Developing Therapeutics for the Prevention of Crystalline Silica-Induced Inflammation by Anti-Depressant Drugs

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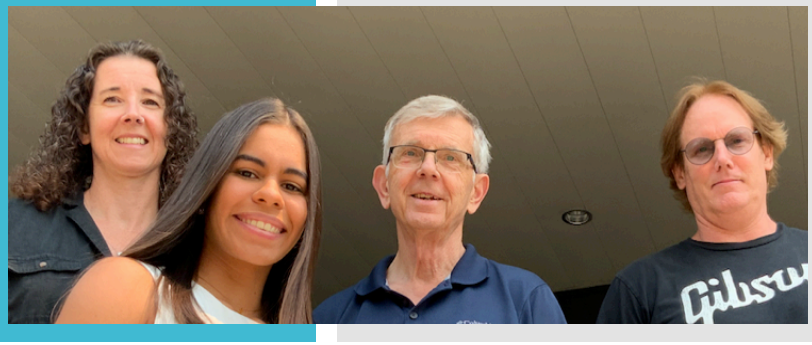

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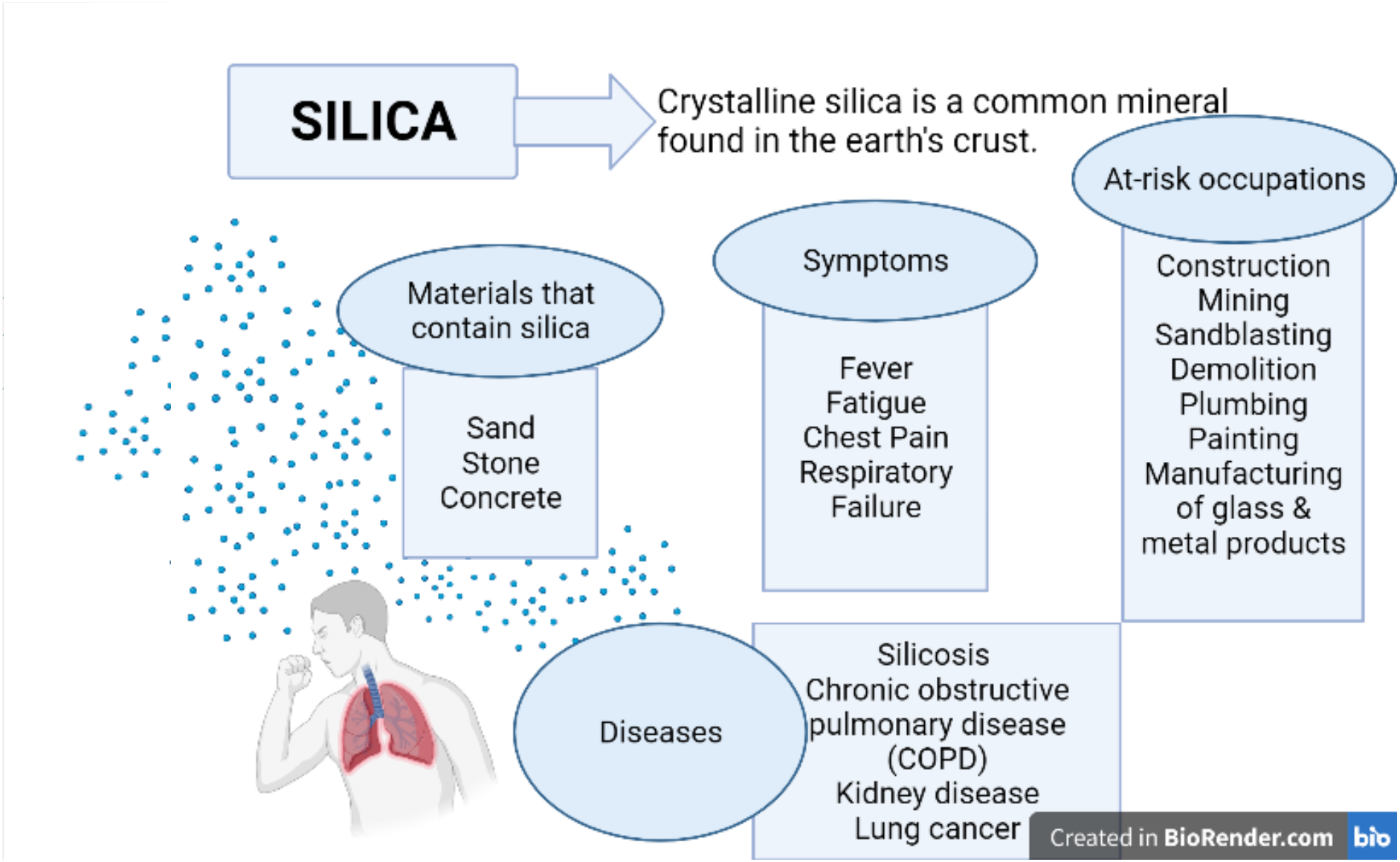
SILICA
Crystalline silica is a common mineral found in the earth's crust.
At-risk occupations
Construction
Mining
Sandblasting
Demolition
Plumbing
Painting
Manufacturing of glass & metal products
Symptoms
Fever
Fatigue
Chest Pain
Respiratory Failure
Materials that contain silica
Sand
Stone
Concrete
Diseases
Silicosis
Chronic obstructive pulmonary disease (COPD)
Kidney disease
Lung cancer
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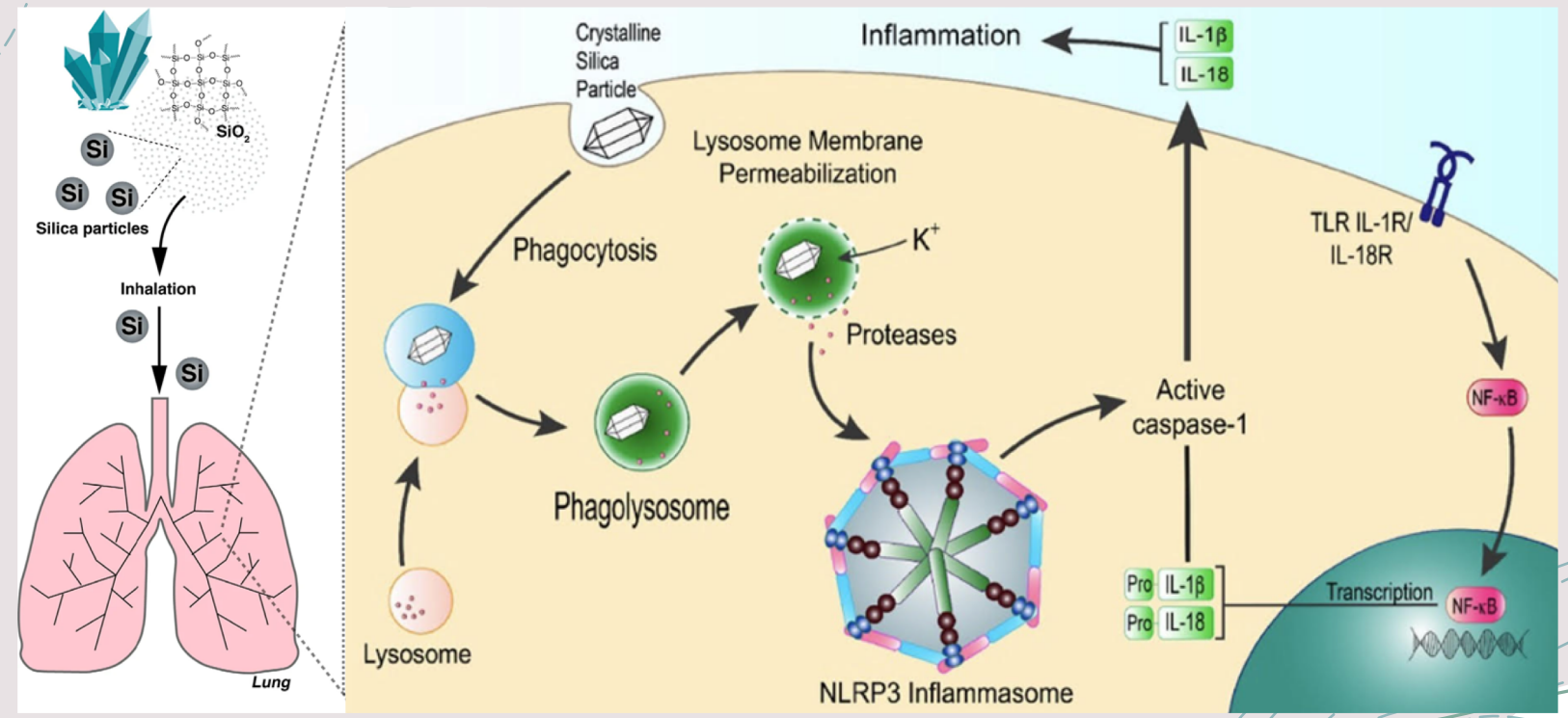
Effects of Silica in Human Body



