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LAY SUMMARY:

This research investigates how intravenous MSCs affect mice's lungs. The Hematoxylin and Eosin (H&E) staining approach allows seeing distinct lung tissue components, providing crucial backdrop for the investigation. The use of this staining technique highlights lung, tissues, bronchioles, veins, arteries, and blood vessels.

MSCs might impact lung function, healing, and immune system response. H&E labelling and IB4 lectin helped discover cell interactions in the lung environment. Lung tissue cells may be located using various stains such as the H&E. This research seeks to understand MSC behaviour and location to treat kidney disease.

MSCs are unique cells that influence our bodies' reactions. MSCs have been shown to impact the immune system and help in healing in previous studies. To maximise their use, you must understand how they function. Ischemia-reperfusion injury (IRI's) effects on the lungs are also examined in this study. Researchers investigate lung tissue from injured and healthy persons to determine how IRI affects the pulmonary environment.

The aim is to know how MSCs interact with lung macrophages. They employ IB4 lectin stains to label cells and specific labelling techniques to view tissue architecture. This research learns a lot about human umbilical cord derived mesenchymal stromal cells (hUC- MSCs) in the lung by combining these strategies.

Thus, to optimise MSC healing, you must know where they travel after intravenous injection. Labelling and unique markers are used to study how MSCs interact with the lung environment. We can improve kidney disease by understanding how these cells interact.

ABSTRACT:

The investigation of the fate of intravenously injected human umbilical cord-derived mesenchymal stromal cells (hUC-MSCs) in mice has substantial significance for the field of regenerative medicine. Using histology and immunofluorescence methods, we investigated the dynamic interaction between mesenchymal stromal cells (MSCs) and the microenvironment of the lung. The use of Hematoxylin and Eosin (H&E) staining in conjunction with lectin IB4 enabled a comprehensive evaluation via microscopic examination, therefore elucidating the relationships between MSCs and lung macrophages. The use of H&E staining facilitated the identification of bronchioles, veins, arteries, and blood vessels, hence augmenting our comprehension of the dispersion of MSCs. The lectin known as IB4 shown a significant affinity towards lung alveolar macrophages (AMs) therefore selectively targeting this essential population during MSC treatment. The use of immunofluorescence facilitated the direct visualisation of interactions between MSCs and macrophages. The use of the three channels (red, DAPI, green) in the fluorescence microscope enabled the precise determination of cellular localisation inside lung tissue. Significant observations on the influence of MSCs in lung tissues, and immunomodulation were obtained. The analysis of results was facilitated using Image J software, which allowed for the quantification of interactions between MSCs and macrophages, as well as the assessment of changes in the composition of immune cells after the administration of MSCs. To summarise the results the hUC-MSC therapy may influence immune cell distribution and number of macrophages. The effects of hUC-MSC injection were compared to control and IRI therapy using Welch Two Sample t-tests. Although no statistically significant changes were detected between these groups, additional research is needed to understand hUC-MSCs' complex biological linkages and therapeutic potential. This work improves our understanding of how hUC-MSCs behave in the lung environment and affect the immune response. Although the first statistical findings did not demonstrate significant differences.



INTRODUCTION:

To enhance the therapeutic efficacy of mesenchymal stromal cell (MSC) treatment, it is essential to get a comprehensive knowledge of the fate of these cells after intravenous delivery. The objective of this work is to investigate the dynamics of mesenchymal stromal cells (MSCs) in mouse lung tissue and their interaction with the surrounding environment. The use of H&E staining enables the observation of anatomical features inside lung tissue, hence enhancing the understanding of the factors involved. The H&E staining technique is used to identify the presence of bronchioles, veins, arteries, and blood vessels (Fischer, et al., 2005) (Zhou & Moore, 2017).

MSCs have the capacity to have an impact on lung functionality, regeneration processes, and immunomodulatory responses. The use of H&E staining and IB4 lectin targeting in the examination of lung alveolar macrophages (AMs) has been shown to contribute to a deeper comprehension of their interactions (Song, et al., 2020). The use of immunofluorescence staining was applied, and subsequent examination was conducted using microscopy techniques. Distinct fluorescent dyes, namely Alexa Fluor 488 and Alexa Fluor 594, were employed to identify and co-localize diverse cellular populations. Additionally, DAPI staining allowed visualization of cell nuclei, facilitating precise. By incorporating this information into the understanding of MSC destiny and behaviour, valuable insights might be gained about their therapeutic potential for lung diseases. MSCs have immunomodulatory and regenerative characteristics, making them potential candidates for regenerative therapy (Song, et al., 2020). Gaining a comprehensive understanding of their ultimate outcome is crucial to maximise the effectiveness of their therapeutic efficacy. The primary objective of this research is to investigate the fate of MSCs after intravenous treatment in mice. Specifically, the study aims to examine the interaction between MSCs and macrophages in the context of IRI. The use of lectin staining has been employed to identify macrophages, whereby isolectin B4 (IB4) demonstrates an affinity for the glycoconjugates present on the surface of macrophages (Sorokin & Hoyt, 1992). The process of lectin staining is used to specifically identify glycan structures that are seen on macrophages inside the microenvironment of the lungs (Nagai-Okatani, et al., 2019).

The use of lectin staining, which relies on the recognition of certain glycan patterns, proves to be a valuable tool in the field of biological study. The present work uses lectin labelling as a method to visualise macrophages inside the microenvironment of mouse lung tissue (Nagai-Okatani, et al., 2019). Our objective is to understand the effects of administering MSCs on lung

function because of their immunomodulatory capabilities. Mesenchymal stromal cells (MSCs) have the potential to have an impact on the microenvironment of the lungs, hence regulating processes such as inflammation and tissue repair. The aim of this study is to examine the interactions between MSCs and the lung environment, with a specific focus on macrophages. To do this, IB4 lectin will be used as a tool for targeting macrophages and endothelial cells. Immunofluorescence and H&E staining techniques provide valuable insights into the examination of anatomical structures and the identification of macrophage populations. The use of this complete strategy contributes to the advancement of our comprehension regarding the dynamics of MSCs inside the environment of the lung. In brief, the optimisation of MSC treatment necessitates a comprehensive comprehension of their subsequent trajectory after intravenous delivery. The primary objective of this research is to investigate the interaction between a specific entity and the environment of the lung. To do this, several methods such as H&E staining for lung tissues and IB4 lectin staining for macrophages are used. Through the examination of the dynamics of MSCs in the context of lung biology, this study makes a valuable contribution to the advancement of regenerative treatments.

METHODOLOGY:

The research holds the following methods:

- H&E staining
- Immunofluorescence
- Microscopy
- Image analysis
- Statistical analysis

1. H&E staining

To identify key structures such as bronchioles, veins, and arteries, frozen (-20°C) sections of mice lung was stained with H&E stain, wherein haematoxylin stained the nuclei and eosin stained the cytoplasm. The stained sections were observed under a microscope. They were then compared with online published images for easier identification of the structures. This step was important for future analysis, as it provided a grasp of the structures and the ability to identify them during immunofluorescence.

2. Immunofluorescence

- Antibodies were used to stain for specific binding analysis.
- The slides prepared with sections of mouse lung tissue were washed with phosphate buffer solution (PBS) in the coplin jar.
- The slides were aspirated, and lung tissues were marked using a DAKO pen by gently drawing circles around the target areas.
- A solution of 90 µL PBS and 10 µL normal goat serum (NGS) was prepared, and the sections were covered with this blocking solution for incubation at room temperature.



- In four labelled Eppendorf tubes, 100 µL of PBS and NGS solution was mixed with 1 µL of primary antibodies (elastin, arginase, heparin sulphate, and laminin), and the tubes were centrifuged for 5 minutes.
- The lung section slides were aspirated, and 100 µL of the primary antibody solutions were pipetted onto the respective sections.
- A wet tissue was placed at the end of the staining slide tray to maintain humidity, and the tray was covered with a lid.
- The slides were refrigerated overnight.

Staining of secondary antibodies for human umbilical cord derived mesenchymal stromal cells hUC-MSCs and detection of Td-Tomato:

- The primary antibody slides were gently aspirated after removal from the refrigerator.
- The slides were placed in a coplin jar filled with PBS solution.
- The secondary antibody solution was prepared in Eppendorf tubes and then centrifuged for 5 minutes.
- The slides were removed from the coplin jar, allowed to dry on a tissue, and 100 µL of secondary antibody solution was added to slides 1, 2, and 4, while slide 3 received 100 µL of PBS.
- The slides were incubated at room temperature.
- A 100 µL solution of DAPI was prepared.
- The slides were washed with PBS and dried, after which 100 µL of the DAPI solution was added to each section and incubated for 5 minutes.
- The slides were mounted with DAKO mounting solution, covered with 22X50mm coverslips, and placed in the refrigerator.
- The slides were observed under a microscope.

Lectin staining for detection of macrophage clustering and their relationship with MSCs, and for counting macrophages in lung tissue with kidney injury:

- New slides of frozen lung sections were taken from the refrigerator
- The slides were washed in a coplin jar filled with PBS and subsequently dried.
- Sections were marked using a DAKO pen.
- A lectin solution was prepared by adding 4 µL of lectin GSL-1 B4 to 796 µL of PBS, resulting in an 800 µL solution.
- The lectin solution was added to marked sections on the slides and incubated overnight in the refrigerator.
- On the next day, the slides were removed from the refrigerator, the lectin solution was aspirated, and the slides were washed in a coplin jar filled with PBS.
- The slides were stained with DAPI solution and incubated for 5 minutes.
- The slides were observed under a microscope. The observation revealed that the lectin bound to macrophages and endothelial cells.

