Insect Cell-Based Cellular Agriculture Platform for Production of Custom Triglycerides

By

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Dedication:

To my parents, Cengiz and Beyhan

My sister, Merve

And to my husband, Yeldar

For their support, patience and guidance.

STATEMENT

I, Asli KAYA, declare that the work presented in this dissertation is original. This work has not been submitted for a degree at any other university, and this research was supported by the Research Training Program.

During the preparation of the thesis the author used Copilot and ChatGPT for the purposes of text enhancement. The use of this generative AI tools includes Introduction, Literature review and Future work parts for paraphrasing, sentence structure, spelling. The author confirms that where text was modified by generative AI, the content was reviewed for possible errors, inaccuracies, and bias. The author takes full responsibility for the submitted thesis and ensures the work is their own and has used generative AI within the parameters of use.

Asli KAYA

July 2025

AUTHORSHIP ATTRIBUTION STATEMENT

I, Asli Kaya, designed and performed the experiments, generated and analysed the data and wrote the manuscripts. Miss Helen Power helped me to establish the cell cultures, and Dr. Nick Proschogo helped me with GC-MS analysis. This work would not be possible without the support and dedication of Dr. Peter Valtchev and Dr. Valeria Messina.

Asli Kaya

July 2025

As supervisor of the candidature upon which this thesis is based, I, Dr. Peter Valtchev, can confirm that the authorship attribution statements above are correct.

Dr. Peter Valtchev

July, 2025

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ABSTRACT

The increasing demand for sustainable and ethical alternatives to traditional animal farming has driven the exploration of cultured meat as a promising solution. However, significant challenges, including gaps in stem cell biology, extracellular matrix formation, scalability, cost of production, and consumer safety, hinder its commercial viability. One of the critical limitations of cultured meat is the absence of structured fat, which is essential for achieving the desired sensory and textural properties.

Insect cells have emerged as an alternative platform for cellular agriculture due to their high cell density, resilience to environmental stressors, and ease of scalability. However, the incorporation of structured fat within insect cell-based systems remains an unaddressed challenge. This study examines a novel method for modifying the lipid composition of insect cells by enriching them with specific free fatty acids. The overall objective was to develop a viable approach to produce triglycerides with a profile resembling that of conventional animal fat, thereby enhancing the sensory and functional properties of cultured meat.

The research focused on understanding the metabolic fate and distribution of supplemented free fatty acids in model insect cells. Preliminary findings demonstrated that insect cells can effectively incorporate and modify fatty acids, making this a viable strategy for improving fat composition in cultured meat systems. Key research questions addressed in this study included the extent of fatty acid incorporation, the biochemical transformations within the cells, and the impact of the chosen fatty acid carrier on cell morphology and viability.

The study investigated a novel strategy for enhancing the fat component in cultured meat by modifying the lipid composition of insect cells. Trichoplusia ni (Hi-5) cells were enriched with exogenous oleic acid (OA) at varying concentrations (0.1, 0.2, and 0.3 mM) to evaluate cell viability, fatty acid uptake, incorporation, and metabolic transformation. Cells were assessed

for viability, morphological changes, and intracellular lipid accumulation using microscopy, flow cytometry and GC-MS lipid profiling.

The results showed that OA loading significantly increased lipid content, particularly at 0.1 mM, which also yielded a uniform lipid uptake pattern. Higher concentrations resulted in reduced cell size and increased granularity, indicating cytotoxic stress and heterogeneity. Lipid analysis confirmed the transformation of OA into other fatty acids, particularly palmitoleic acid, highlighting active metabolic processing within insect cells.

These findings demonstrated that insect cells can be tailored through fatty acid supplementation to produce lipid profiles resembling animal fat. This approach offers a promising avenue for overcoming the fat-related limitations of cultured meat, bringing the field closer to developing nutritionally and sensorially complete meat alternatives. Future work should focus on optimising fatty acid combinations, improving delivery systems, and scaling up the process for commercial applications.