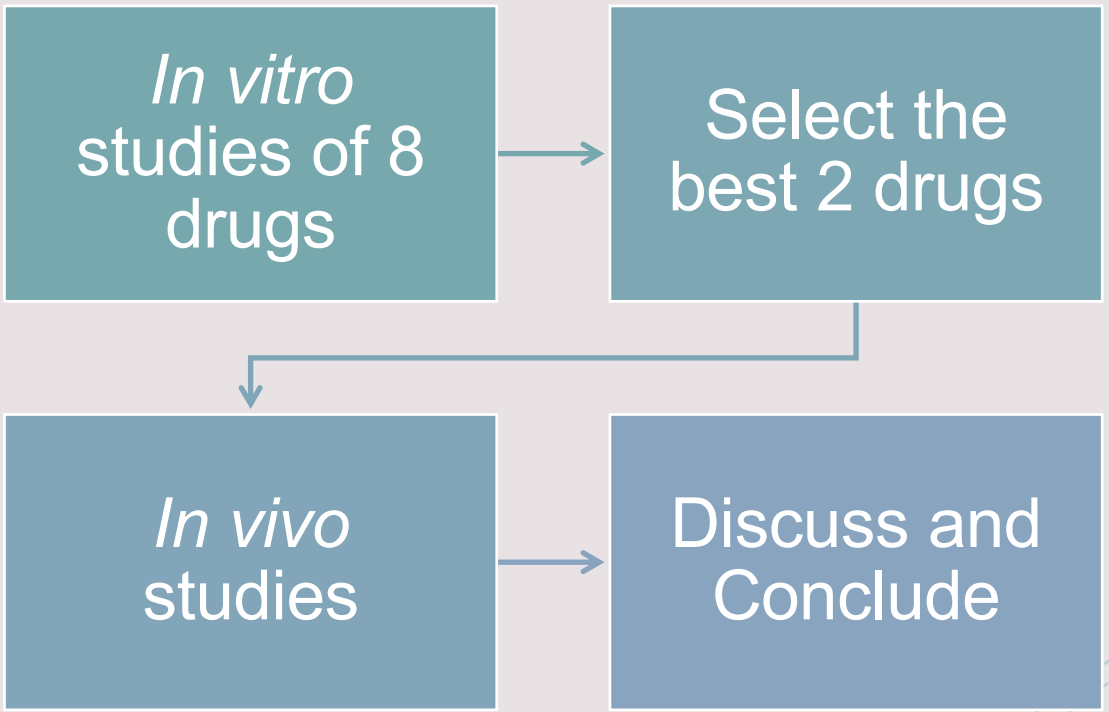
Problem

Inadequate treatments for Chronic inflammatory diseases

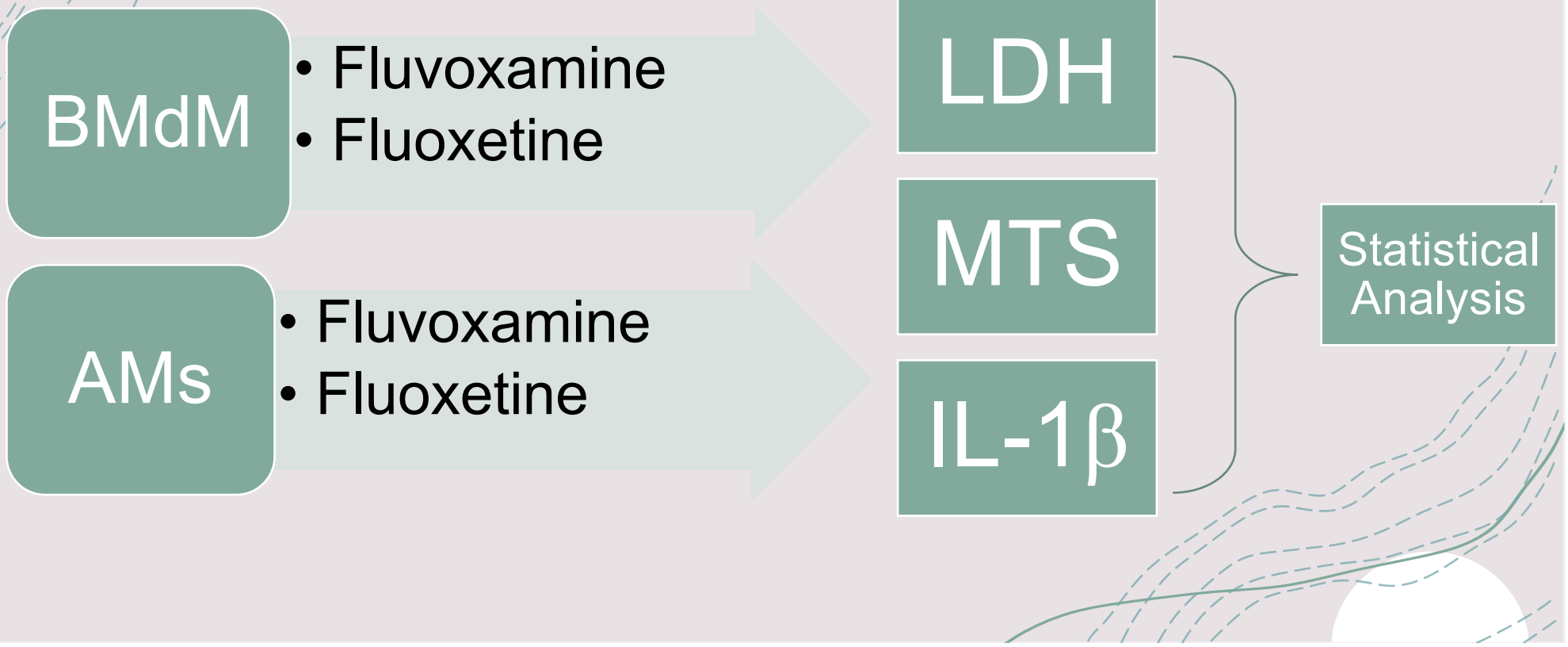
Goal

Test novel therapeutics based on proposed mechanism

Research


In vitro studies of 8 drugs
Select the best 2 drugs
In vivo studies
Discuss and Conclude

