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CES LOPE DE VEGA

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Contribution of the adipose tissue extracellular matrix in obesity associated metabolic diseases and fibrosis.

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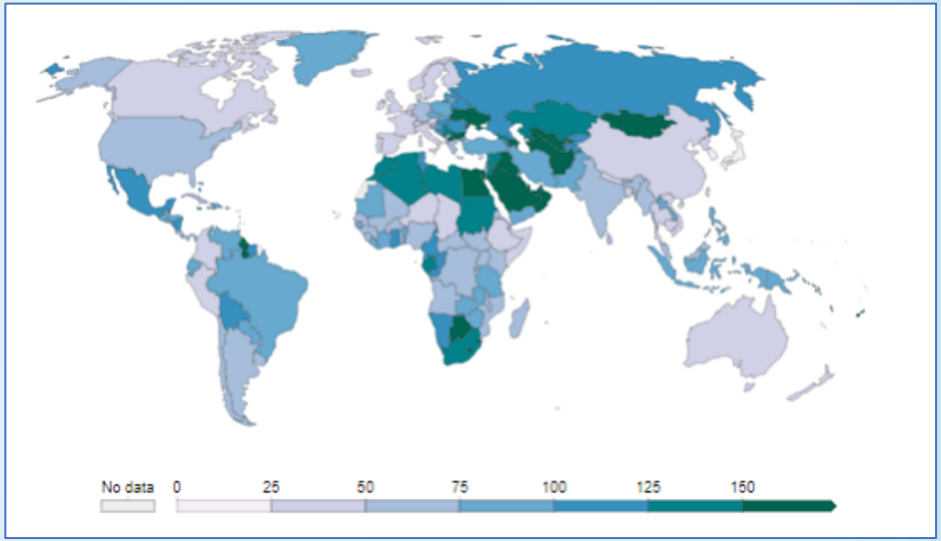
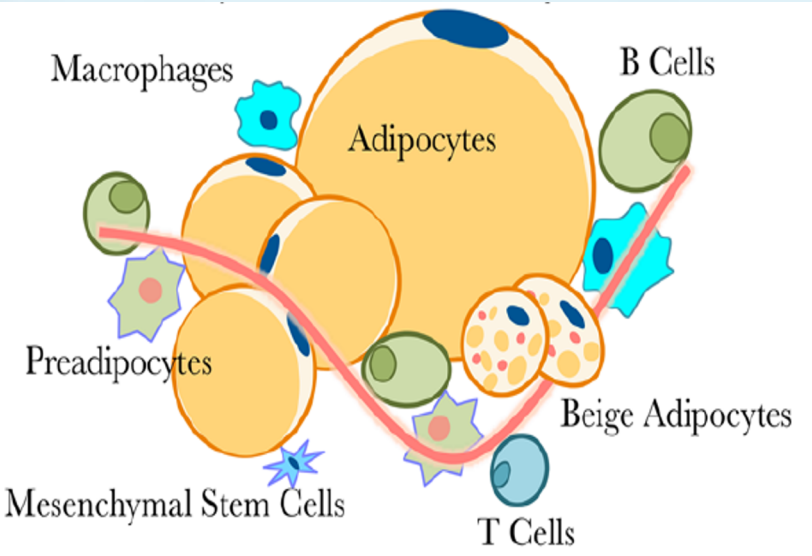
1. INTRODUCTION



