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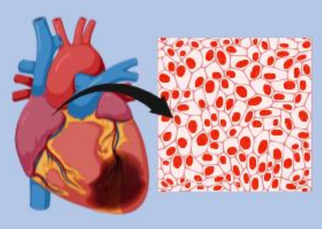
Unveiling the Metabolic Tango: Exploring the Interplay of Lipid Droplets and Mitochondria Through a New Staining Technique

Introduction



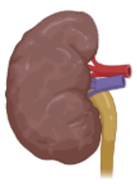
- Cardiovascular and metabolic diseases pose some of the biggest health challenges researchers are currently facing. Heart and circulatory diseases on average cause a death every 3 minutes and 5.8 million people in the UK have diabetes (British Heart Foundation, 2025).
- Lipids are organic molecules made of fatty acids and glycerol molecules. They can be found in the food we digest. Lipids can form droplets which can collect excess lipids to allow energy to be stored. Mitochondria can use these lipid droplet reserves to release energy for cellular processes.
- In diseased heart tissue, these interactions are thought to be dysfunctional and potentially contribute to the pathology of these diseases such as diabetes. When these processes are disrupted, issues such as lipid accumulation can occur which can cause an excess of fat to build up in the heart which can damage heart function and worsen disease progression.
- Research into these interactions is limited by current techniques - mitochondrial tracking stains such as MitoBrilliant exist solely for use in living cultured cells which prevents the visualisation of mitochondria in non-living patient tissue. Therefore, to improve the understanding of disease it would be beneficial to visualise lipids and mitochondria in patients which supports the need for a new co-staining method.

Patient A
Degenerative Aortic Stenosis
53 years old, 30.5 BMI.

Patient B
Endocarditis, smoker
39 years old, 26.8BMI.

Patient C
Endocarditis, AV replacement, smoker, asthma
40 years old, BMI 23.9.

Patient D
Aortic Stenosis, Diabetes, Hypertension
67 years old, 32.9 BMI.

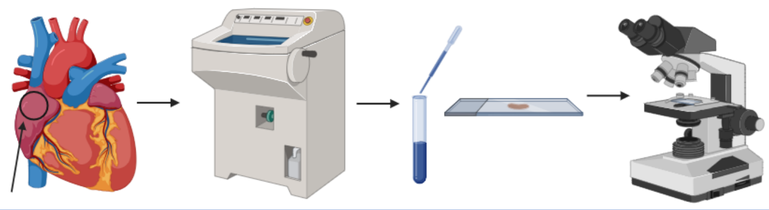


Patient E
CKD stage 3, Diabetes, Pacemaker
74 years old, BMI 37.6.

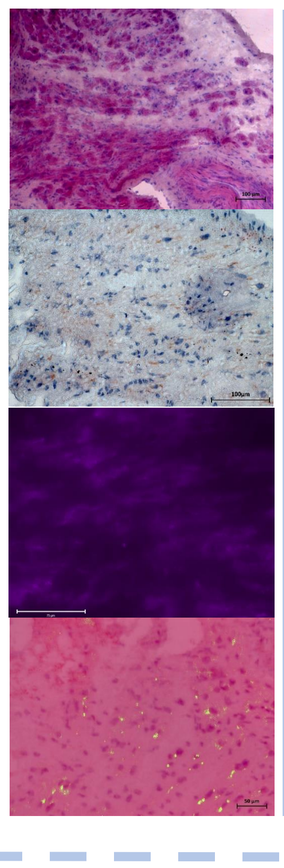
Research Aims

1. To develop a protocol suitable for staining non-living, frozen tissue with MitoBrilliant or MitoTracker
2. To investigate differences in heart tissue between a specific patient cohort selected for age, BMI and health conditions

Methods



- Samples of the right atrial appendage were taken from 5 female patients undergoing heart surgery and frozen in OCT at –80°C.
- Sections of 10µm were cut using a cryostat and cells were stained with 3 different optimised protocols: H&E, Oil red O and MitoBrilliant. The MitoBrilliant staining technique was developed through a series of optimised testing.
- Cultured human ventricular fibroblasts were stained with Oil red O.
- Slides were imaged using the Zeiss AX10 microscope on Zen imaging software at multiple magnifications and the EVOS microscope for x400 magnification.
- MitoBrilliant was imaged using the Cy5 filter and at a wavelength of 662nm on the Zen microscope.