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Chapter 1. Introduction

The increasing global population, climate change, and environmental sustainability concerns have added significant pressure on traditional livestock farming and the food industry. The livestock industry is a major contributor to greenhouse gas emissions, deforestation, and excessive water usage, making it an unsustainable option for meeting future protein demands. To address this issue, alternative protein sources and sustainable food production systems are gaining attention[1].

Cultured meat, also known as lab-grown or cell-based meat, is produced through the in vitro cultivation of animal cells, bypassing the need for traditional animal farming. This innovative method has been explored in various species, including bovine, porcine, and insects[2].

Despite its potential as a sustainable alternative to conventional meat production, the development of cultured meat faces several significant challenges, including limited understanding of stem cell biology, difficulties in forming the extracellular matrix, issues with scalability, high production costs, and concerns regarding consumer safety[3]. Consumer acceptance also remains low, largely due to the absence of fat-driven sensory attributes that contribute to the tenderness, juiciness, and flavor of meat products. Because fats play a crucial role in enhancing these sensory qualities (serving as key drivers of texture, juiciness, and the transport of aroma and flavor components) addressing these limitations is essential for the successful commercialisation and widespread acceptance of cultured meat[4].

Insect cell culture is emerging as a promising alternative because of high-cell density, no need for heating, resilience to pH changes, shear and osmotic stress and easy scalability. There is a large, unbridged gap between cells grown in culture and structured tissues such as muscle, and the most significant missing factor is the lack of the fat component that is essential for the textural and sensory properties of meat, such as mouth feel and flavour. The co-culture of

adipocytes and muscle cells is complex, and further research is required to understand the structured extracellular matrix and distribution of fat tissue[3].

Implementing cell culture technology in the food industry presents a sustainable and cost-effective method for providing high-protein nutrition to a growing global population. This approach can significantly reduce the environmental impacts associated with traditional livestock farming, including greenhouse gas emissions, land use, and water consumption. By decreasing reliance on conventional animal agriculture, cell culture technology has the potential to enhance food security and promote a more sustainable and resilient food system[1].

Objective:

The objective of this research was to investigate the possibility of modifying the present fatty acid profile in insect cells. Exogenous fatty acids can be integrated into insect triglycerides without extensive modification, preserving specific structural diversity. These fatty acids would then be transformed into other fatty acids with distinct chemical structures and functionalities, which might result in enhancing the sensory and textural properties of cultured meat products. This approach aims to improve the overall quality and consumer acceptance of cultured meat by addressing the sensory attributes typically associated with traditional meat products in future.

Research Question:

Can the incorporation of exogenous fatty acids into insect cells alter the fatty acid profile in a way that might improve the sensory and textural qualities of cultured meat?

Hypothesis:

Introducing exogenous fatty acids into insect cells will modify their triglyceride composition, leading to the production of structurally diverse and functionally distinct fatty acids that can enhance the sensory and textural properties of cultured meat, thus increasing its resemblance to traditional meat products.

Rationale for the choice of oleic acid:

Oleic acid (OA) is a monounsaturated fatty acid that serves as an excellent model compound for research. Cells can convert OA into a wide range of other fatty acids through desaturation, elongation, and saturation pathways, making it a foundational precursor for more complex lipid species. Its biological relevance extends beyond metabolism as OA is the most abundant monounsaturated fatty acid found in animal fats, where it strongly influences key sensory attributes of meat, including mouthfeel, juiciness, and overall flavor perception. This makes it particularly valuable for studies related to food science, fat composition, and sensory quality.

In addition to its metabolic and sensory importance, OA is highly compatible with albumin-based delivery systems. Albumin naturally binds fatty acids, and OA has a well-established affinity for several of its major binding pockets, including FA2, FA4, FA5, and FA7. This strong and predictable binding behavior ensures efficient transport and controlled release in biological systems. Compared with many other fatty acids, OA also exhibits relatively low cytotoxicity, which makes it safer and more practical for in vitro experiments where maintaining cell viability is essential. Altogether, these characteristics position OA as a versatile, physiologically relevant, and experimentally convenient fatty acid for a wide range of scientific applications.