3. Microscopy:

An electron fluorescent microscope, model LEICA DM 2500, was used to observe each slide. The microscope had illumination techniques such as brightfield and darkfield. During the experiment, three channels (red, green, and DAPI) were utilized. This methodology facilitated the visualization and analysis of direct interactions between administered MSCs and macrophages within the lung microenvironment.

4. Image analysis:

- The software ImageJ was downloaded.
- The acquired images from the fluorescent microscope were launched in ImageJ.
- To merge images for observation, the path was IMAGE > MERGE CHANNELS > CHOOSE THE IMAGE COLOR FILTERS (e.g., red, green, or DAPI).
- It was ensured that each image was saved in the same format, such as JPEG.
- The cell counter tool was used via PLUG IN > ANALYZE > CELL COUNTER > INITIALIZE > CHOOSE TYPE (e.g., TYPE 1) > RESULTS.
- The results were saved in Excel format by going to FILE > SAVE AS > EXCEL.

- The results were saved in Excel for subsequent statistical analysis.

5. Statistical analysis:

- Downloaded the results from Image J in excel format.
- Developed bar charts based on cell count i.e., number of macrophages in each section of the mouse lung, its average and standard deviation.
- Used R studio for analysis performed the Welsch two sample test and obtained p-values.


RESULTS:

1. Identification of key anatomical features in the mouse lung.

To be able to determine how the hUC-MSCs interact with host lung cells, it was first necessary to be able to identify the key structures in the mouse lung. To this end, frozen lung sections were stained with H&E and observed using light microscopy. Using light microscopy, tissues such as bronchioles(B), arteries(A), veins(V) were observed. (Refer to Fig.1). Understanding the location and morphology of bronchioles, arteries, veins, and vessels is crucial for future analyses and interpretation of experimental data referring to lung physiology and pathology.

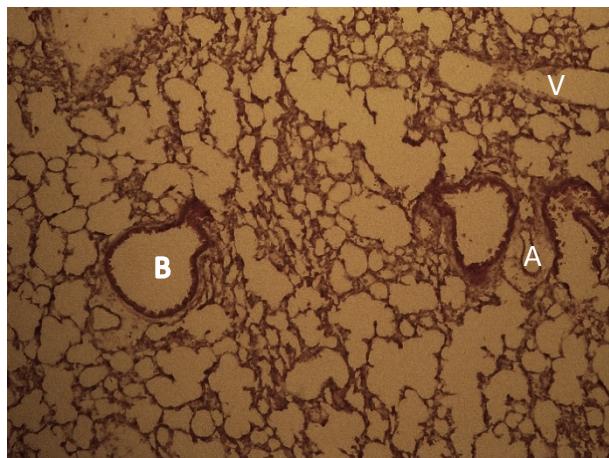


Figure 1. Identification of key anatomical features in the mouse lung.

Important structures of the lung tissue segment of the mouse are depicted and labelled in the figure. The location of bronchioles (B), arteries (A), veins (V), are indicated on the figure.

2. Establishing the degree of autofluorescence in the mouse lung and assessing the suitability of secondary antibodies.

To be able to determine the degree of autofluorescence in the mouse lung and evaluating autofluorescence and validating the suitability of secondary antibodies ensures the accuracy and dependability of experimental results. This experiment examined mouse lung tissue  autofluorescence. This was crucial for assessing autofluorescence's effect on experimental  accuracy and dependability. To separate delivered MSC signals from background  autofluorescence, secondary antibodies must be validated. This aims to improve lung  microenvironment data processing and interpretation between MSCs and host cells in the lung  microenvironment (Refer figure 2).



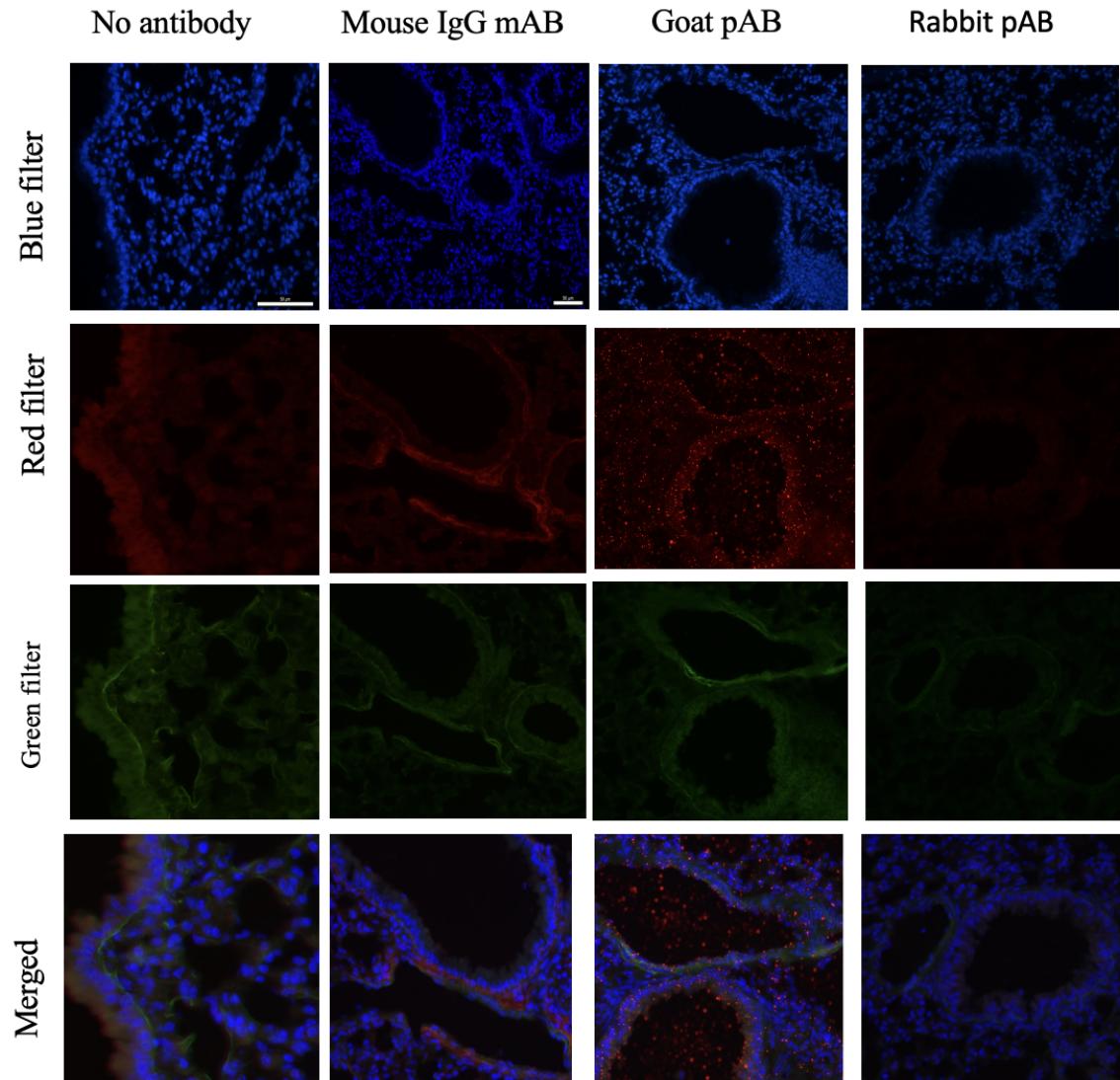


Figure 2 Establishing the degree of autofluorescence in the mouse lung and assessing the suitability of secondary antibodies.

The fluorescence images display the red, blue, and green channels. To visualize specific cellular populations, the secondary antibodies are labelled with distinct fluorophores, such as red (Fluor 594) and blue (DAPI) a nuclear stain. The merged image depicts the degree of autofluorescence as well as stained antibodies in the mouse lung. The following images were observed at low power of 20X and high power of 40X. Scale bars, 50

3. Identifying hUC-MSCs in the mouse lung.

To be able to determine the tracking of the cells and their migration after being injected intravenously and to understand if the MSCs reach the lung tissue. The detection of hUC-MSCs in the mouse lung has been employed for various research objectives. These objectives involve the tracking of their migration patterns following intrave*nous* injection, evaluating their localization at specific predetermined sites within the lung tissue, observing their timescale, and identifying potential variations in their population changes. Furthermore, the primary objective of this study was to explain the intricate interactions that occur between hUC-MSCs and different components of the lung, such as macrophages and the extracellular matrix. The objective of this observation was to make anticipated results regarding the therapeutic outcomes of hUC-MSCs in terms of their survival and potential effects the cells expressed Td-tomato reporter. The methodology employed for the identification of human umbilical c*onnective* derived mesenchymal stromal cells (hUC-MSCs) within the mouse lung is demonstrated in Figure 3. The findings of this study p*rovide* valuable insights into the spatial arrangement of hUC-MSCs within the pulmonary t*issue*. The investigation encompasses the spatial positioning of these entities in relation to specific target sites, their dynamic interactions with neighbouring cellular components, and the observed changes over time in cell populations. This can be observed by referring to Figure 3.

