New Stemsation:

A COMPREHENSIVE ANALYSIS OF ADIPOSE STEM CELLS

DOCTOR OF PHILOSOPHY FACULTY OF SCIENCE UNIVERSITY OF TECHNOLOGY SYDNEY

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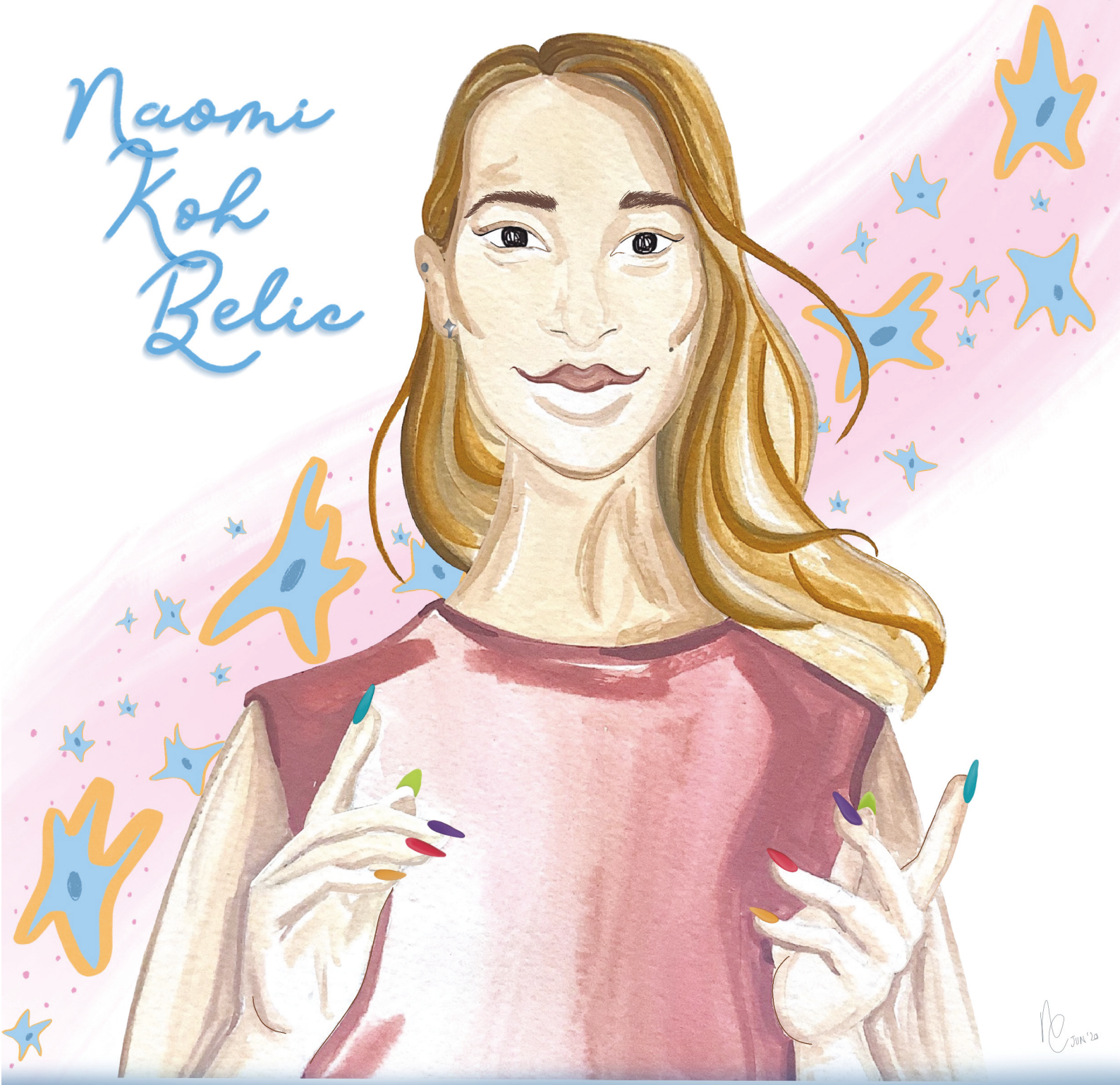

I, Naomi Koh Belic, declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Science Faculty at the University of Technology Sydney. This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research is supported by an Australian Government Research Training Program.