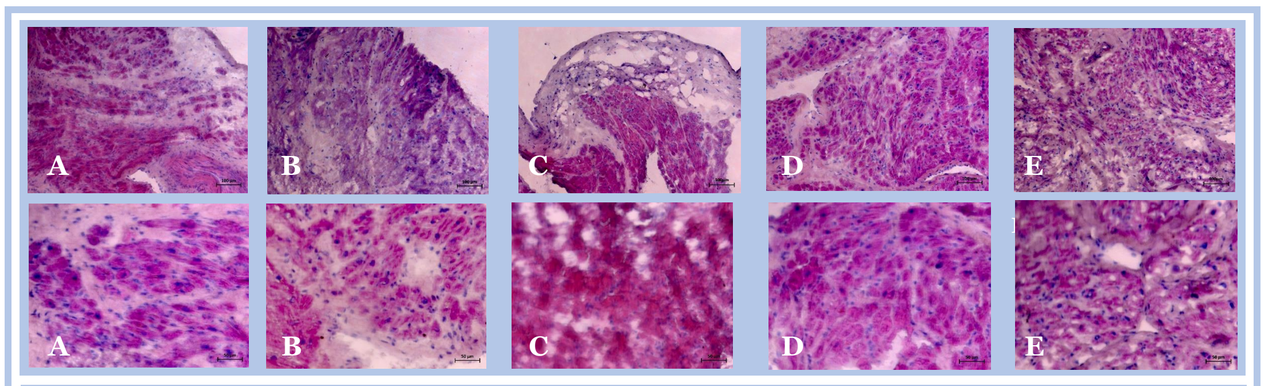
Hematoxylin & Eosin (H&E) Staining
- 1 minute Hematoxylin, cold water rinse, 0.25% HCL in ethanol for 3 dips, cold water rinse, hot water 2 minutes, 1 minute eosin, 70%, 95%, 95% ethanol for 10-15 seconds each and 5 minutes in 100% ethanol. Mounted with DPX.

Oil Red O Staining
- 4% PFA for 10 minutes, 2 minutes propylene glycol, 30 minutes with 60 Oil red O, PBS rinse and 30 seconds with Gills Hematoxylin. Mounted with DPX.

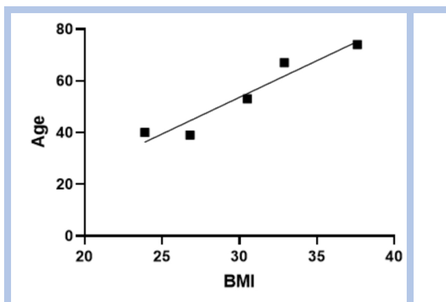
MitoBrilliant (MB) Staining
- The final technique involved: 4% PFA for 10 minutes, 3 minutes PBS, 10 minutes with 0.2% Triton-X, 3 minutes PBS, 30 minutes with 200nM MitoBrilliant incubated at 37 ,3mins PBS, VectaShield for mounting and immediate imaging.

Co-Staining
- Steps 1-6 of MB staining were combined with steps 2-5 of Oil red O staining. Slides were mounted with VectaShield and imaged immediately.

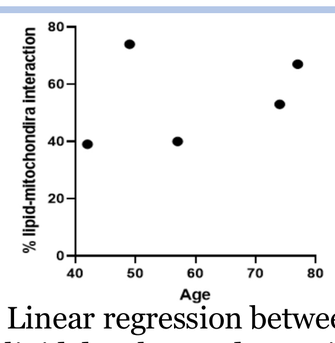
Results



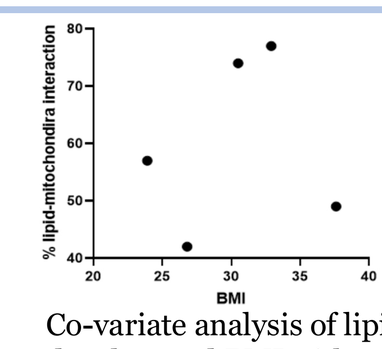
- H&E staining shows us nuclei (blue), cytoplasm & the extracellular matrix (pink), this combination allows us to observe an overall structure of patient heart tissue providing insight into the abundance of cells and allowing us to check for any obvious damage.
- Patient samples C & E display signs of damage potentially due to defrosting/incorrect freezing. This therefore allows us to be prepared for what other stains may change within the tissue.



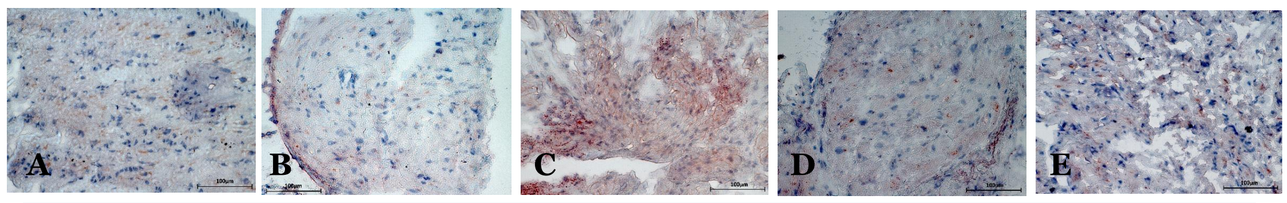
Linear regression between age and BMI. R2 = 0.9228.