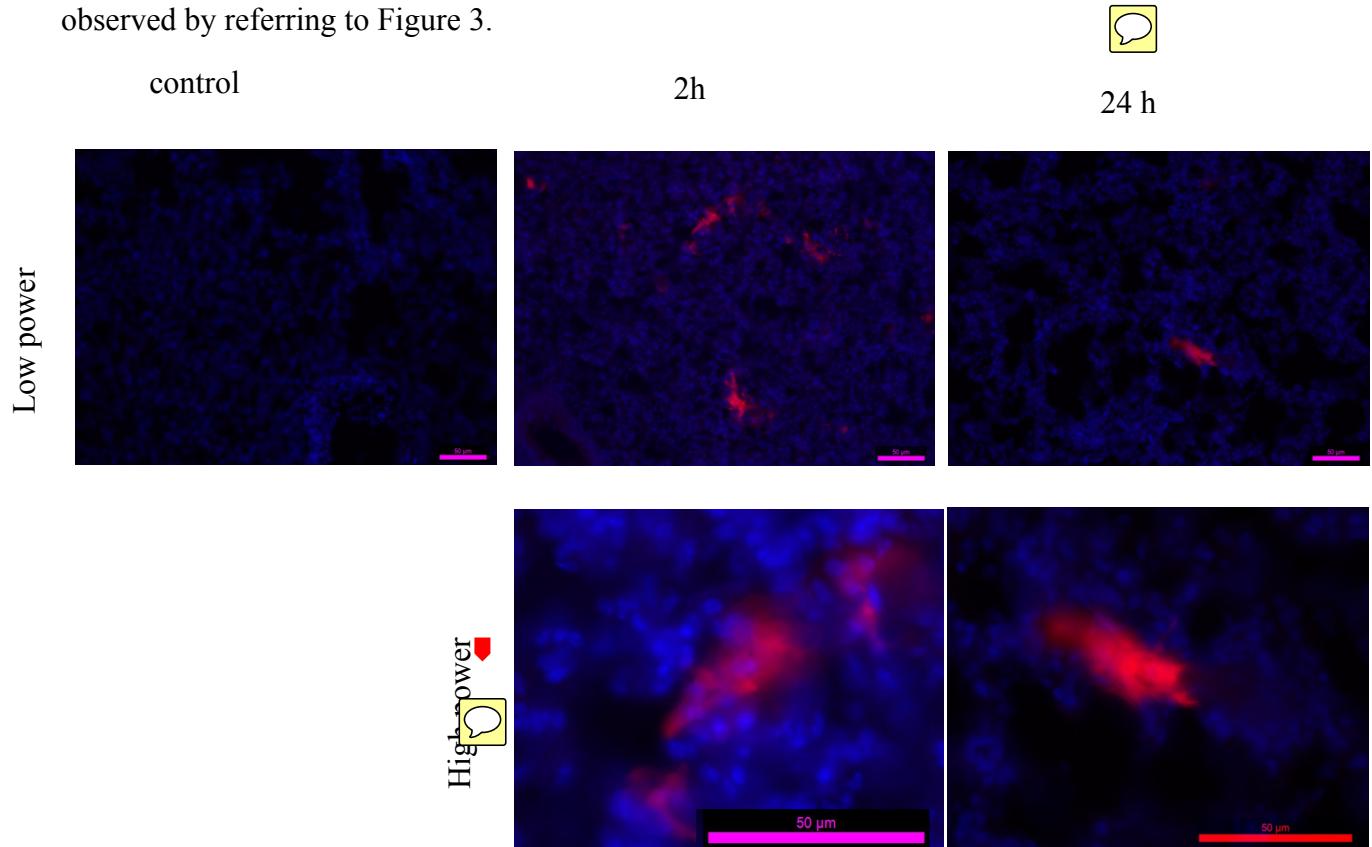


Figure 3 Detection of TdTOMATO-expressing hUC-MSCs in frozen sections of mouse lung.

Lungs from the following animals were analysed: (i) control animals where no cells were administered; (ii) animals where lungs were isolated 2h after TdTomato+ hUC-MSCs were administered IV; (iii) animals where lungs were isolated 24h after TdTomato+ hUC-MSCs were administered IV. Using an exposure time of 1.2s, no red fluorescence was present in the control lungs, but red fluorescence hUC-MSCs were present in samples at 2h and 24h post administration. The observation revealed that between 2 and 24 hours, a proportion of cells had died and were expressing TdTomato Analysis using lower power (20x) objective showed that there were more cells in the 2h samples. Analysis using high power (40x) objective showed that nuclei were present in the red fluorescent cells, indicating that they were likely to be viable. Scale bars, 50 μm.



4. Detection of macrophages clustering and showing their relationship with the MSCs

The objective of this step was to evaluate the immunomodulatory effects and consequences of macrophage clustering around hUC-MSCs with the purpose of identifying the related immunomodulatory features, investigating novel targeted therapeutics, and observing if the macrophages cluster around the MSCs or not.



The lung interaction between hUC-MSCs and macrophages was a major focus. IB4 lectin a fluorescent labelling marker was used to detect whether the macrophage cluster around the hUC-MSC and their interaction in lung tissue. Certain antibodies targeted macrophages, whereas others targeted hUC-MSCs. This technology allowed the co-localization of different cellular populations to study their spatial relationships. The fluorescent images obtained by this method provide light on whether the macrophage show clustering around hUC-MSCs. Figure (4) shows experiment outcomes.

The images in Figure (4) shows this interaction well. It co-localizes macrophages (green channel) and hUC-MSCs (red or another channel), allowing us to study their intricate interactions. These findings suggest that macrophages' proximity to hUC-MSCs may modulate immunity. Readers can easily grasp the interplay between cell types in Figure (4), which may have substantial consequences for immune responses and potential treatment methods. Thus, macrophage clustering and their interaction with hUC-MSCs provide a novel



perspective on these cells' immunomodulatory effects in the lung environment. This suggests that hUC-MSC therapeutic potential must include the cellular environment and interactions.

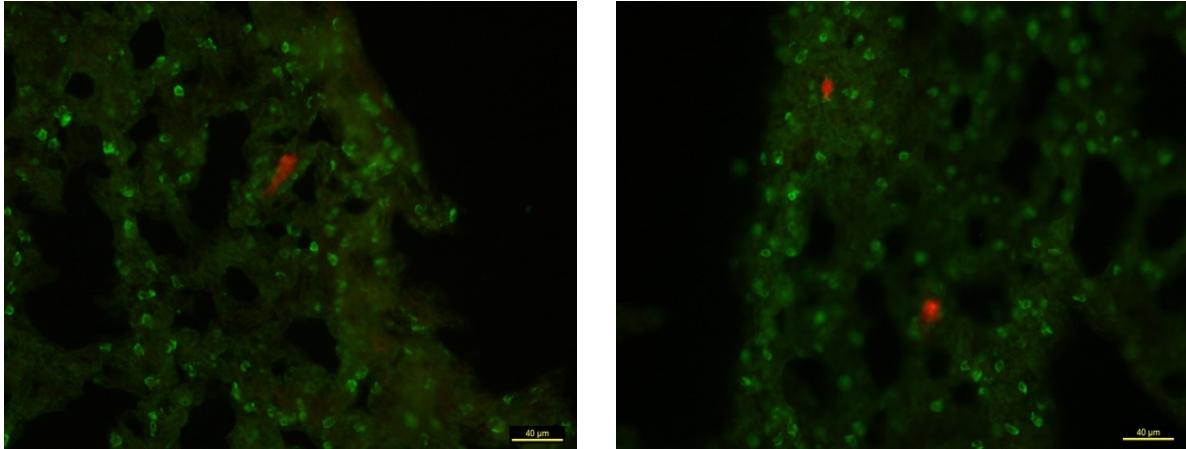


Figure 4 Detection of macrophages clustering and showing their relationship with the MSCs.

These images depict no clustering of macrophages. Red signals for hUC-MSCs and green dots for macrophages serve a role in immunofluorescence staining. This labelling method uses fluorophores with varied emission spectra to distinguish cell types or components. The hUC-MSCs are labelled with a fluorophore that exhibits red fluorescence when activated by specified wavelengths of light. This colour helps identify and monitor hUC-MSCs in tissue. Macrophages are labelled with a fluorophore that exhibits green fluorescence. This colour allows macrophage identification and localisation in the microenvironment. Macrophage presence and distribution may be detected and analysed using green fluorescence. In conclusion, red and green signals in the pictures distinguish and visualise hUC-MSCs and macrophages, allowing them to interact and move inside lung tissue. All observations were made using a 20X microscope objective. The scale bars are 40 μ m.

5. Detection of number of macrophages in the lung tissue having kidney injury and to observe the count of the macrophages.

To be able to determine the interactions between the host-cell. This phase evaluated the number of macrophages in lung tissue with kidney damage and examined how human cells vary it. This



study investigated host cell interactions and human cell impacts on local immune response. To measure macrophage count in lung tissue, IB4-lectin staining was used for detection. Macrophages may be identified by lectins, proteins that bind to carbohydrates. The staining helped identify and quantify tissue macrophages. The analysis findings are shown in Figure (5) The photos show kidney-injured lung tissue macrophage counts. Green signals indicate identified and measured macrophages. Welch Two Sample t-tests compared experimental group results. The analysis included three groups: IRI-with-cells vs. Control, IRI-with-cells vs. MSCs, and Control vs. MSCs. The t-test results reveal impact of intravenous MSCs from human umbilical cords in mice and MSC administration's effects.

In conclusion, the statistical analysis and quantification of macrophages in lung tissue with kidney damage help us understand host cell interactions and human cell administration's immunomodulatory effects.

