manual hematoxylin and eosin staining of mouse tissue sections.. *Cold Spring Harb Protoc.,* 2014(6), pp. 655-8.

Chapel, A. et al., 2003. Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. *J. Gene Med.,* Volume 5, p. 1028–1038.

Cona, L. A., 2023. *Types of Mesenchymal Stem Cells (MSCs).* [Online]   
Available at: https://www.dvcstem.com/post/mscs  
[Accessed 03 5 2023].

Cona, L. A., 2023. *What are mesenchymal stem cells (MSCs)?.* [Online]   
Available at: https://www.dvcstem.com/post/what-are-mesenchymal-stem-cells#:~:text=Mesenchymal%20stem%20cells%20(MSCs)%20are%20adult%20stem%20cells%20isolated%20from,)%2C%20and%20umbilical%20cord%20tissue  
[Accessed 03 05 2023].

Cox, G., Crossley, J. & Xing, Z., 1995. Macrophage engulfment of apoptotic neutrophils contributes to the resolution of acute pulmonary inflammation in vivo.. *Am J Respir Cell Mol Biol.,* 12(2), pp. 232-237.

Devine, S. M. et al., 2003. Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates.. *Blood ,* Volume 101, p. 2999–3001..

Eggenhofer, E. et al., 2012. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. *Front Immunol,* 26(3), p. 297.

Eirin, A. et al., 2017. Mesenchymal stem cell-derived extracellular vesicles attenuate kidney inflammation.. *Kidney Int,* Volume 92, p. 114–124..

French, L. E. et al., 1996. Fas and Fas ligand in embryos and adult mice: ligand expression in several immune-privileged tissues and coexpression in adult tissues characterized by apoptotic cell turnover. *J Cell Biol ,* 133(2), pp. 335-43.

Jackson, J. S. et al., 2010. Homing of stem cells to sites of inflammatory brain injury after intracerebral and intravenous administration: a longitudinal imaging study.. *Stem Cell Res. Ther,* Volume 1, p. 17.

Jin, S. Z. et al., 2012. Ex vivo-expanded bone marrow stem cells home to the liver and ameliorate functional recovery in a mouse model of acute hepatic injury.. *Hepatobiliary Pancreat. Dis. Int.,* Volume 11, p. 66–73.

Joerger-Messerli, M. S. et al., 2018. Extracellular vesicles derived from Wharton’s jelly mesenchymal stem cells prevent and resolve programmed cell death mediated by perinatal hypoxia-ischemia in neuronal cells.. *Cell Transpl.,* 27 (1), pp. 168-180.

Koike, C. et al., 2014. Characterization of amniotic stem cells.. *Cell. Reprogr.,* 16 (4), pp. 298-305.

Kraitchman, D. L. et al., 2005. Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction.. *Circulation ,* Volume 112, p. 1451–1461.

Liechty, K. W. et al., 2000. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep.. *Nat. Med.,* Volume 6, p. 1282–1286.

Liu, X. B. et al., 2012. Angiopoietin-1 preconditioning enhances survival and functional recovery of mesenchymal stem cell transplantation.. *J. Zhejiang Univ. Sci. B,* Volume 13, p. 616–623.

Lv, Y.-T.et al., 2013. Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism.. *J. Transl. Med.,* 11 (1), pp. 1-10.

Marmotti, A. et al., 2012. Minced umbilical cord fragments as a source of cells for orthopaedic tissue engineering: an in vitro study.. *Stem Cells Int.,* Volume 2012, p. 326813.

Mauer, A. et al., 1960. Leukokinetic studies ii. A method for labeling granulocytes in vitro with radioactive diisopropylfluorophosphate (Dfp). *J Clin Invest.,* 39(9), p. 1481–1486.

Miyahara, Y., Nagaya, N., Kataoka, M. & al., e., 2006. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction.. *Nat Med.,* Volume 12, pp. 459-465.

Nagamura-Inoue T, H. H., 2014. Umbilical cord-derived mesenchymal stem cells: Their advantages and potential clinical utility.. *World J Stem Cells,* 6(2), pp. 195-202.

Norooznezhad, A. H. et al., 2022. Human placental mesenchymal stromal cell‐derived exosome‐enriched extracellular vesicles for chronic cutaneous graft‐versus‐host disease: A case report.. *J. Cell. Mol. Med. ,* 26 (2), pp. 588-592.

ONI, 2021. *What is immunofluorescence?.* [Online]   
Available at: https://oni.bio/nanoimager/super-resolution-microscopy/immunofluorescence/  
[Accessed 04 05 2023].

Özmert, E. & Arslan, U., 2020. Management of retinitis pigmentosa by wharton’s jelly-derived mesenchymal stem cells: Prospective analysis of 1-year results.. *Stem Cell Res. Ther. ,* 11 (1), pp. 1-17.

Parekkadan, B. & Milwid, J. M., 2010. Mesenchymal stem cells as therapeutics. *Annu. Rev. Biomed. Eng.,* Volume 12, p. 87–117.

Payandeh, M. et al., 2019. Human placenta-derived mesenchymal stromal cells transfusion in a critically ill infant diagnosed with coronavirus disease 2019 (COVID-19): A case report.. *Transfus. Apher. Sci.,,* Volume 103454.

Pereira, R. F. et al., 1995. Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice.. *Proc. Natl. Acad. Sci. U.S.A.,* Volume 92, p. 4857–4861.

Peters, T., Sindrilaru, A., Hinz, B. & al., e., 2005. Wound-healing defect of CD18(−/−) mice due to a decrease in TGF-beta1 and myofibroblast differentiation.. *EMBO J.,* 24(19), p. 3400–3410.

Pipino, C. et al., 2013. Placenta as a reservoir of stem cells: An underutilized resource?. *Br. Med. Bull.,* Volume 105, pp. 43-68.

Saeedi, P., Halabian, R. & Fooladi, A. A. I., 2019. A revealing review of mesenchymal stem cells therapy, clinical perspectives and Modification strategies.. *Stem Cell Investig,* Volume 6, p. 34.

Sonali, J. & Dihua, Y., 2017. Immunofluorescence. *Basic Science Methods for Clinical Researchers,* pp. 135-150.

Ullah, M., Liu, D. D. & Thakor, A. S. J. I., 2019. Mesenchymal stromal cell homing: Mechanisms and strategies for improvement. *iScience,* Volume 15, p. 421–438.

Wang, J. et al., 2022. Mesenchymal stem cells: A new therapeutic tool for chronic kidney disease.. *Front Cell Dev Biol.,* 4(10), p. 910592.

Weng, S.-C.et al., 2015. Expression of decoy receptor 3 in kidneys is associated with allograft survival after kidney transplant rejection. *Scientific Reports,* Volume 5, p. 12769.