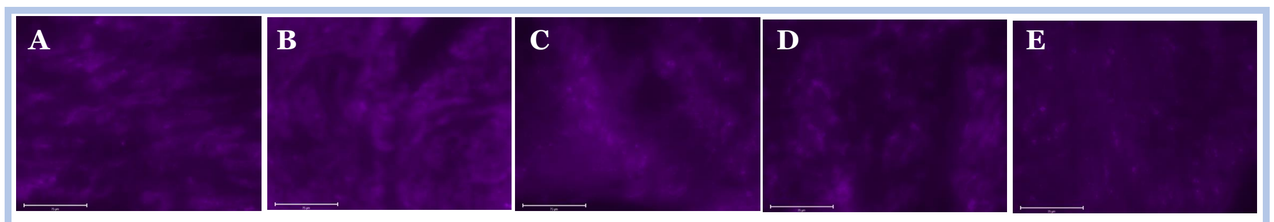


Linear regression between lipid droplets and age with BMI as a co-variant. R2= 0.

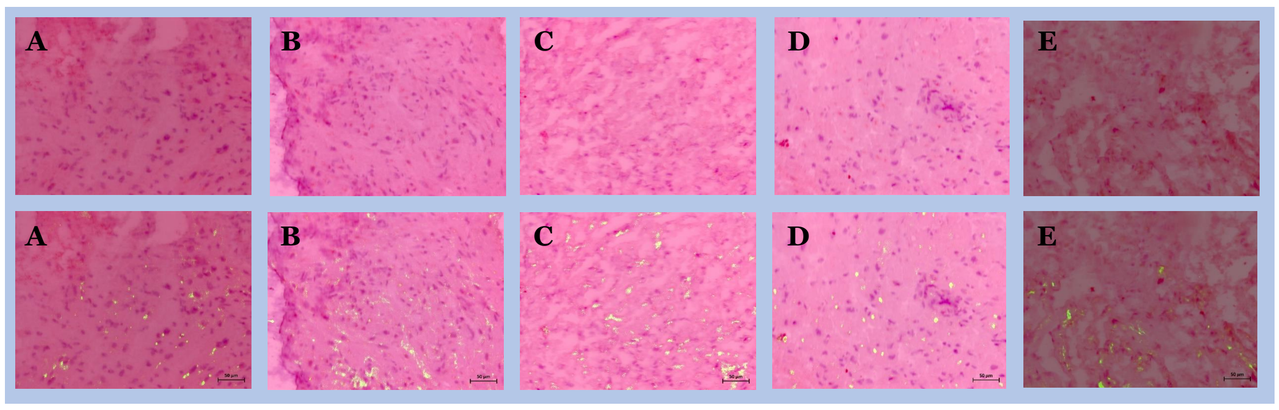


Co-variate analysis of lipid droplets and BMI with age accounted for. R2 = 0.09666.

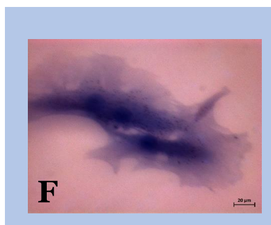




The images above show x400 magnification images of MitoBrilliant with visible mitochondrial staining, this will allow the density of mitochondria to be studied in tissue.



- Images collected from patient samples that had been co-stained using the newly developed protocol can be used to determine the relationship between lipid droplets and mitochondria.
- The amount of red (lipid droplets) were marked and counted, then the image with MitoBrilliant was overlaid to count the amount of areas where a lipid droplet coincided with or were in contact with mitochondrial staining.



- Images A-E are from patient samples that were co-stained then viewed in x400 magnification and imaged for Oil red staining.
- Image F is an image of a cultured ventricular fibroblast stained with Oil red O after fixation.
- C is the most abundant in lipid droplets with hundreds visible.
- Samples C,D and E all had over 200 lipid droplets present in the images selected, sample A had 87 and sample B had 85 visible.

The cell in F also had a large amount of lipid droplets present, these were small and not clustered unlike many of the patient samples which had multiple areas of dense lipid droplets. However sample C whilst abundant in lipid also displayed the individual smaller lipid droplets like F.

Conclusion & Discussion

- Mitochondrial tracking stains are suitable for fixed/non-living tissue and can provide great insight into mitochondria in patient samples.
- This allows greater testing in clinical and therapeutic research into more than just cardiovascular diseases.
- Due to a limited sample size (n=5) these results may require further testing to support conclusions drawn & results are only applicable to females
- As all samples have a heart condition and/or are on cardiac relevant medications, there is no control group to compare these findings to at the present.
- Damage was noted in some of the tissue samples due to faulty original freezing and/or defrosting during the cutting process which may have weakened results
- Additionally many factors such as BMI and age may simultaneously contribute to lipid density therefore it is difficult to draw conclusions on one singular factor