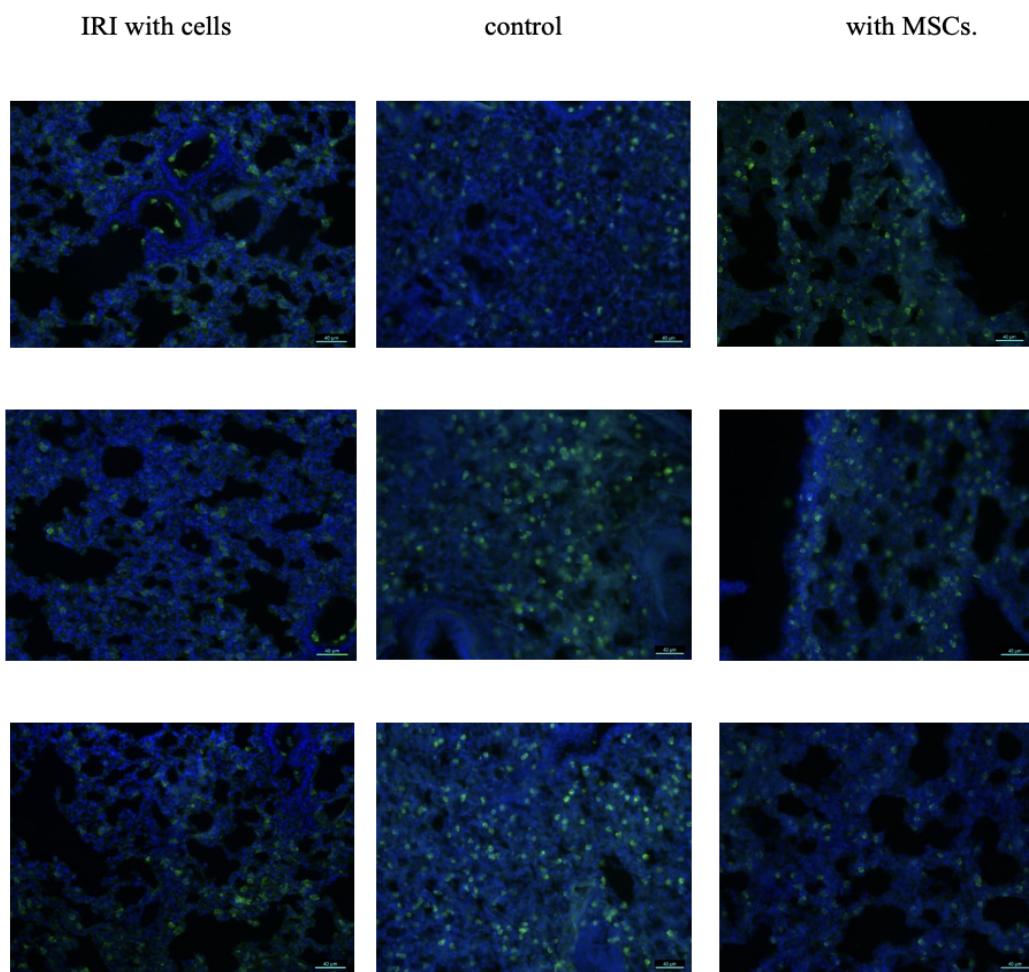


Figure 5 Detection of number of macrophages in the lung tissue having kidney injury and to observe the count of the macrophages.

The figure above depicts the distribution of macrophages (represented by green dots) stained with IB4 lectin within the DAPI-stained lung tissue microenvironment (shown in blue). Each sample group is represented by three different images in each group. Exposure periods for nuclei and monocytes are specified: nuclei were exposed for durations spanning from 510 to 140 ms, whereas monocytes were exposed for 140 ms. All images were captured at a 20X magnification, and scale bars=40 μ m, the image represents animals from 3 sample groups (i) animals exposed to ischemia-reperfusion injury (IRI) with cell administration, (ii) control animals without cell administration, and (iii) animals treated with Mesenchymal Stromal Cells (MSCs).

STATISTICAL ANALYSIS

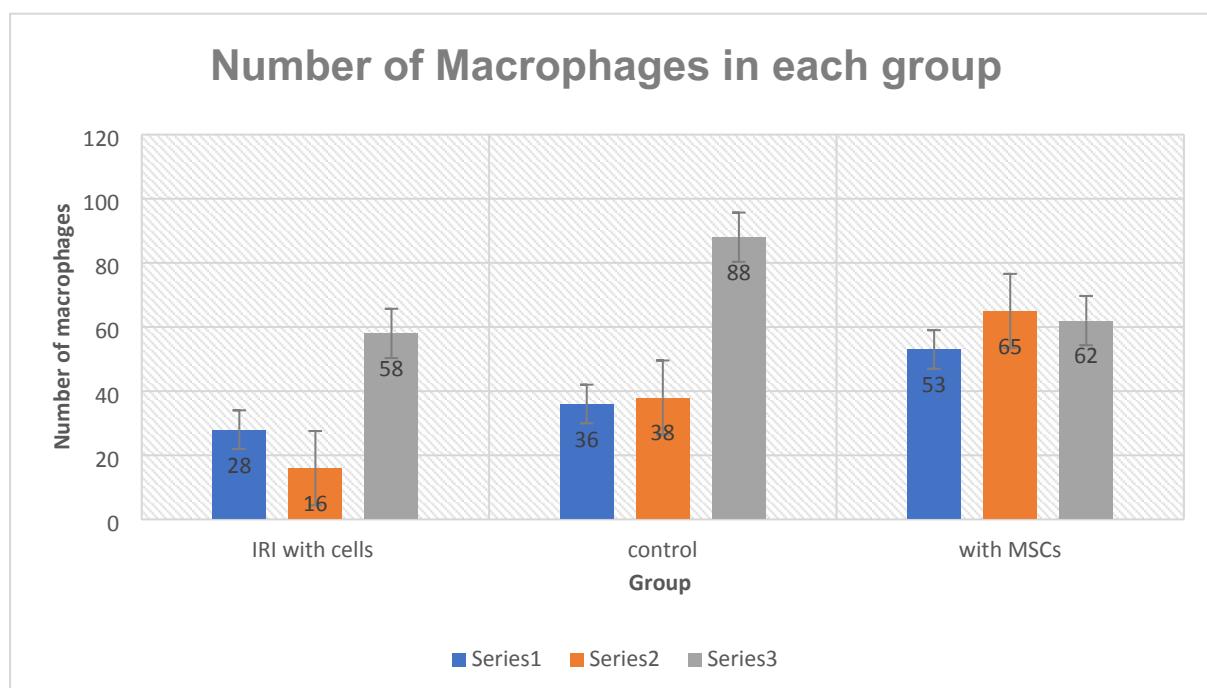


Figure 6 Bar chart detection of number of macrophages in the lung tissue having kidney injury and to observe the count of the macrophages.



This bar chart depicts the collective number of macrophages in each of the groups from all the images. Each sample group is represented by three distinct images pasted in (figure 5). On X-axis the groups are present and on Y-axis values of the number of macrophages present in every section. In figure 6, Series 1 indicates 1st section of mouse lung for each of the group which is seen in blue, series 2 indicates the 2nd section seen in orange and series 3 depicts 3rd section of the mouse lung for the three respective groups i.e (i) animals exposed to ischemia-reperfusion injury (IRI) with cell administration, (ii) control animals without cell administration, and (iii) animals treated with Mesenchymal Stromal Cells (MSCs) that is seen in grey. The error bars in the chart illustrates standard deviation in the data points within each group smaller. Smaller error bars indicate less variability.

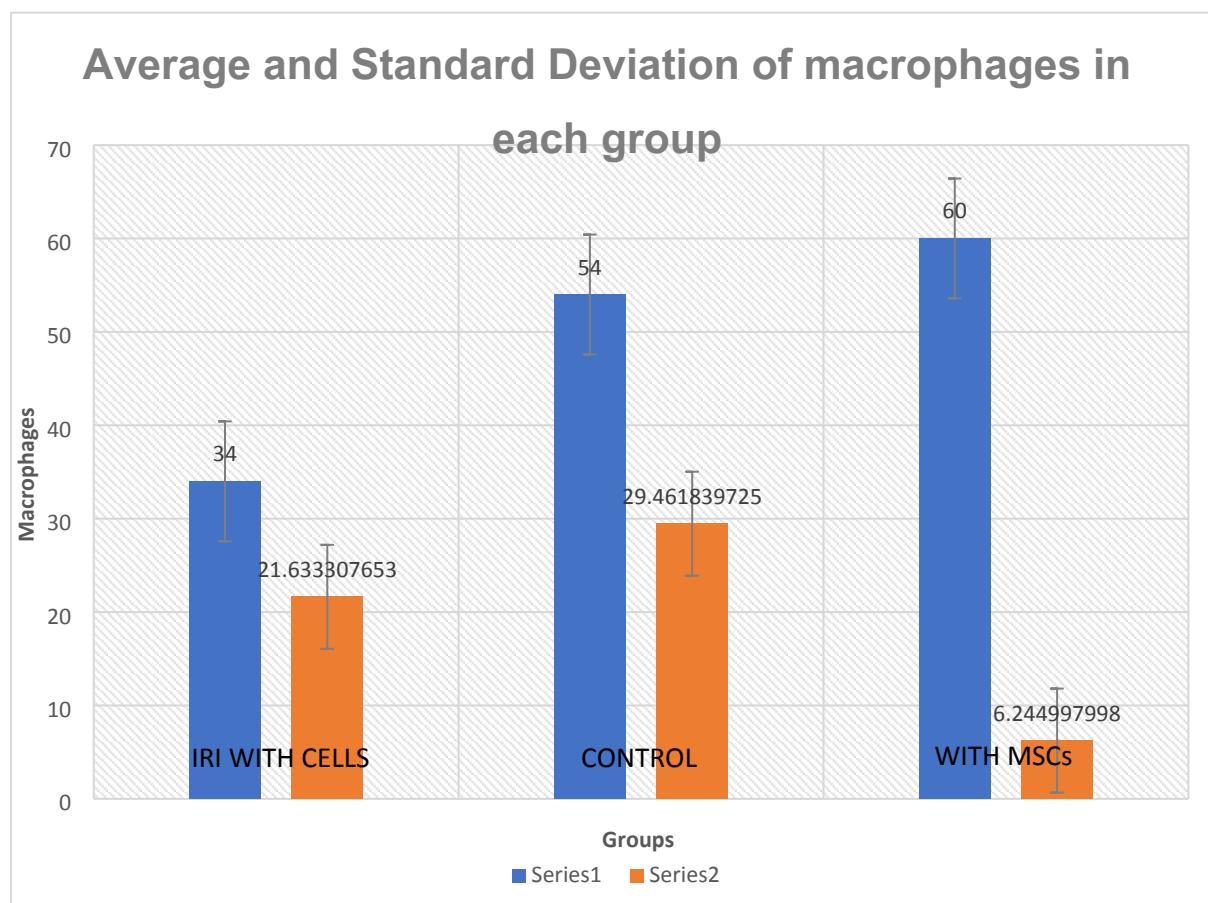


Figure 7: Average and standard deviation of macrophages in each group



The bar chart shown in Figure 7 illustrates the average and standard deviation values of each group, represented by the blue colour, as well as the standard deviation values of each group,

