

Introduction

Why study fat tissue?

- Obesity and metabolic diseases are major public health concerns.

How does fat tissue expand?

- Adipocytes = fat cells
 - Preadipocytes, mature adipocytes, hypertrophic adipocytes.
- Adipocyte hyperplasia (cell proliferation)
- Adipocyte hypertrophy (cell enlargement)

What is fibronectin (FN)?

- An extracellular matrix (ECM) protein that is involved in several cellular processes.
- Two forms: cellular FN and plasma FN.
- Fibronectin is present in the extracellular matrix of adipocytes.

What do we not know?

- The contribution of plasma versus cellular FN used for assembling the ECM of adipocytes.

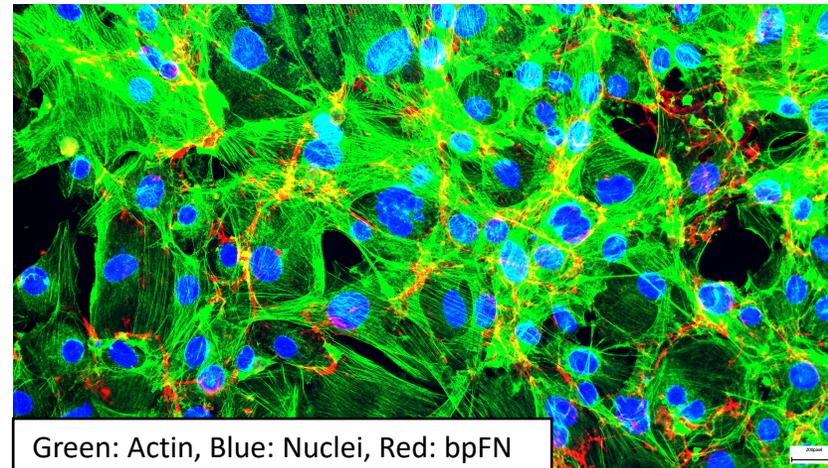
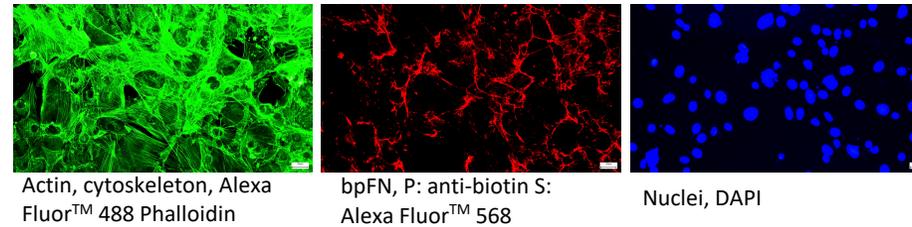
Methods

General Plan:

- Biotinylate bovine plasma FN to quantify the plasma FN matrix in preadipocytes, mature adipocytes, and hypertrophic adipocytes.
- Immunofluorescent staining and imaging
 - Biotinylated plasma FN, actin network, fat droplets, and nuclei will be stained using primary and secondary fluorescent antibodies.
- Both images and networks will be quantified using CellProfiler.

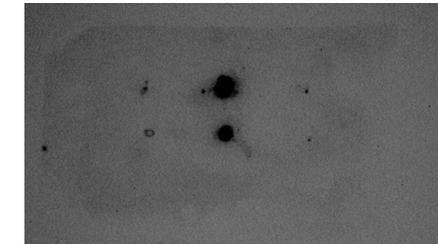
It is hypothesized that adipocyte differentiation and hypertrophy will alter the fibronectin matrix assembly, changing the matrix from cellular fibronectin to plasma fibronectin.

Preadipocytes (NC9 + bpFN)

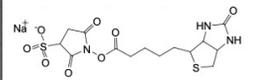


Expectations: Observe an increased plasma FN assembly in hypertrophic adipocytes –indicative of the initiation of fibrosis

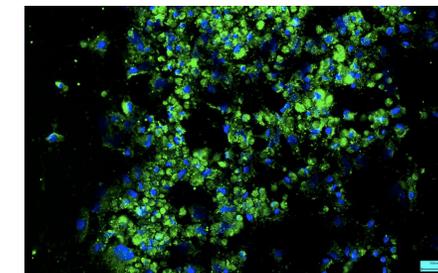
Results



Dot Blot: L→R : (-), bpFN, (+)



EZ-Link-Sulfo-NHS-Biotin
- Water soluble
- Conjugates to FN without altering protein function.



Cultured 3T3-L1 preadipocytes:

Green: BODIPY (Fat droplets)
Blue: DAPI (nuclei)

Future Directions

- Characterization of adipocyte morphology using Bodipy to analyze lipid droplet size.
- These experiments will help expand our understanding of circulating plasma FN in obesity and its potential relations to metabolic diseases.

Acknowledgements

- Kaartinen Lab (Dentistry, Medicine)
- Office of Science Education
- University Advancement at McGill