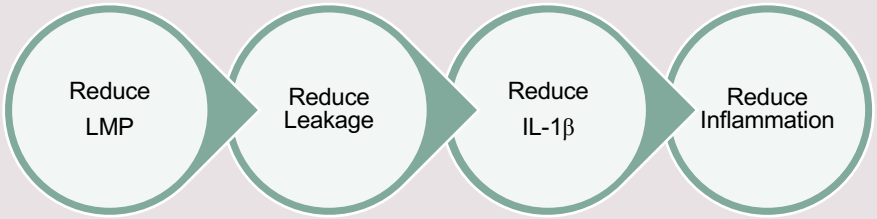
In vitro studies of two drugs



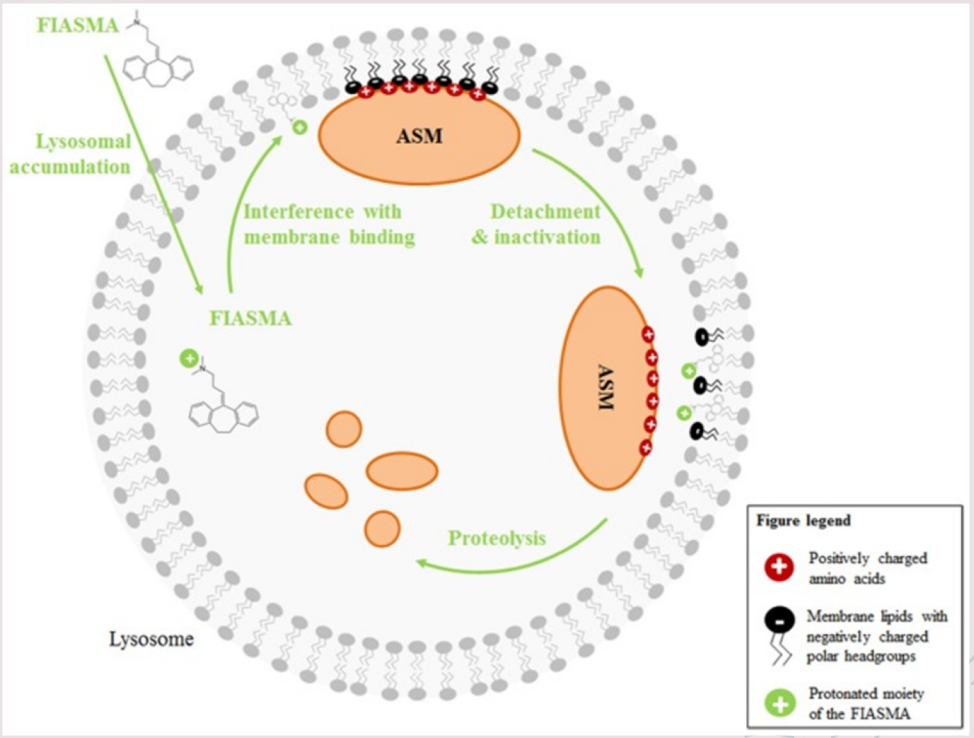
Hypothesis

Antidepressant drugs will decrease the silica-induced inflammatory response in Murine Bone Marrow derived Macrophages (BMdM) and Alveolar Macrophages (AMs) with minimum cytotoxicity.

How drugs help reduce inflammation?

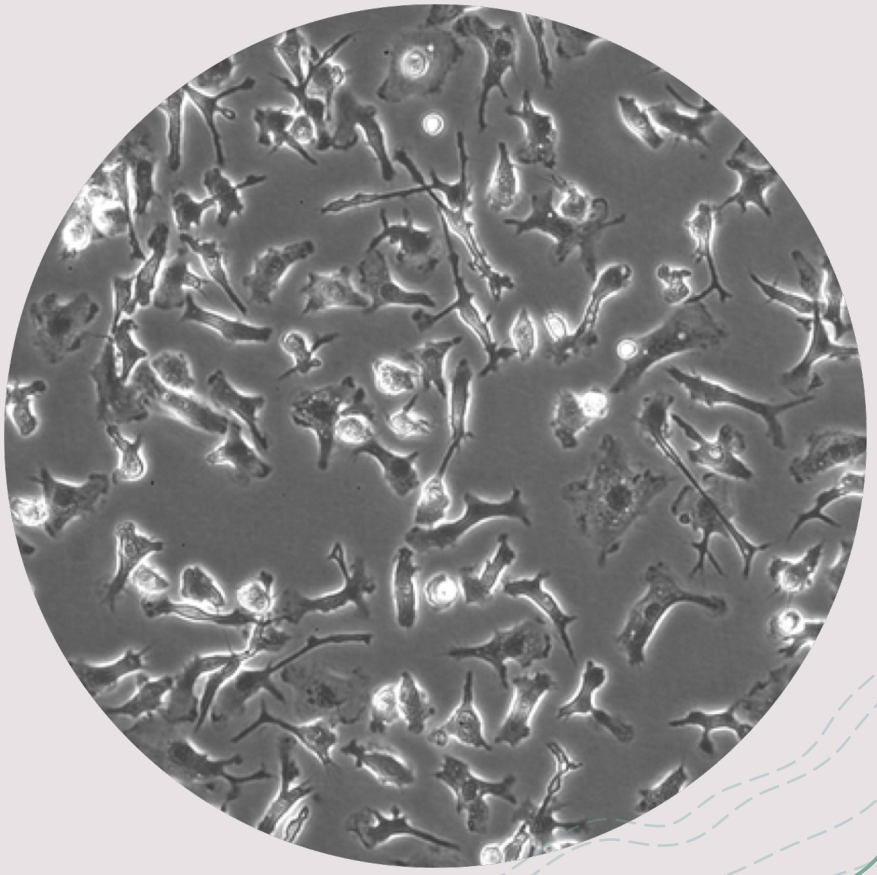


Functional Inhibitors of Acid Sphingomyelinase (FIASMA)