represented by the orange colour. The error bars in the graph represent the standard deviation of the data points  in each group. Broader error bars are indicative of more uncertainty or variability within the dataset, while narrower error bars imply more exact readings. However, the graphic displays a substantial number of broader bars. Here series1 (in blue) indicates average number of macrophages in each group and series 2 (in orange) indicates the standard deviation of macrophages of all the three images in each group (refer figure 5) for reference.

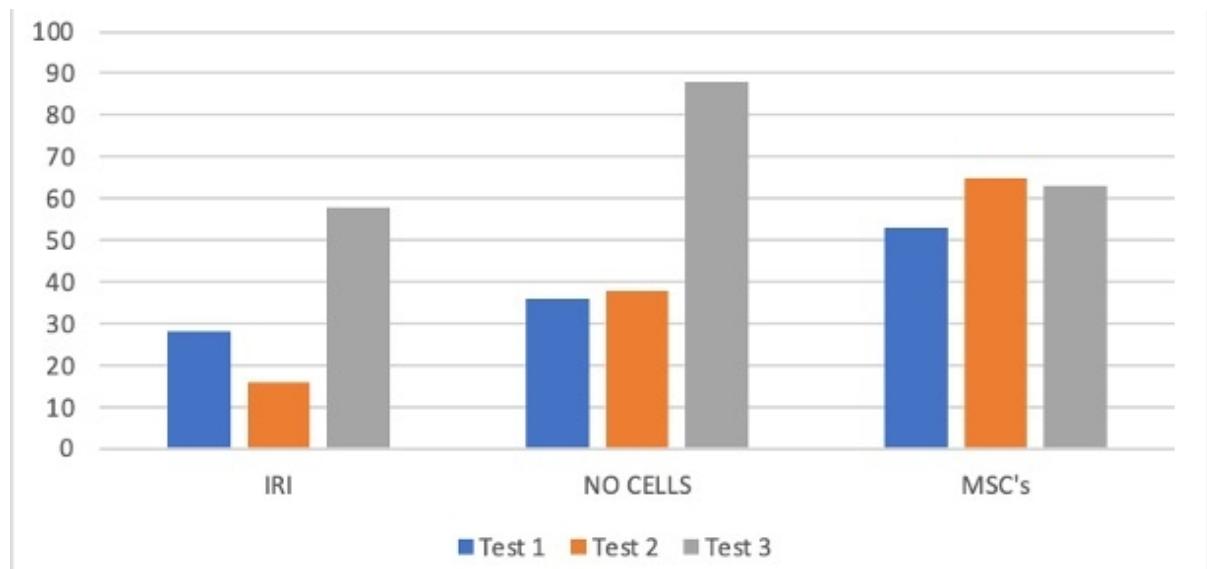


Figure 7 bar chart representing analysis from welch two sample tests.

This bar chart illustrates the number of macrophages present in every group and a single image and test conducted on them shown in figure (5). on X-axis we have the groups and tests conducted individually and on Y-axis we have the values that show the number of macrophages.

Analysis

The findings from the Welch Two Sample t-tests conducted on the three groups (IRI-with-cells vs. Control, IRI-with-cells vs. With MSC's, and Control vs. With MSC's) offer valuable insights into the outcomes of intravenous administration of mesenchymal stromal cells (MSCs) generated from human umbilical cord in mice.

Comparison of IRI-with-cells and Control:

The t-value obtained was -0.94774, whereas the corresponding p-value was 0.4014. The obtained p-value of 0.4014 exceeds the predetermined significance level of 0.05, suggesting that there is insufficient evidence to support the presence of a statistically significant disparity in means between the IRI-with-cells group and the Control group. These findings indicate that there were no statistically significant differences observed in the outcomes of mice following the intravenous injection of IRI-with-cells and Control treatments.

Comparison of IRI with cells and IRI with mesenchymal stromal cells (MSCs):

The t-value obtained from the analysis was -2.021, while the corresponding p-value was found to be 0.1613.

The obtained p-value of 0.1613 exceeds the predetermined significance level of 0.05, indicating that there is insufficient evidence to support the presence of a statistically significant difference in means between the IRI-with-cells group and the With MSC's group. This finding suggests that there is no substantial difference in the outcomes of intravenous administration of mesenchymal stromal cells (MSCs) generated from human umbilical cord in mice, compared to the treatment using ischemia-reperfusion injury (IRI) with cells.

The comparison between control and with MSC's.



The t-value obtained was -0.36377, whereas the corresponding p-value was 0.7482.

Interpretation: The obtained p-value of 0.7482 exceeds the predetermined significance level of 0.05, indicating that there is insufficient evidence to support the presence of a statistically significant difference in means between the Control group and the With MSC's group. This

suggests that the delivery of mesenchymal stromal cells (MSCs) generated from human umbilical cord did not yield substantial variations in comparison to the Control therapy.

Considering the t-test findings, there is insufficient evidence to indicate a significant divergence in the outcomes of MSCs obtained from the human umbilical cord after intravenous administration compared to the other treatments (IRI-with-cells and Control). These findings suggest that the administration of mesenchymal stromal cells (MSCs) did not result in significant alterations in the number of macrophages in results  when compared to the alternative treatments. Moreover, additional studies and experiments may be necessary to attain a more comprehensive comprehension of the impacts of MSC administration in mice.

DISCUSSION:

The objective of this research was to gain insight into the outcomes of intravenous injection of mesenchymal stromal cells (MSCs) in mice, with a particular focus on examining their interaction with lung tissue and macrophages. The study used a range of methodologies, such as Hematoxylin and Eosin (H&E) staining, immunofluorescence, microscopy, and image analysis, to investigate the behaviour of MSCs within the microenvironment of the lung. The use of H&E staining facilitated the observation of crucial anatomical components inside the lungs, including bronchioles, arteries, veins, and blood vessels, so establishing a framework for later investigations. The study used immunofluorescence methods to monitor the movement of hUC-MSCs and examine their interactions with various components of the lung. The findings of the study demonstrated the concurrent existence and spatial overlap of hUC-MSCs and macrophages, suggesting the possible occurrence of immunomodulatory impacts. The use of lectin helps to locate macrophages in the lung tissue, hence providing valuable insights into the importance of their interactions. The statistical analysis conducted in this research included the use of Welch Two Sample t-tests to evaluate the impact of MSC treatment on lung macrophage populations. The findings of the study revealed that there were no statistically significant disparities seen between the Ischemia-Reperfusion Injury (IRI) group treated with hUC-MSCs and the Control group. These results show that the administration of hUC-MSCs did not induce significant modifications in the count of the macrophages as compared to the initial state. Moreover, there were no notable disparities in the statistical outcomes seen between the groups receiving IRI-with-cells and With MSCs, suggesting that the administration of hUC-MSCs did not have a significant effect on the results when compared to IRI alone. Comparable findings were seen when comparing the Control group with the group administered with MSCs. In general, the research offers significant contributions to our understanding of the interaction between hUC-MSCs and lung tissue in a mouse experimental paradigm. Although there were no notable disparities found across the groups, these results enhance our comprehension of the behaviour of human umbilical cord-derived mesenchymal stromal cells (hUC-MSCs) inside the lung environment. The complete methodology used in this work, which encompasses histological staining, immunofluorescence, microscopy, and statistical analysis, contributes to a deeper understanding of mesenchymal stromal cell (MSC) destiny and their potential immunomodulatory effects. Nevertheless, it is crucial to acknowledge that more investigation may be necessary to comprehensively clarify the effects of hUC-MSC administration and their therapeutic capabilities in lung-related conditions. The

results of the study highlight the intricate nature of interactions involving mesenchymal stromal cells (MSCs) in the lung system, hence emphasising the need for ongoing research in the  fields of regenerative medicine and immunomodulation.

APPENDICES:

Figure 3.5 Detection of number of macrophages in the lung tissue having kidney injury and to observe  the count of the macrophages images with cell count results.