1. INTRODUCTION

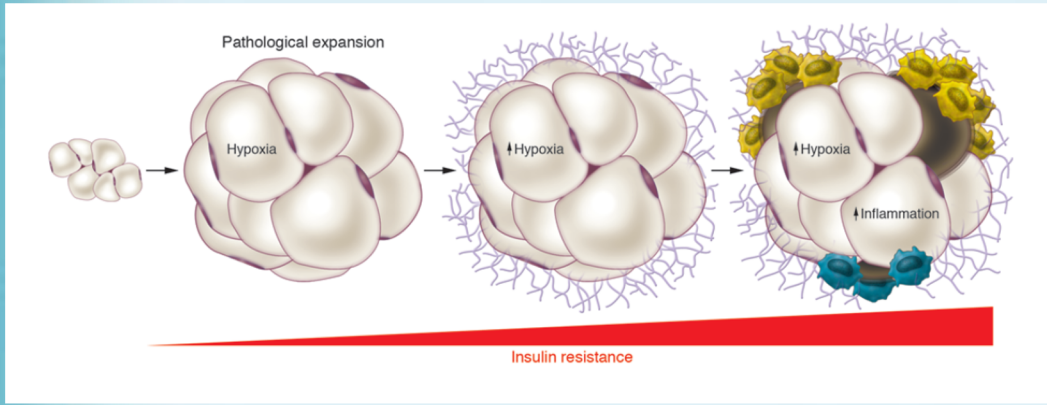
ADIPOSE TISSUE



- Function of this endocrine organ:
  - Energy storage as lipids in the adipocytes
  - To release adipokins
  - Energy homeostasis as a thermal insulator.
- Cell types:
  - preadipocytes (adipogenesis)
  - among others
- Characterized by a high degree of expandability:

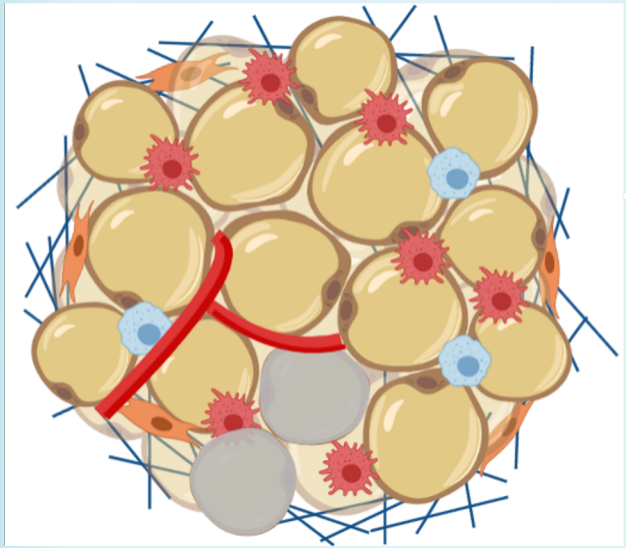
1. INTRODUCTION

ADIPOSE TISSUE PATHOLOGY



- This expandability may become saturated during obesity.
- Then, it induces toxic responses triggering insulin resistance, type 2 diabetes or cardiovascular disease due to lipids are redirected to peripheral organs.
- Extracellullar matrix is increased in AT growth

Extracellular Matrix: the great unknown structure



EXTRACELLULAR MATRIX

Main components

- **Glycoprotein**
- **Collagens**
- **Proteoglycans**
  - Decorin, ***lumican,*** etc.

Extracellular matrix- associated proteins

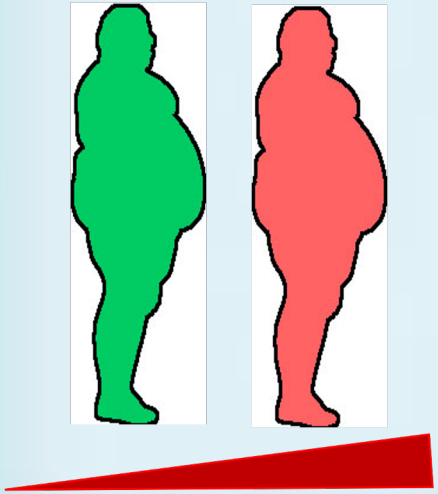
MAIN FUNCTION IN AT

- **to regulate cell behaviour**
- **to maintain adipocyte differentiation, function and survival**

1. INTRODUCTION

Obeses

normoglycaemic insulin resistant



FIBROSIS

- Fibrosis is an excessive accumulation of extracellular matrix components, especially collagen
- In the adipose tissue of obese people, there are many more fibrous areas that are associated with reduced plasticity causing metabolic dysfunction.

LUMICAN

- EM Protein which is involved in the formation of collagen fibrils.
- Increases levels in AT of insulin resistant-obese individuals as compared to normoglycemic obese subjects

2. OBJECTIVES

Main objective:

- To analyze the extracellular interaction with adipocytes through the development of a 3D culture system that allows the development of conditions associated with obesity and insulin resistance.

Others objectives:

- To learn the basics of confocal microscopy
- To analyze in more detail into basic marking applications
- To perform the practice and know the laboratory materials.
- To quantify images and analysis of results.