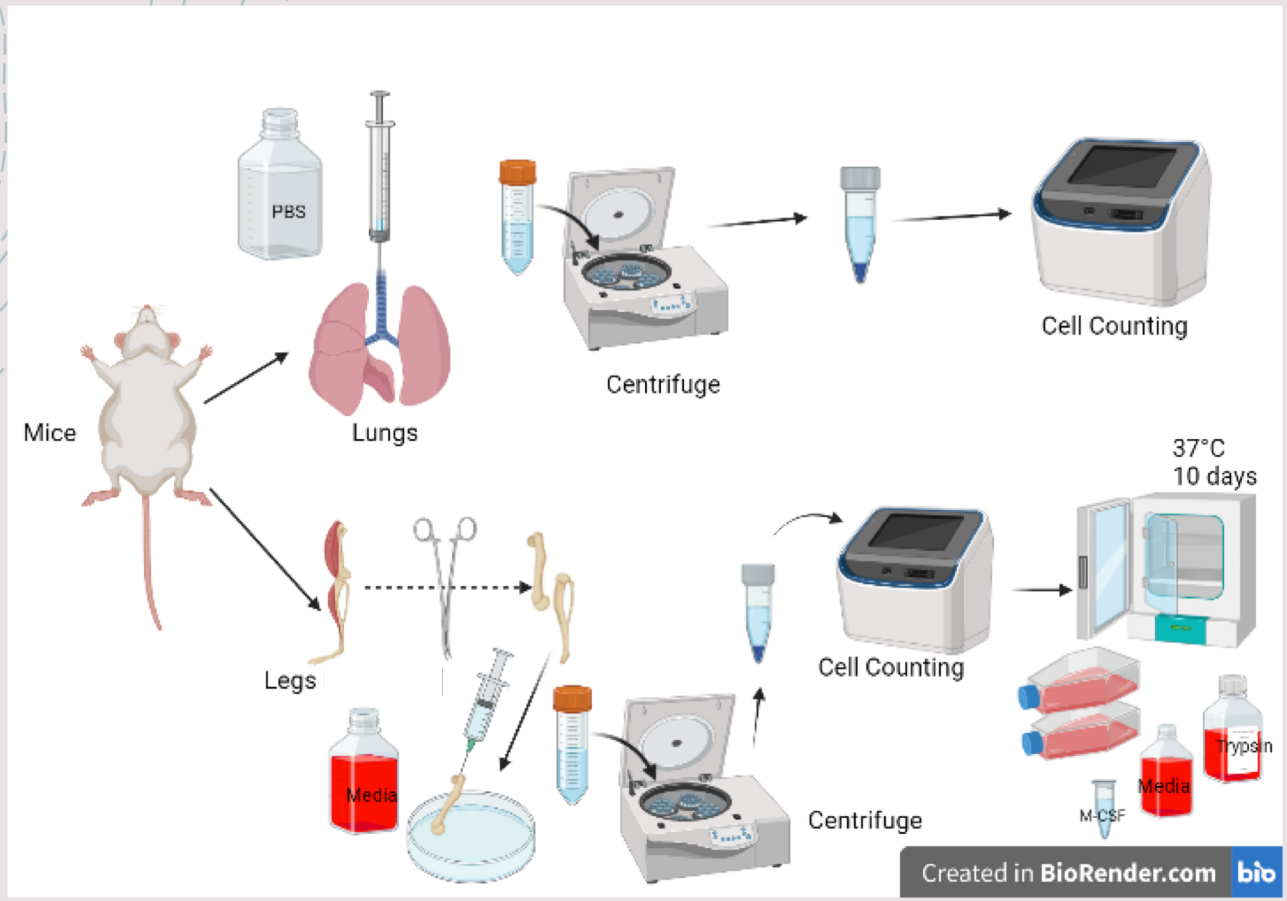


Methods

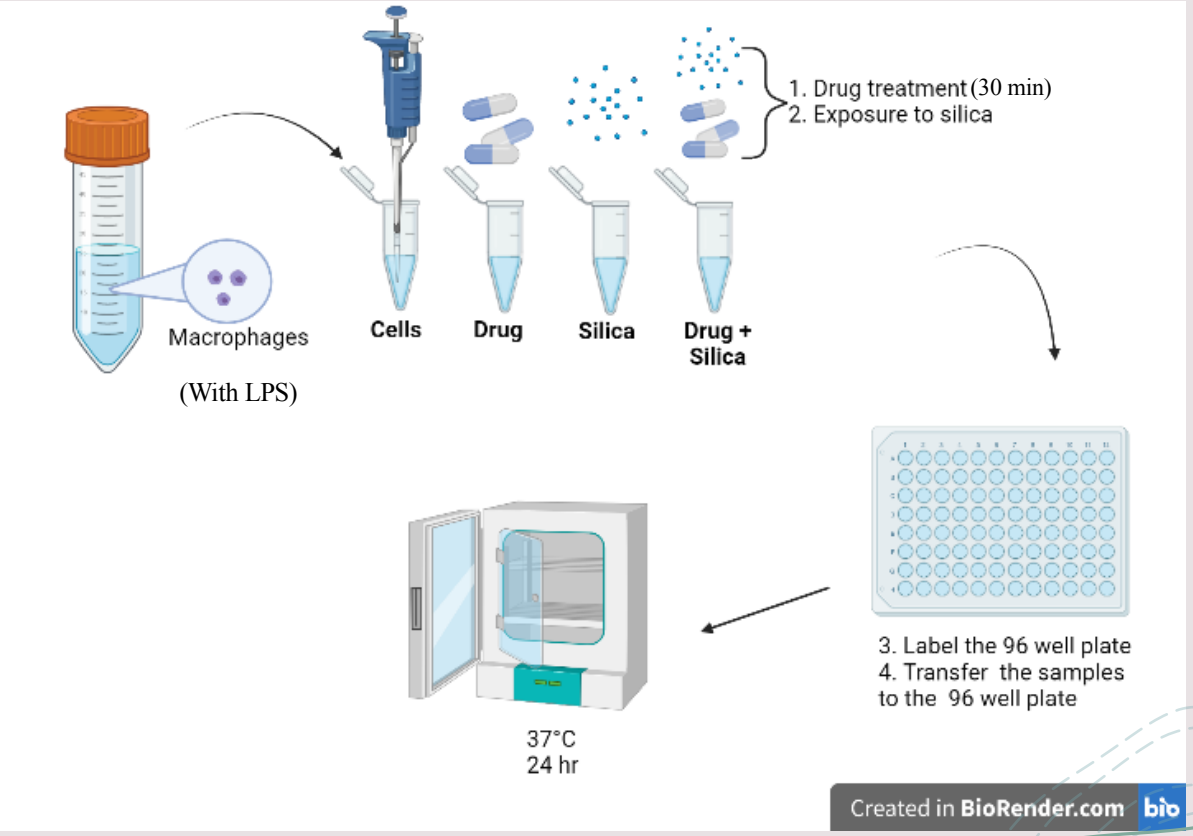
- Isolation and culture of bone marrow derived macrophages
- Isolation and culture of alveolar macrophages
- Cell treatments
- *In vitro* assays



Isolation and culture of BMdM and AMs

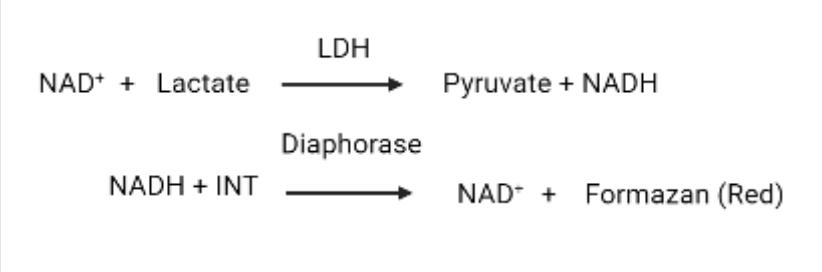


Cell Treatments

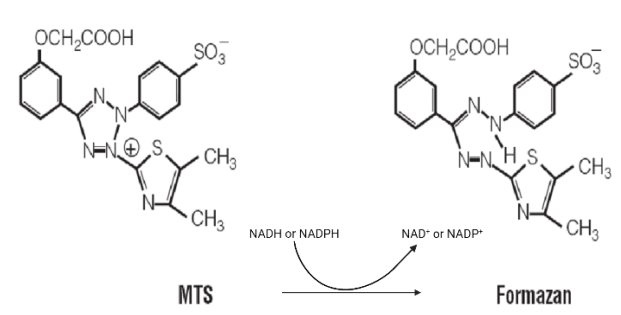


Lactate Dehydrogenase (LDH) Assay

- Quantitatively measures lactate dehydrogenase (LDH)
- LDH is a stable cytosolic enzyme that is released upon cell lysis.
- Results in the conversion of tetrazolium salt (INT) into red formazan.
- The amount of color formed is proportional to the number of lysed cells.