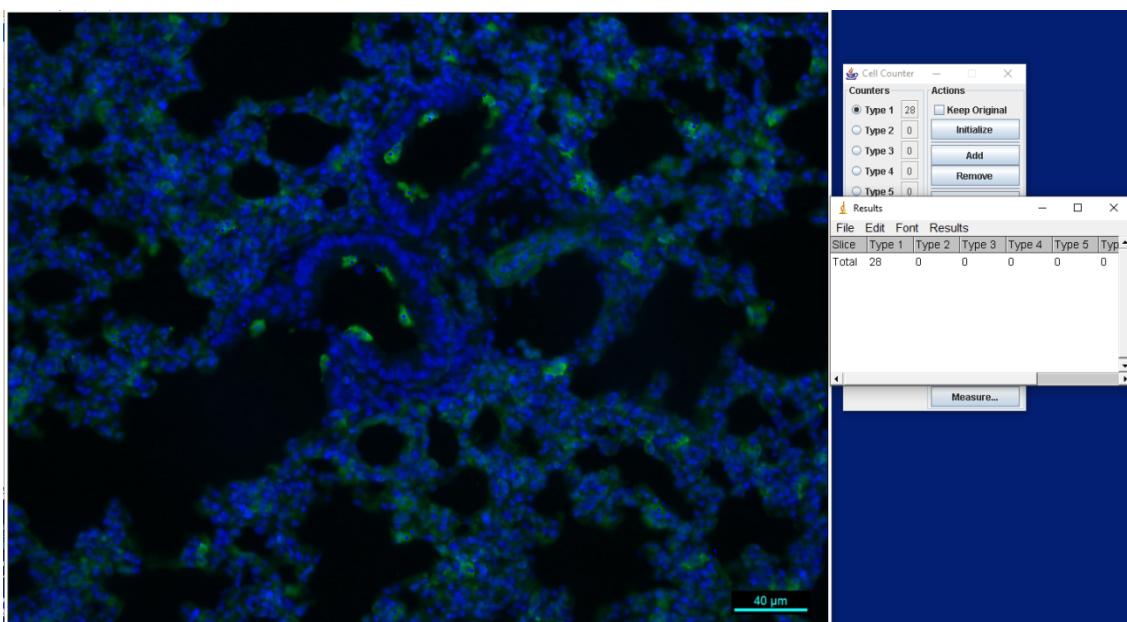


Fig1: cell count for IRI with cells image 1

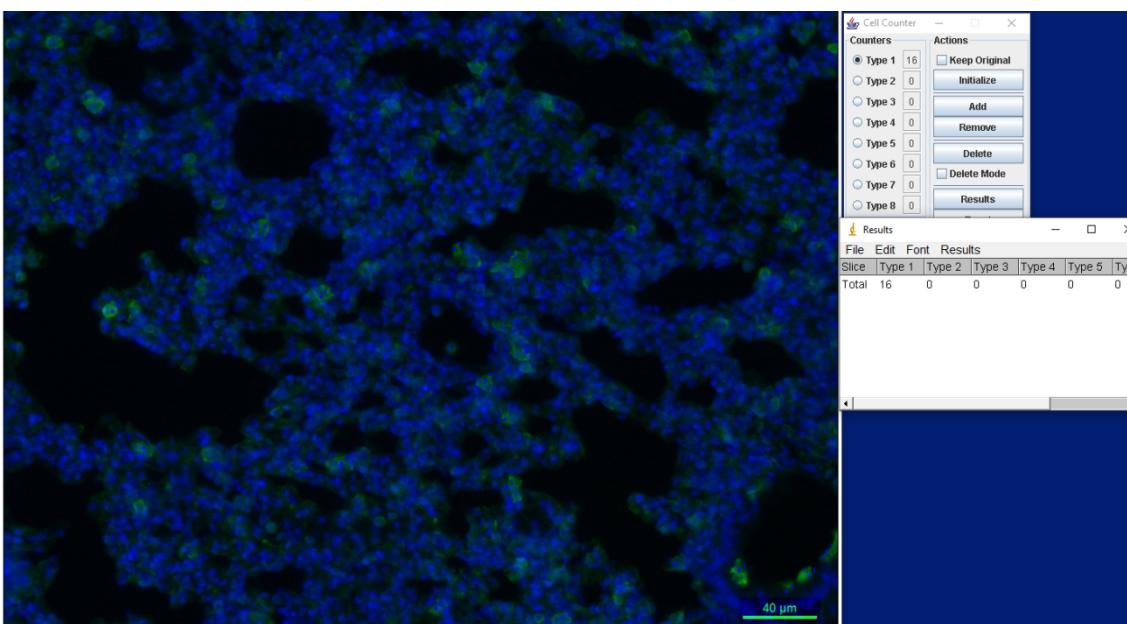


Fig2: cell count for IRI with cells image 2

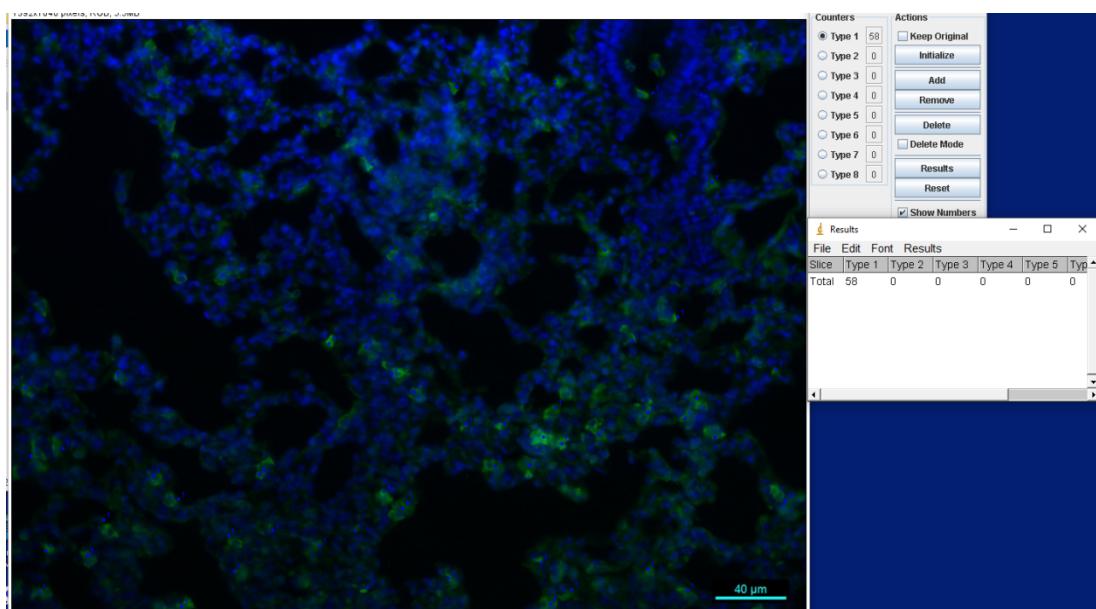


Fig3: cell count for IRI with cells image 3

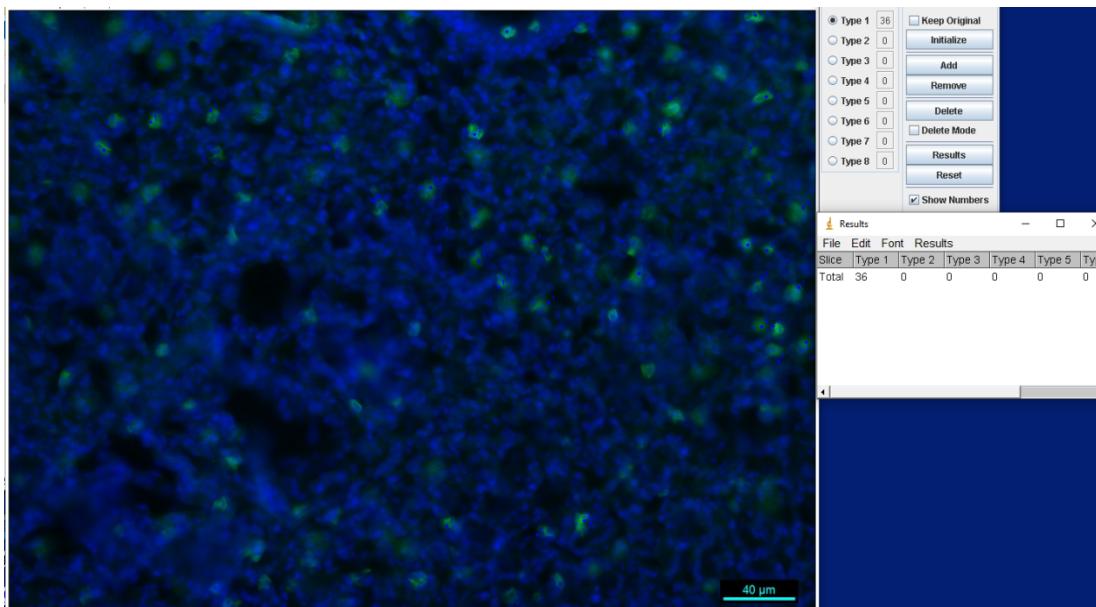


Fig4: cell count for control image 1