Hypothesis:

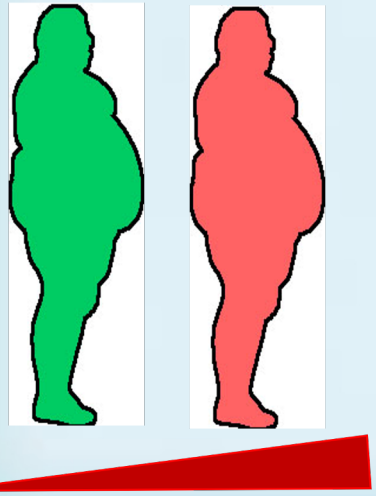
LUM could have a key role in the dysfunction of AT and thus, could be an important factor in the development of metabolic diseases associated with obesity.

3. Materials and methods

Obeses

normoglycaemic insulin resistant

NG **IR**



collagen I + Limucan

In vitro model, simulating adipose tissue from normoglycaemic or healthy patients.

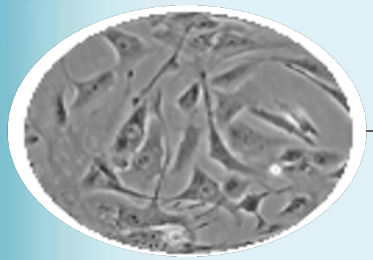
Cultures with collagen I alone or with collagen I + lumican.

3T3-L1 cell line, trying to reproduce the conditions found in patients.

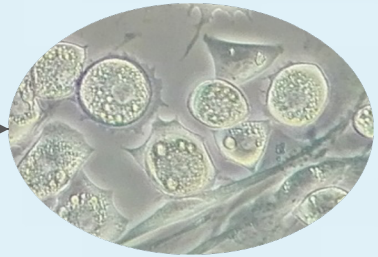
3.- Materials and methods

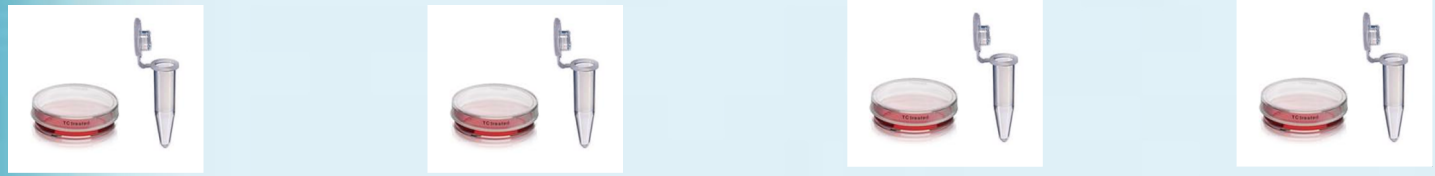
- **CELL DIFFERENTIATION-ADIPOGENESIS**

FIBROBLAST



ADIPOCYTES





Day 0 Day 3 Day6 Day 9

3.- Materials and methods

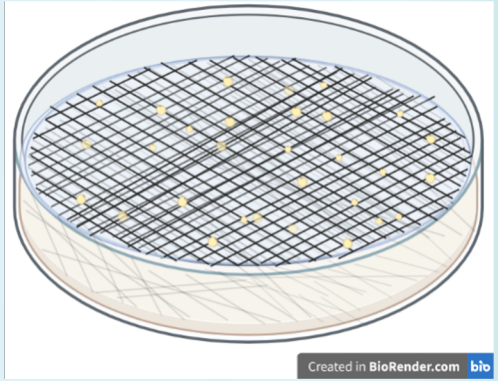
3D CULTURE PREPARATION

These three-dimensional cultures are usually grown in bioreactors, small capsules in which cells can grow in spheroids or 3D colonies.