MTS Assay



3-(4,5-dimethylthiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolum, inner salt (MTS)

MTS tetrazolium compound is bioreduced by cells into colored formazan product.

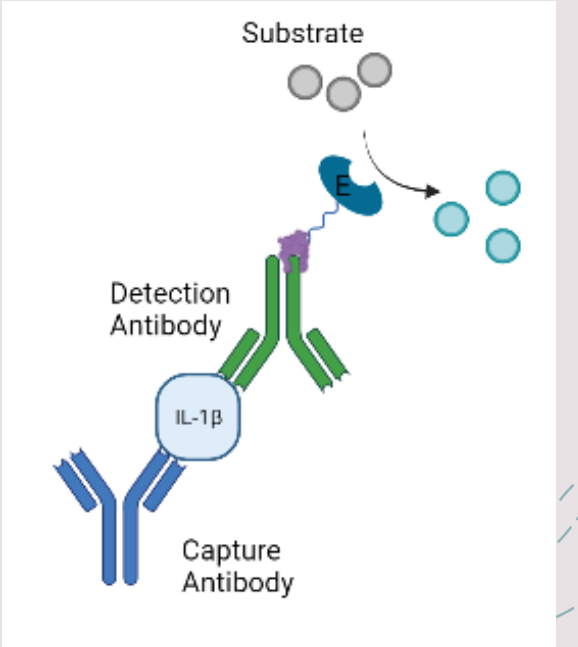
This conversion is presumably accomplished by NADPH or NADH

This is produced by dehydrogenase enzymes in metabolically active cells (mitochondria).

The quantity of formazan product is directly proportional to the number of living cells in culture (Cells viability).

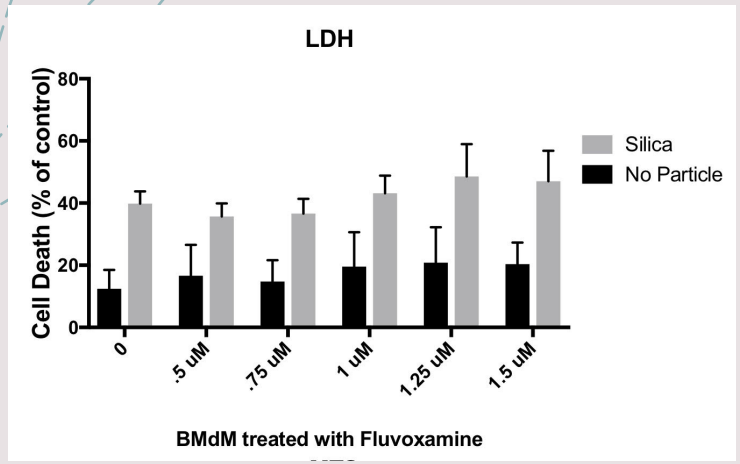
Enzyme-Linked ImmunoSorbent assay (ELISA) technique for IL-1β

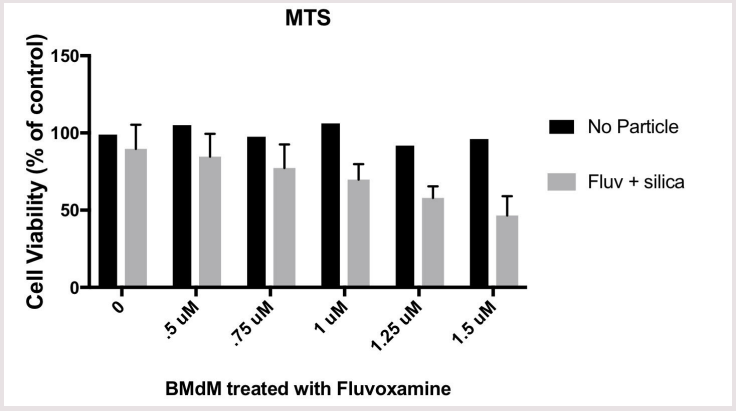
- Immobilized antigen is detected by an antibody bound to an enzyme capable of generating a color change
- A sandwich ELISA measures antigen between two layers of antibodies
- Streptavidin- HRP
- Enzyme = Horseradish Peroxidase (HPR)