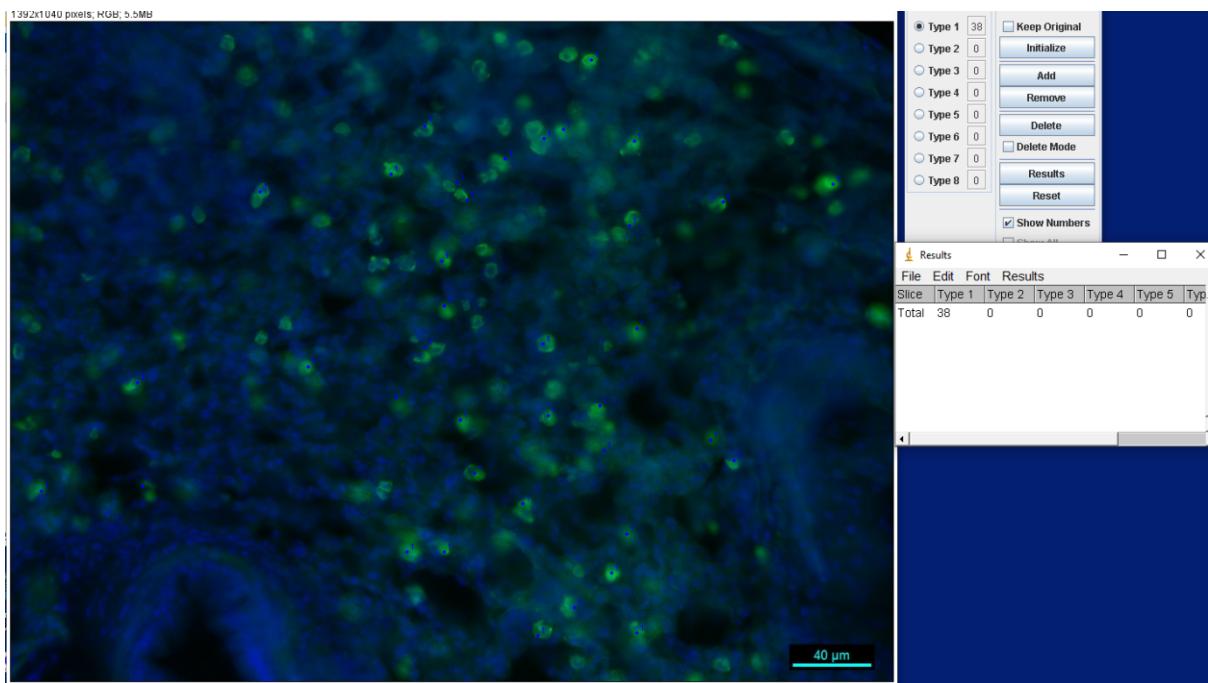


Fig5: cell count for control image 2

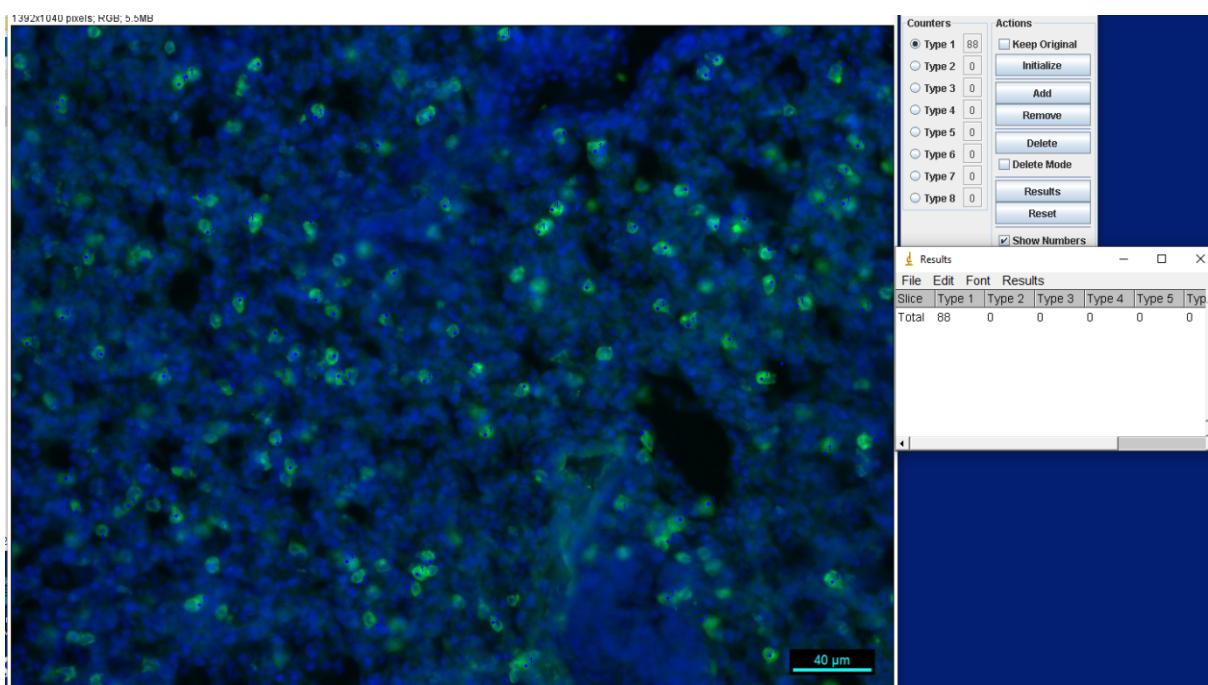


Fig6: cell count for control image 3

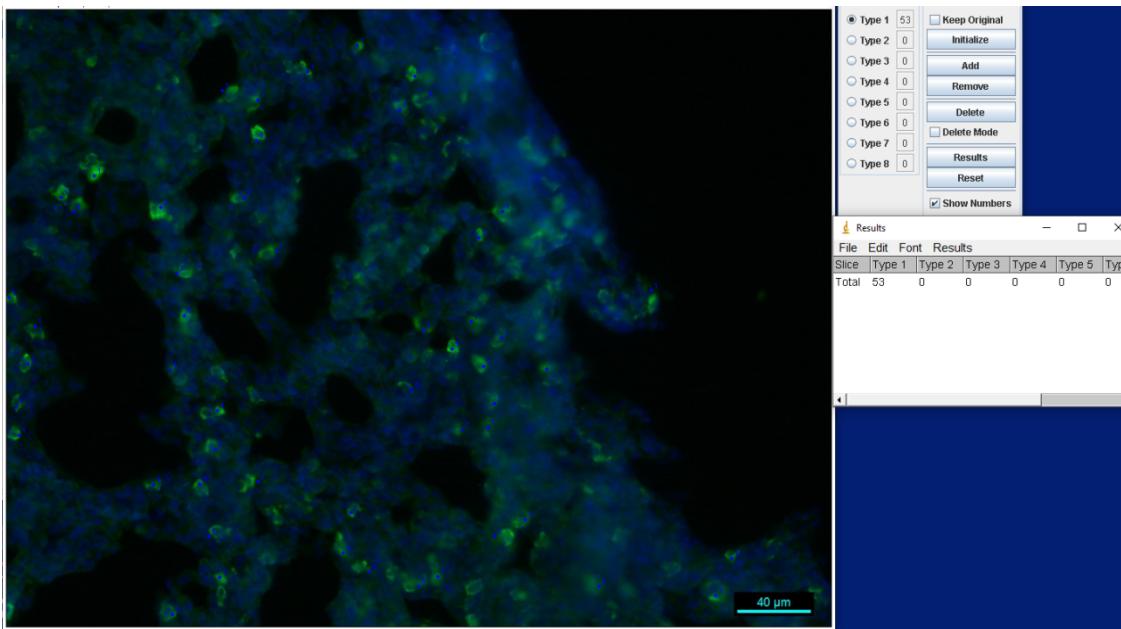


Fig7: cell count for with MSCs image 1.

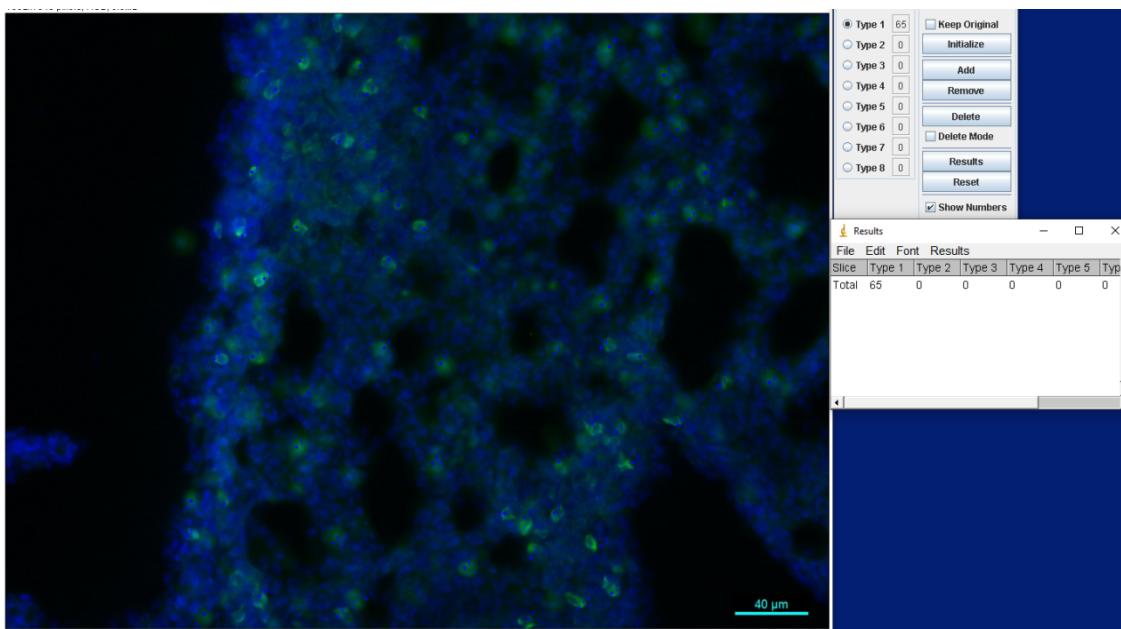


Fig8: cell count for with MSCs image 2.