This thesis is divided into four main sections:

Chapter 1 offers an extensive review of traditional agriculture, highlighting the necessity for innovative techniques in alternative food production. It examines cellular agriculture and cultured meat, with a focus on the potential integration of insect cells to enhance the functionality of cultured meat products.

Chapter 2 details the experimental design, materials, and methods employed in the research.

Chapter 3 presents the results and discussion, encompassing all the research conducted in this thesis.

Chapter 4 concludes the thesis, providing recommendations for future research and outlining potential directions for further investigation.

1.1. Literature review

Introduction

The dramatic growth of the global population, coupled with decreasing land availability, poses significant challenges to sustaining a viable future. Traditional agriculture is confronted with numerous issues, including pesticide and fertiliser contamination, depletion of groundwater levels, soil degradation, and a declining interest among the younger generation[4] .While the use of crop residues as biomass can enhance soil fertility and crop productivity, the management of nitrogen compound pollution remains inefficient. Consequently, these practices contribute to rising ecosystem temperatures and altered species distributions, with some species facing the risk of extinction[5] . Addressing these challenges requires innovative and sustainable agricultural practices to ensure food security and environmental health for future generations.

When referring to conventional agriculture in the context of alternative production techniques, it typically encompasses practices that are unsustainable and environmentally damaging. These practices are often characterised by large-scale operations, increased greenhouse gas emissions, and the dominance of major agricultural corporations. This comparison highlights the need for more sustainable and environmentally friendly agricultural methods[6].

There is a pressing need for alternatives to traditional animal farming because of the ethical, environmental, and sustainability concerns(**Figure 1.1.**)[7] , and projected future demand for high-protein food. In addition to consistent challenges with traditional agriculture, there is hope for a novel technological solution. The most important is cellular agriculture, a novel food production technology using in vitro cell culture[8]. Fundamentally, using this technology, scientists can produce cultured meat or muscle tissue outside the organism. This process starts with the extraction of stem cells from the animals. Then, these cells are cultured in a bioreactor

or culture tubes with the required nutrients and oxygen at the optimum temperature. Over time, the cells grow, divide and differentiate into tissue resembling animal meat[9] .

Although progress has been made with cultured meat during the last two decades, there is still more research and improvements to be carried out in the field, such as scaling-up problems due to high costs, cell differentiation limitations and the inability to produce fat, which is a crucial element in meat sensory specifications[9,10].

Furthermore, eating farmed animal meat or its meat products causes animal-borne diseases and antibiotic resistance in the human body[11]. Antibiotic overuse in food-producing animals, especially poultry, has fueled the rise of antibiotic-resistant bacteria and resistance genes that spread through the environment and food chains, threatening ecosystems and human health[12]. These resistant pathogens undermine treatments, prolong illnesses, increase healthcare costs, and could cause up to 10 million deaths annually by 2050. Researchers are investigating alternatives such as essential oils, nano-antibiotics, lactic acid bacteria, bacteriocins, predatory bacteria, and bacteriophages, which show promise in reducing reliance on conventional antibiotics and supporting safer, sustainable food practices[13]

According to the Food and Agriculture Organisation (F.A.O.) report, by 2050, the population will increase to ten billion, with an increase in food demand by 50 % in developing countries. Today, the number of malnourished people is around 815 million, and by 2050, this can increase to two billion. Developments in cellular agriculture could reduce those risks and increase consumer acceptance over time[14].