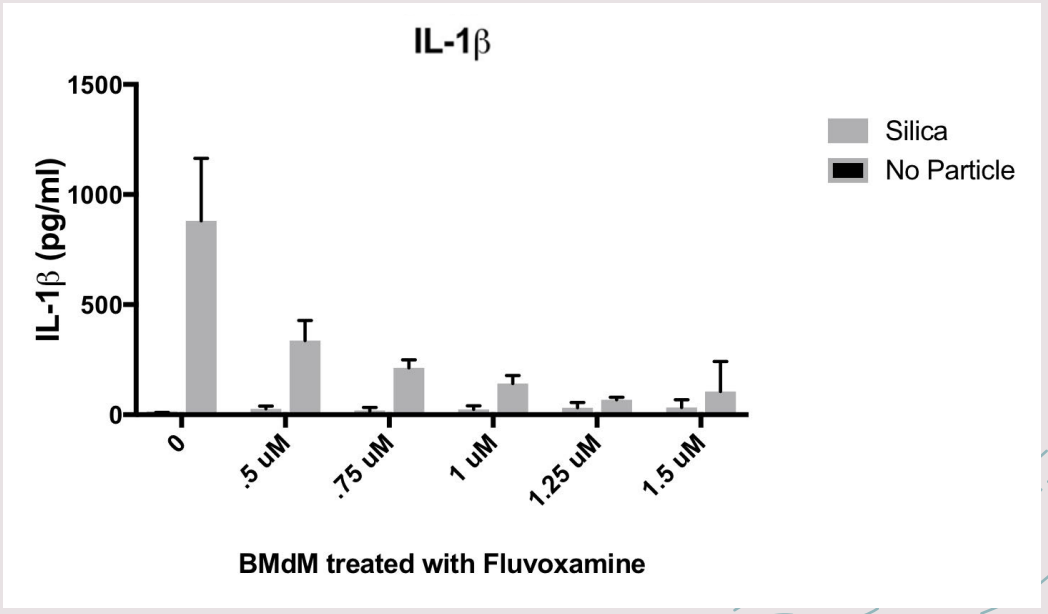


Results

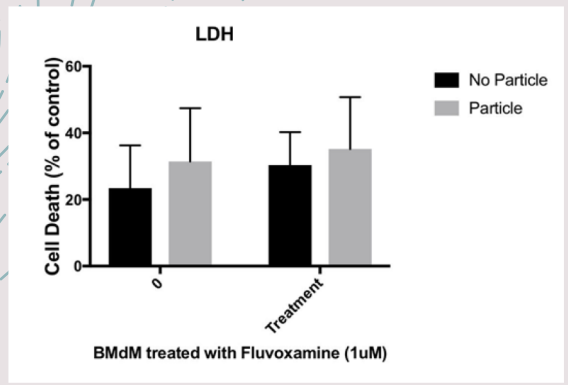
BMdM Fluvoxamine Dose Response

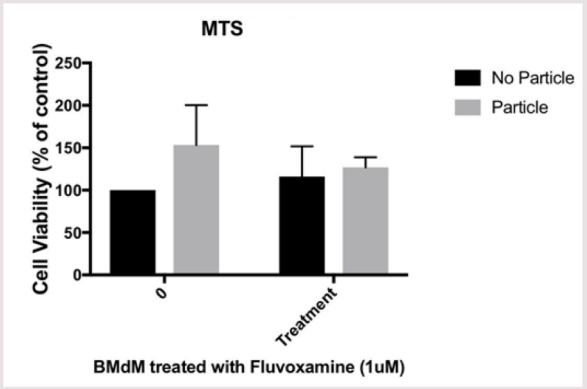


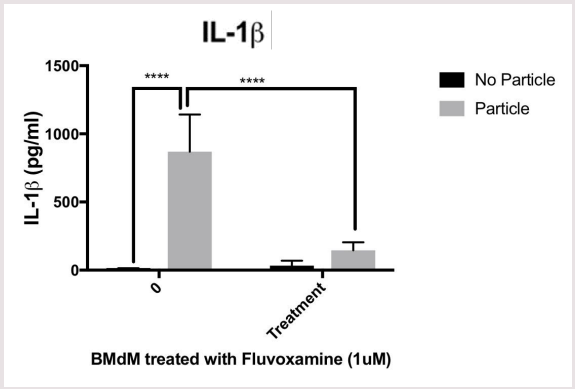


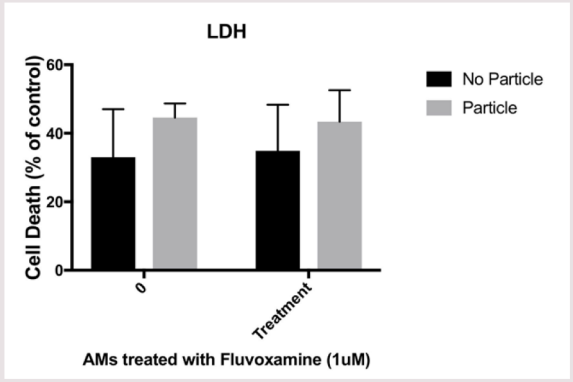


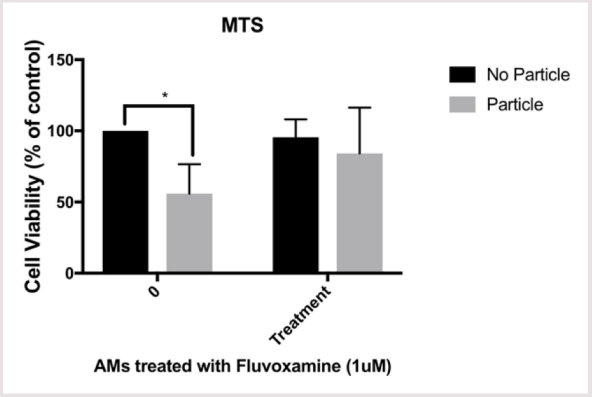
BMdM & AMs Fluvoxamine (1μM)