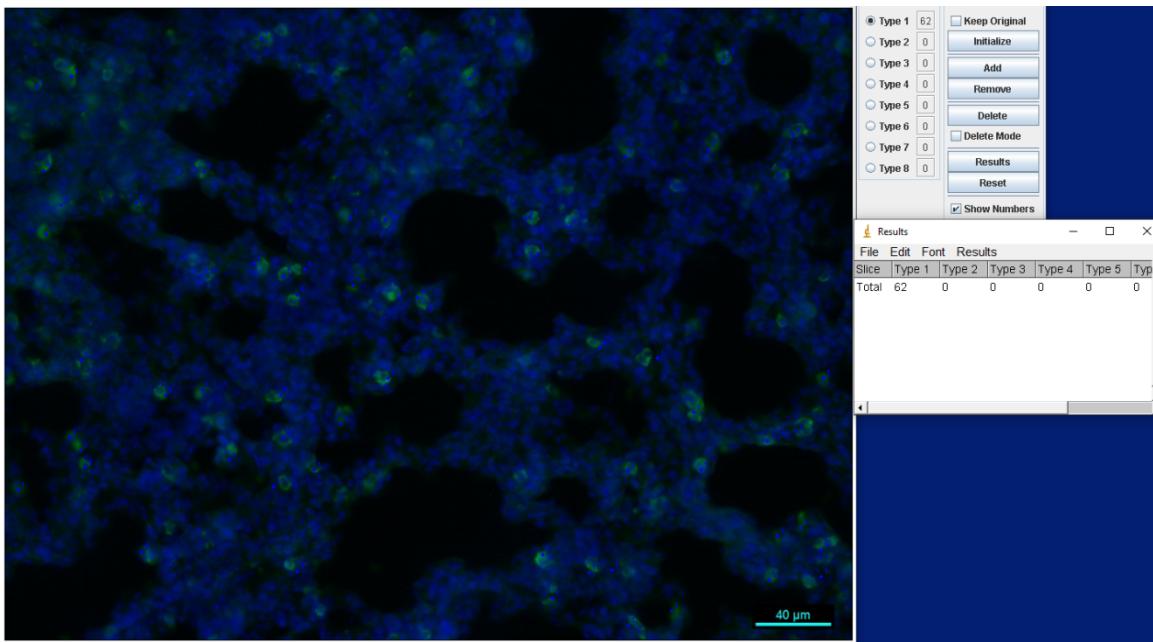


Fig9: cell count for with MSCs image 3.

Figure 6&7 : data from Image J in excel format

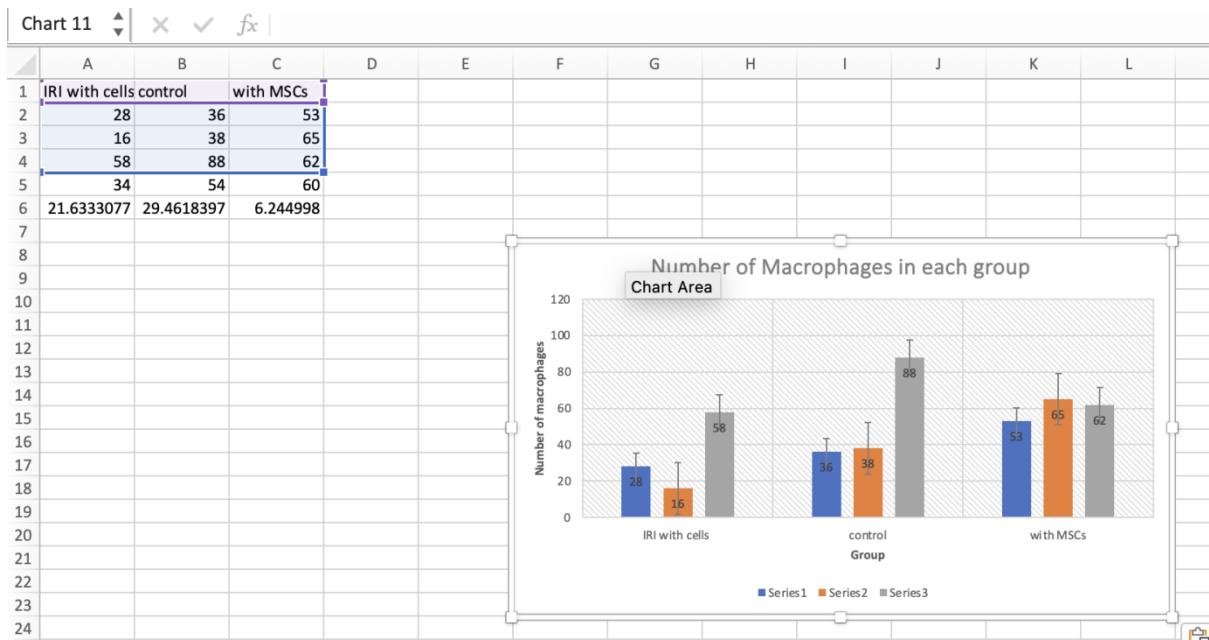


Fig10: bar chart that explains number of macrophages in each section of the mouse lung.

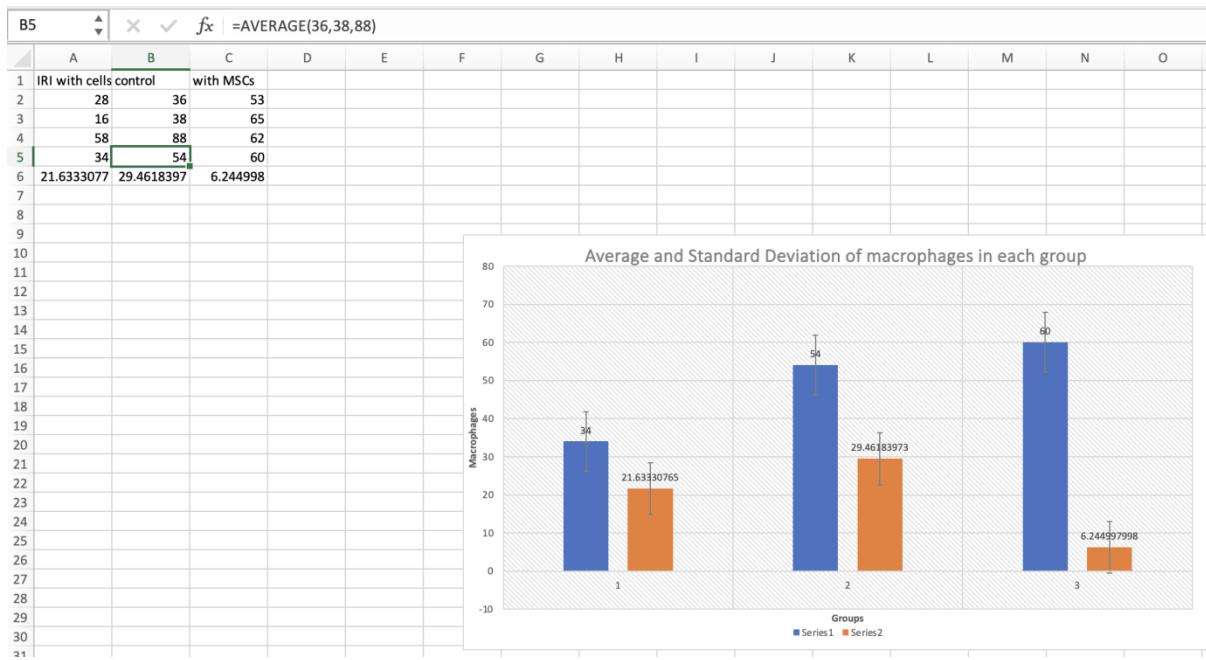


Fig11: bar chart showing the average number of macrophages in each group.

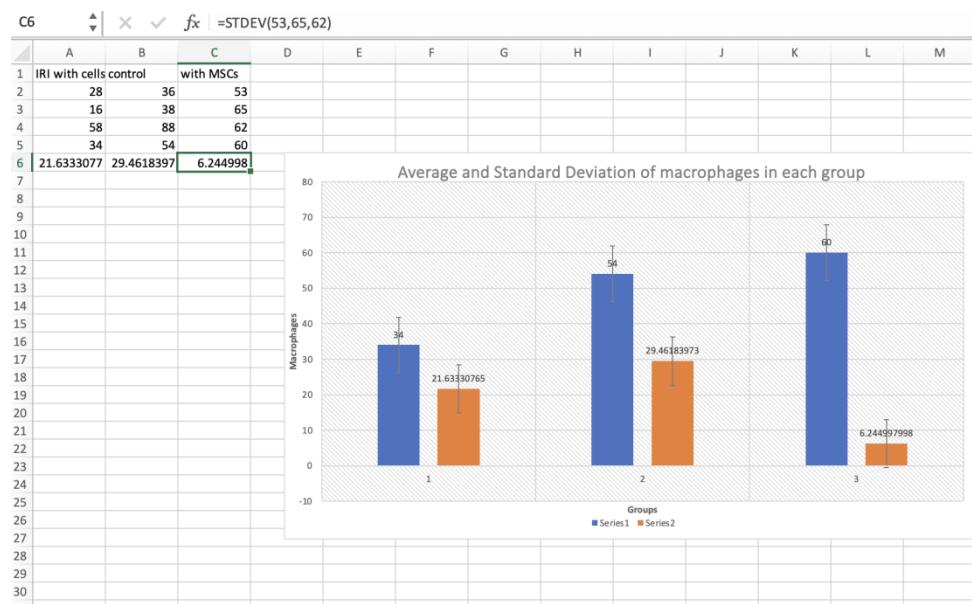


Fig12: bar chart showing the standard deviation of the number of macrophages in each group.

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