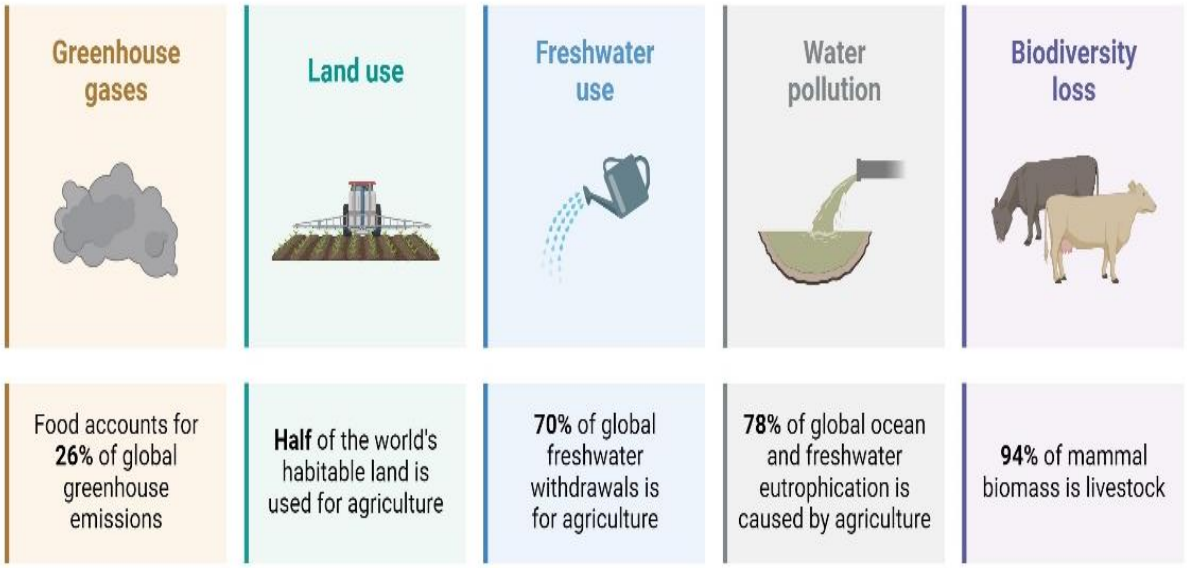

Figure 1. 1. Environmental effects of traditional meat production (Created in BioRender.com)[7,15].

1.2. Challenges in Cultured Meat Production

The term 'cultured meat' is becoming very popular because of public concerns about factory meats and the environmental effects of traditional agricultural processes. Cultured meat is obtained from some species' in vitro cell culture process instead of \farming physical animals (**Figure 1.2**). This in vitro process involves isolating adipose cells and muscle tissues from animals, culturing them in a proper serum-free growth medium, and differentiating the tissue with a scaffold system[9] .