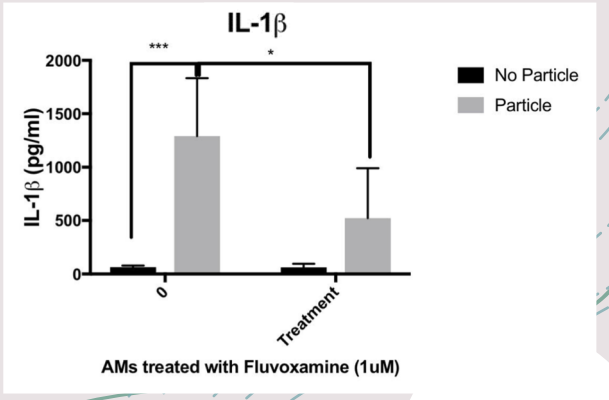




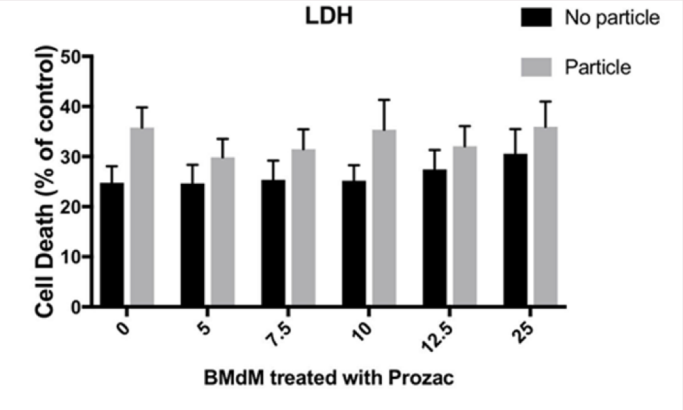


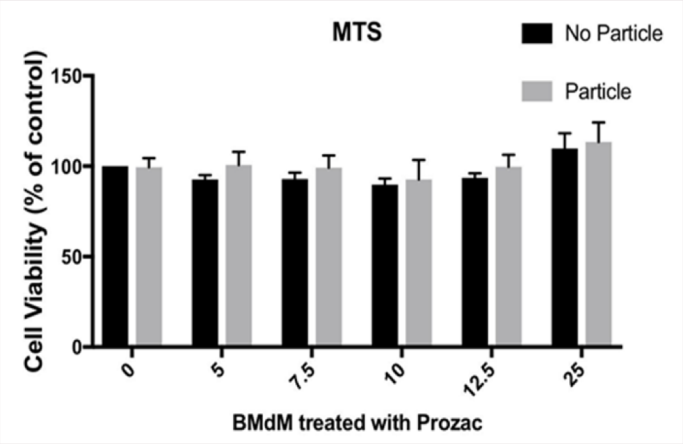


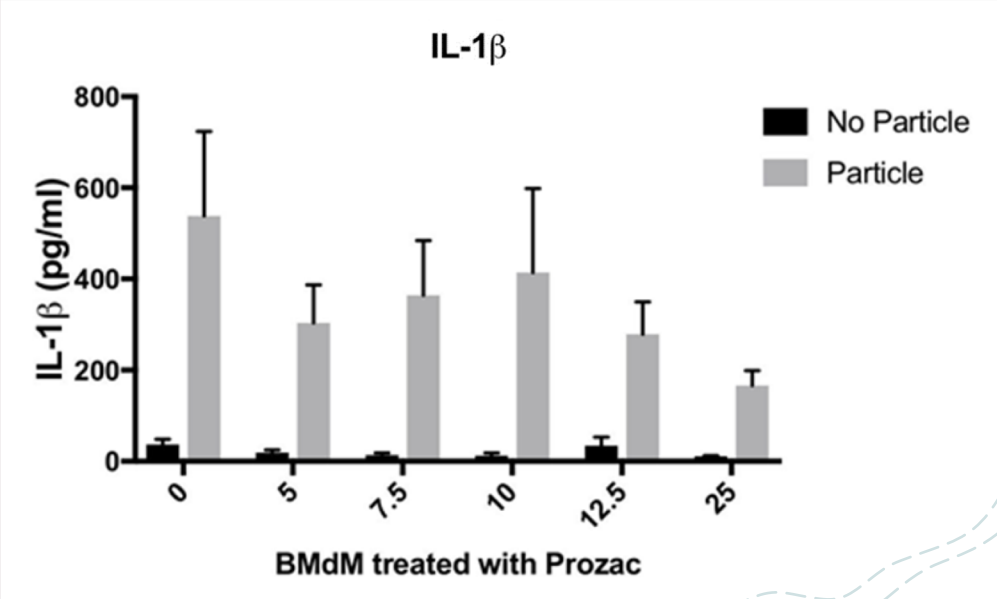




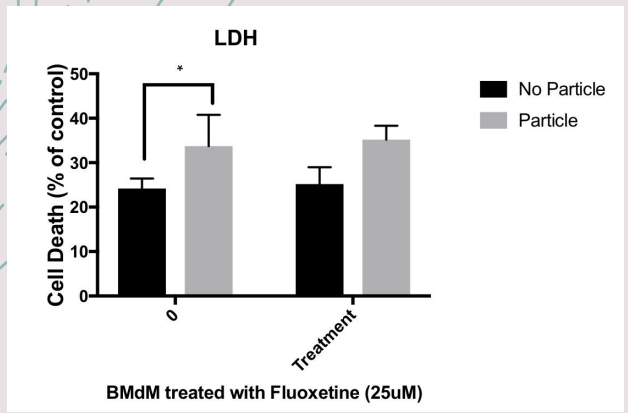
BMdM Fluoxetine Dose Response

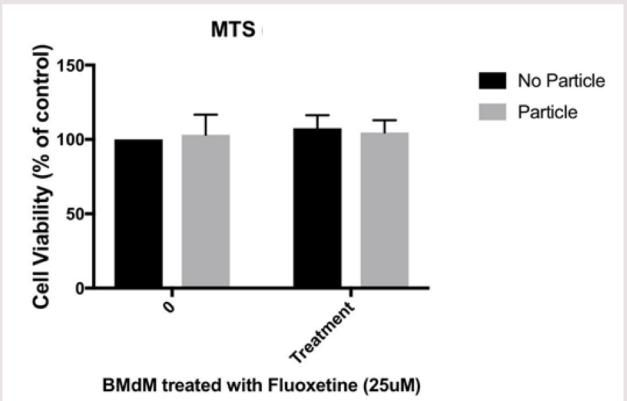


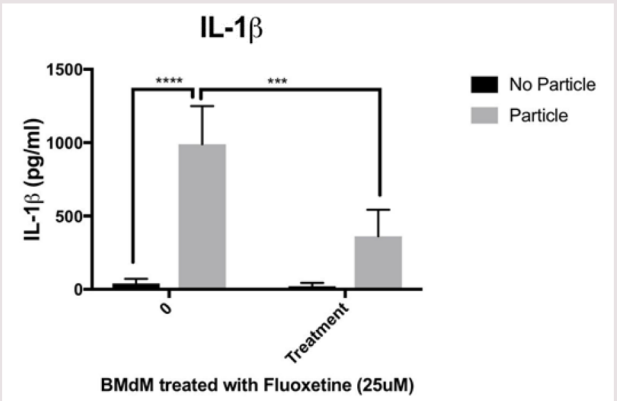


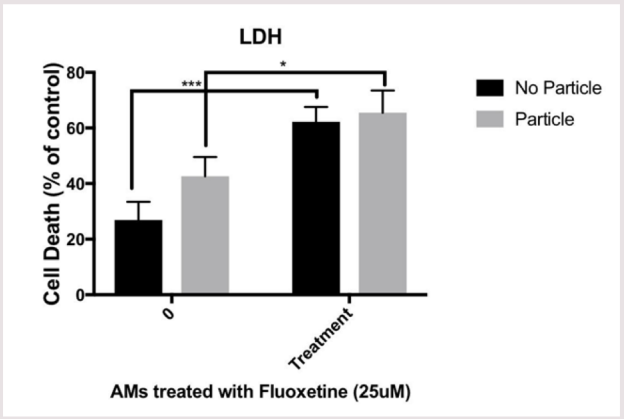


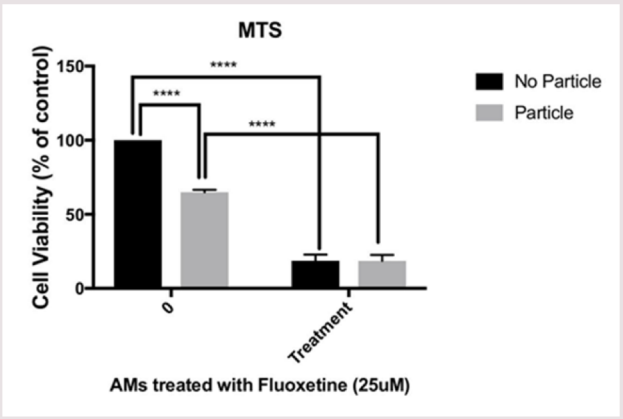
BMdM & AMs Fluoxetine 25μM

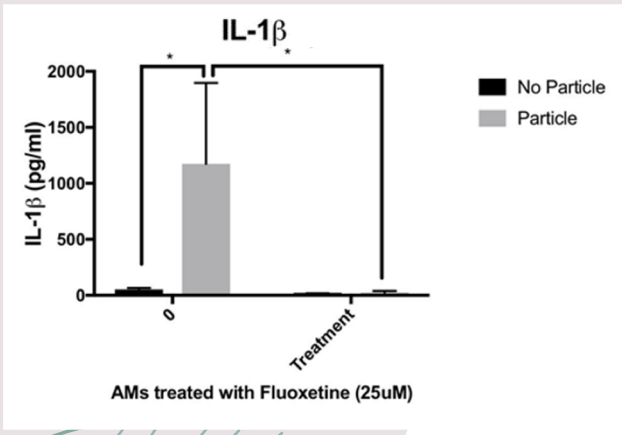




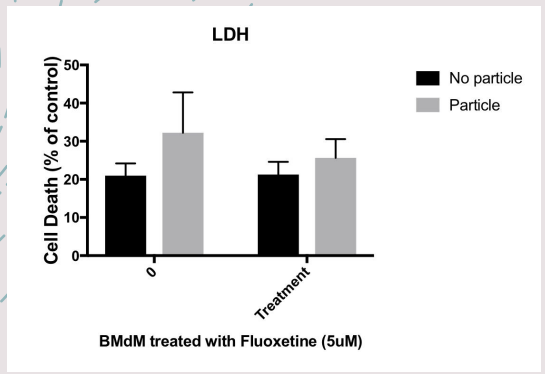


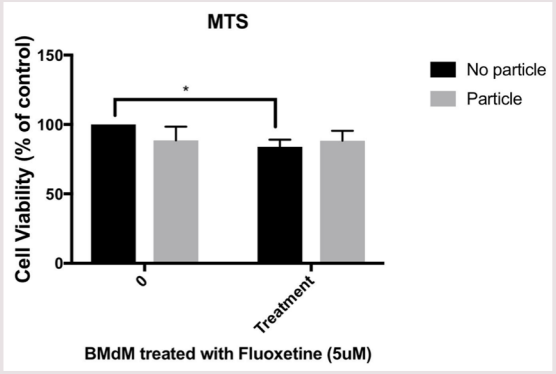


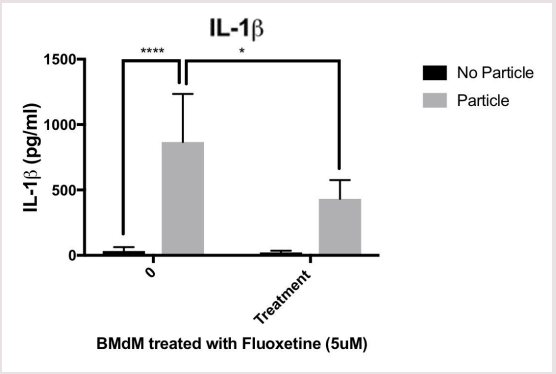


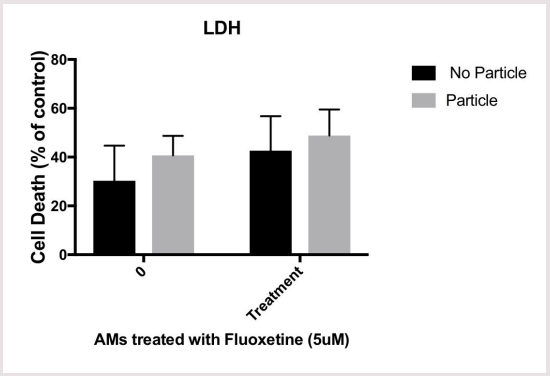


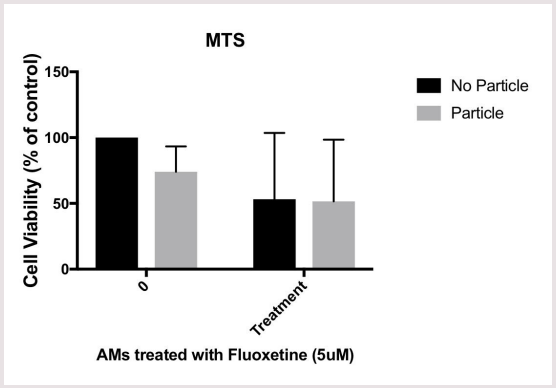
BMdM & AMs Fluoxetine 5µM

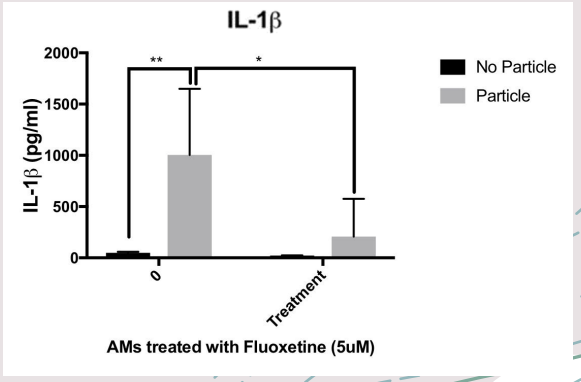












Conclusions

- Both treatments reduced crystalline silica-induced inflammation in mice immune cells without greatly increasing cytotoxicity
- BMdM and AMs are different
- The FDA approved drugs could be repurposed as a treatment for chronic inflammatory diseases
- Additional studies were needed to make sure that the proposed mechanism of the drugs is directly reducing the LMP
- Human immune cells

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References

Beckmann, N., Sharma, D., Gulbins, E., Becker, K. A., & Edelmann, B. (2014). Inhibition of acid sphingomyelinase by tricyclic antidepressant and analogs. *Frontiers in Physiology* .

BioRender. BioRender App. (n.d.). https://app.biorender.com/illustrations/60f5fb1ca1283400a52eee82.

Biswas, R., Trout, K. L., Jessop, F., Harkema, J. R., & Holian, A. (2017). Imipramine blocks acute silicosis in a mouse model. *Particle and Fibre Toxicology*.

Burmeister, R., Rhoderick, J. F., & Holian, A. (2019). Prevention of crystalline silica-induced inflammation by the anti-malarial hyfrochloroquine. *Inhalation Toxicology*, *31*. https://doi.org/http://doi.org/10.1080/08958378.2019.1668091