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Over the last eight years that I've been at the University of Technology Sydney my life has been tumultuous. But there has always been one place that's remained with me through it all. I've always found comfort on a tiny red plastic stool, waiting under the dusty grapes hung up on the roof, littered with Christmas decorations that have never been taken down, the giant deer rug staring into my soul… sometimes my mind wanders, and I remember the round aquarium on the wall that housed a dead goldfish for years. I'm never left with my thoughts for long before my wobbly table is piled high with steamed egg and chive dumplings, hand sliced noodles with fragrant spicy sauce and special braised eggplant. The trifecta that I fell in love with when I had first moved to Sydney at the big age of 17. Chinese Noodle House has seen me through my highest highs while I sloppily scoffed dumplings with a plastic cup full of wine in hand surrounded by friends, and in my absolute lowest of lows when it offered a place where I could come on my own and find joy in the food in front of me. Thank you for offering stability amongst your chaos and for always keeping my belly and heart full.

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Abstract

Stem cells are defined by their ability to self-renew and differentiate into multiple cell types. Because of this, they have potential to repair or replace damaged tissue and are of great interest for regenerative medicine which is advancing at an astonishing rate. However, patient hope is also being exploited by predatory clinics offering unproven stem cell treatments. These treatments have little to no scientific evidence of safety, let alone efficacy and are detrimental to scientific progression. The growth of unproven autologous stem cell treatments can be attributed to the realisation that liposuction can be used to extract stem cells. As adipose stem cells are being used in both proven and unproven treatments it is vital to understand how they function. This project successfully characterised the proteome of adipose stem cells through the analysis of the whole cell lysate, membrane bound fraction, extracellular vesicles and select secreted cytokines. Isolation and cryopreservation techniques were also investigated as there is a lack of standardisation in these areas. When comparing traditional and xeno free media for the isolation and expansion of adipose stem cells, there was a distinct shift in the proteome, and this is largely associated with proteins involved in metabolism. Furthermore, the cytokine profiles were wildly different, and the absence of vital stem cell cytokines suggest that traditional media may be preferential, however substantially more research is required to validate this. It was revealed in this study that cryopreservation also causes a shift in the adipose stem cell proteome, albeit not as distinct as the one observed from different media. This proteomic shift also manifests in the metabolome with evidence of particular pathways being altered after cryopreservation, but again this requires further validation. Metabolites involved in the kynurenine pathway were further investigated as this pathway accounts for the metabolism of more than 95% of tryptophan. This is the first study to confidently demonstrate that the kynurenine pathway of tryptophan metabolism is activated by IFN-γ in human adipose stem cells. Lastly, because it is the responsibility of scientists to ensure that science is accessible for everyone, the use of digital media for scientific education was explored. The knowledge from this project can be adapted to produce better scientific educational video content, and should be employed to educate the broader community about stem cell therapies, as it is known that patients use digital media to access unproven treatments.

Abbreviations

ASC adipose stem cell

BMSC bone marrow stem cell

DMEM Dulbecco's modified eagle medium

ESC embryonic stem cell

FBS fetal bovine serum

FGF fibroblast growth factor

FSC fetal stem cell

G-CSF granulocyte colony-stimulating factor

GM-CSF granulocyte macrophage colony-stimulating factor

HSC hematopoietic stem cell

IDO 1 indoleamine 2,3-dioxygenase

IFN interferon

IL interleukin

IP interferon gamma-induced protein

KMO kynurenine 3-monooxygenase

LC-MS/MS liquid chromatography coupled with tandem mass spectrometry

MCP monocyte chemotactic protein

MIP macrophage inflammatory protein

NSC neural stem cell

PGDF platelet-derived growth factor

RANTES regulation on activation normal T cell expressed and secreted

TDO tryptophan 2,3-dioxygenase

TNF tumour necrosis factor

VEGF vascular endothelial growth factor