Collagen type I

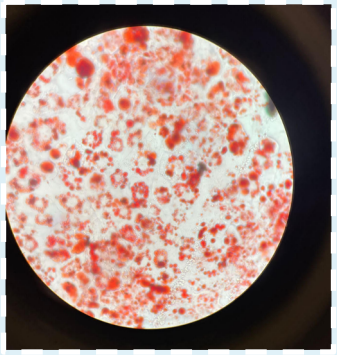
+

Lumican



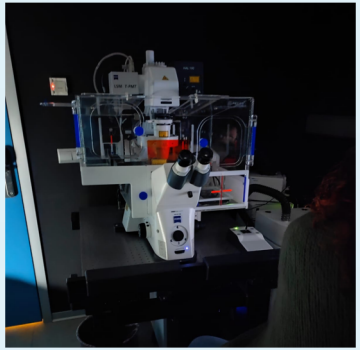
3. Materials and methods

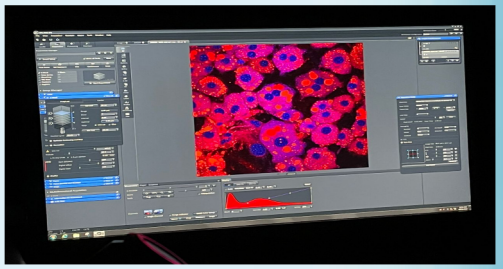
DYE



Oil Red-O dyes lipids in red, while the cell nuclei are counterbalanced in blue by means of a solution of DAPI.

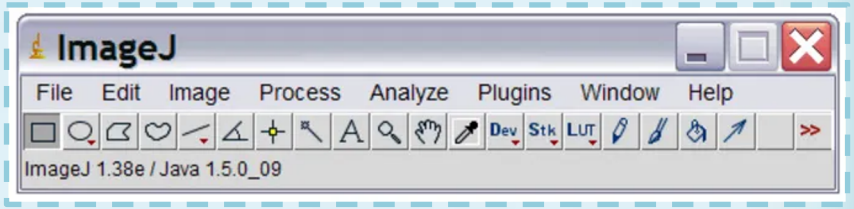
CONFOCAL MICROSCOPE





The confocal microscope is a microscope that employs an optical imaging technique to increase contrast and/or reconstruct three-dimensional images using a spatial pinhole to eliminate out-of-focus light or lens flare in specimens that are thicker than the focal plane.