Cali Silica Powder. Kelp4less. (2018). https://www.kelp4less.com/shop/cali-silica-powder/.

Department of Labor logo UNITED STATESDEPARTMENT OF LABOR. Silica, Crystalline - Overview | Occupational Safety and Health Administration. (n.d.). https://www.osha.gov/silica-crystalline.

EZ-LDH. DoGenBio. (n.d.). http://www.dogenbio.com/shop/item.php?it_id=1491191620.

Harvest Mouse 3. Pet mice blog. (2016, January). https://petmiceblog.co.uk/wp-content/uploads/2016/01/Harvest-Mouse-3.jpg.

Human PEDF ELISA Kit (ab246535). Abcam. (2021, July 20). https://www.abcam.com/Human-PEDF-ELISA-Kitb246535.html?msclkid=9ea1837f d86317433739e3874abb0ab5&utm_ source= bing &utm_medium =cpc&utm_campaign=AMER_US_ KITASS_QPA_Desktop_BMM&utm_ term=%2Bpedf+%2Belisa&utm_content=QPA_PEDF_pedf+elisa&gclid=9ea1837fd8631743373 9e3874abb0ab5&gclsrc=3p.ds.

IL-1 beta Antibodies. Anti-IL-1 beta Antibodies | Invitrogen. (n.d.). *https://www.thermofisher.com/antibody/primary/target/IL-1+beta?ef_id=cdea4ea4bb541fbe4fd32e5c2a590ce7%3AG%3As&s_kwcid=AL%213652%2110 %217662235431965 5%217662240987763 0&cid=bid_pca_aup_r01_co_cp1359_pjt0000_bid000 00_0se_bng_bt_pur_con.*

Laboratory Supplies. (n.d.). https://5.imimg.com/data5/BP/YO/MY-331489/laboratory-supplies-500x500.jpg.

Mouse Hepatic Macrophages from C57BL/6. Sciencell Research Laboratories, inc. (n.d.). *https://www.sciencellonline.com/products-services/primary-cells/hematopoietic/mouse-hepatic-macrophages-2258.html.*

MTSassay. Abcam. (2018). *https://www.abcam.com/products?keywords=MTS%2Bassay&&msclkid=11fb8fb9b6861cdab3d 1ef4126c1bafd&utm_source=bing&utm_medium=cpc&utm_campaign=AMER_US_KITASS_N PK_Desktop_BMM&utm_term=%2Bmts+%2Bcell+%2Bproliferation+%2Bkit&utm_content=N PK_mts_mts+cell+proliferation+kit&gclid=11fb8fb9b6861cdab3d1ef4126c1bafd&gclsrc=3p.ds.*