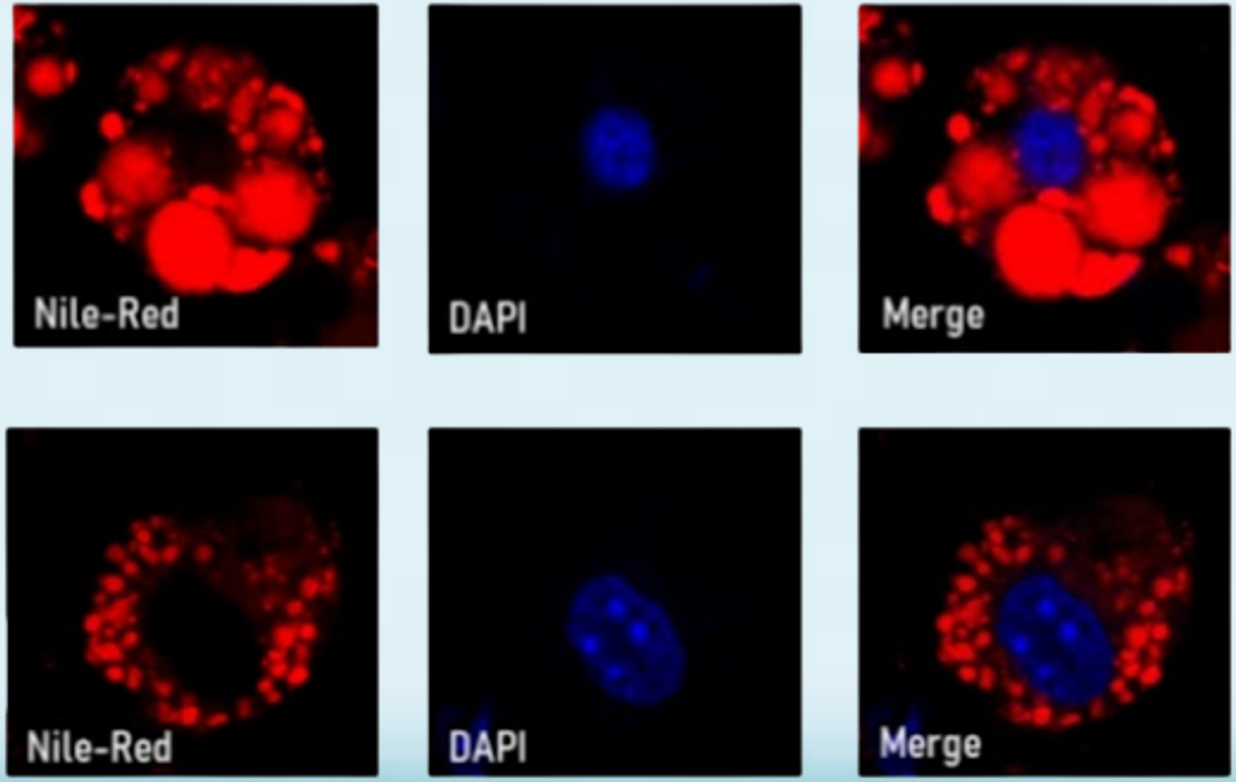
3. Materials and methods



ImageJ software was used to determine the amount of lipids per cells. The mean gray value of each treatment (with lumican vs w/o lumican) was compared using GraphPad Prism 5 applying t-student test and showing in a graph.

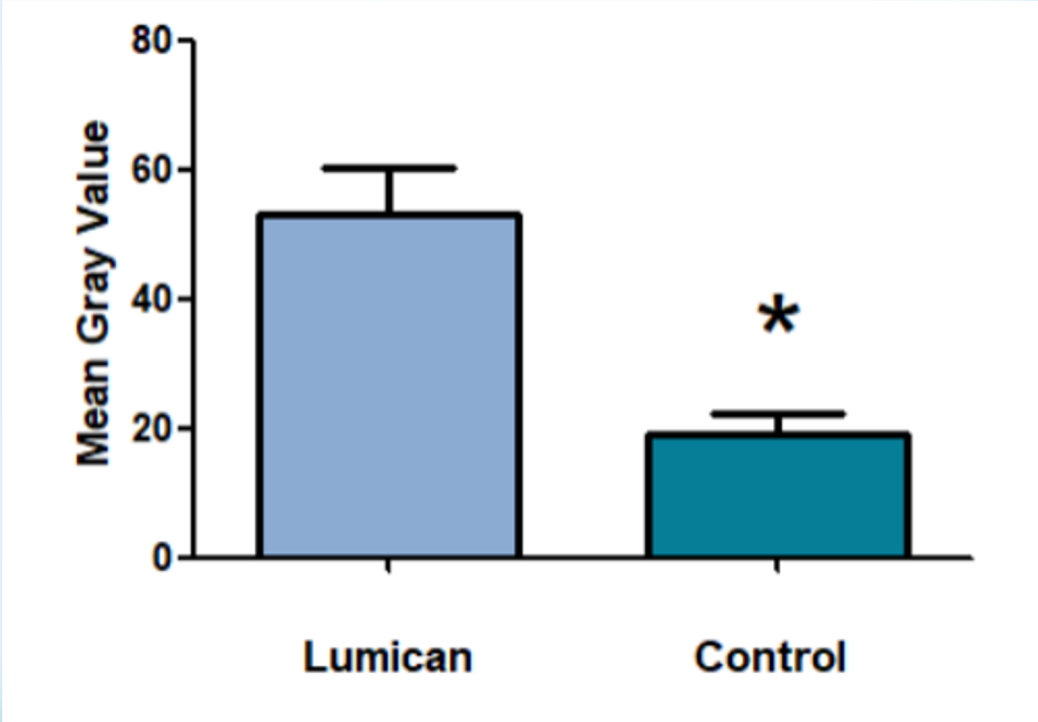
4. Results

LUMICAN



4. Results

In our results, when we stained the lipids with Nile Red, we observed that the presence of lumican impaired lipid droplet accumulation as you can see in the confocal images, and In fact, as you can see in the graph, the total lipid content was significantly reduced in response to the lumican.



5. Conclusions

In conclusion, the results obtained in this work indicate that:

1. 3D culture through the use of collagen matrices allows the growth of fibroblasts and their subsequent differentiation into mature adipocytes including the extracellular component, which is absent in conventional models (2D).
2. The combination of collagen matrices together with PGs, such as lumican, make it possible to generate modulable 3D models that make it possible to reproduce, in part, the pathophysiological conditions and to study the impact that the extracellular matrix exerts on cell function in pathologies such as fibrosis.
3. High concentrations of lumican, as occurs in the adipose tissue of obese individuals with insulin resistance, alters the accumulation of lipids in adipocytes.

Our results support the idea that lumican concentration imbalance, as occurs in obesity and insulin resistance, could contribute to the pathogenic effects of fibrosis on adipocyte function.

Acknowledgements

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