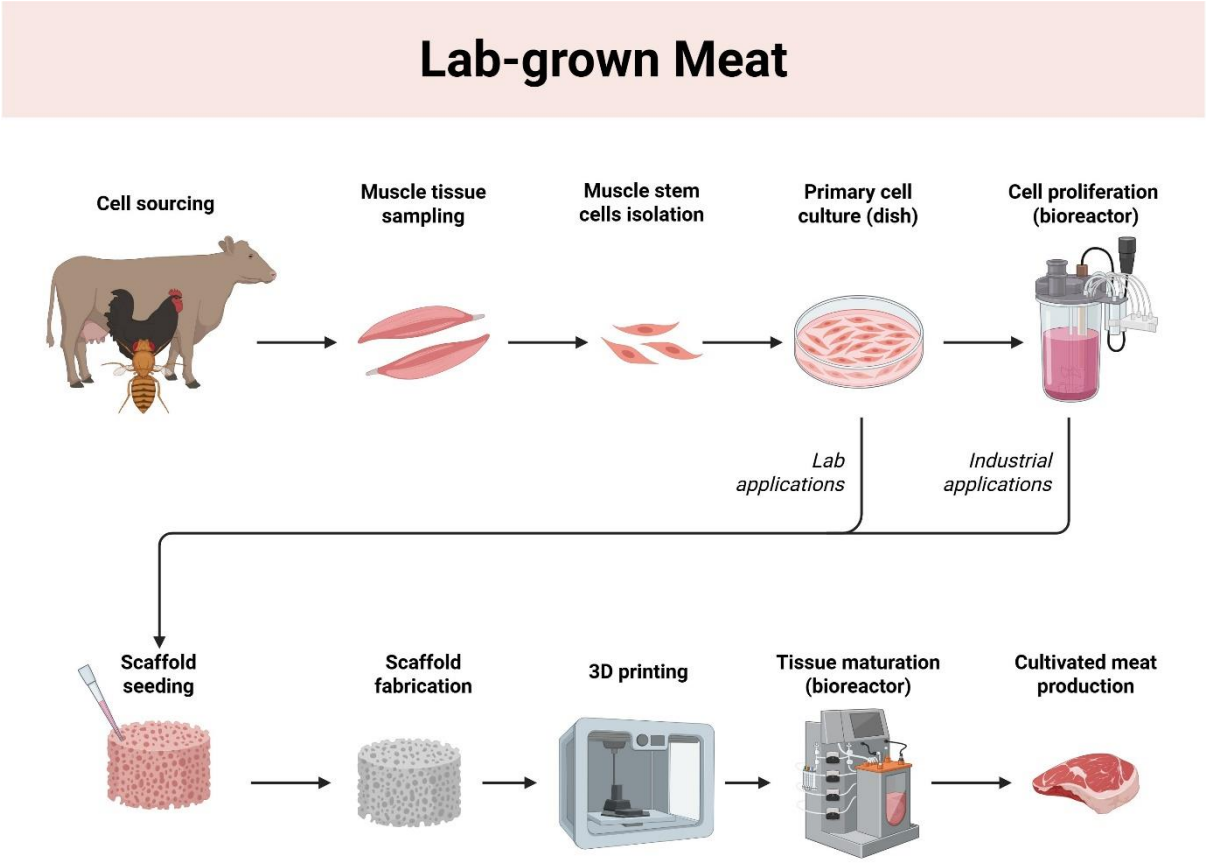

Figure 1. 2. Summary of the lab-grown Meat Production (Created in BioRender.com).

The first lab-grown meat was reported by Professor Mark Post at the University of Maastricht in Amsterdam in 2013. In 2016, the world's first cultured meatball was produced by Memphis Meat[16] . **Table 1.1** shows different types of meat sources that are being used for cultured meat production in different countries. The cultured meat industry is expected to share 40 % of the global meat market in 2040, with a decrease in the cost component[17] .

Table 1. 1. Several types of meat sources are being used for cultured meat production in different countries[17,18].

Source	Company	Product	Establishment Year	Country
Chicken	Just	Chicken Nugget	2019	USA
	Memphis Meat	Chicken Tender	2017	USA
	Peace of Meats	Chicken Nugget	2020	Belgium
	Future Meat Tech.	Shawarma	2019	Israel
Duck	Memphis Meat	Nugget	2019	USA
	Gourmet	Foie Grass	2020	France
Beef	Mosa Meat	Burger	2013	Netherlands
	Memphis Meat	Meat Ball	2016	USA

The transition from conventional animal slaughtering to cultured meat production results in significant environmental benefits, including a reduction in greenhouse gas emissions by approximately 76%, decreased deforestation, and a lower water footprint[19]. However, this shift necessitates the development of specialised bioreactor designs, optimisation of production processes, and stringent control of conditions such as temperature, pH, pressure, gas levels, cell density, and cell viability[15].

Despite these advancements, the production of cultured meat remains costly, often up to four times more expensive than traditional meat. Additionally, the regulatory framework for cultured meat is still evolving and requires further refinement[15]. Moreover, establishing a cultured meat market in countries with established traditional meat industries poses significant challenges. These include overcoming cultural preferences, economic barriers, and the need for substantial investment in infrastructure and technology[7,15].

Consumer acceptance of cultured meat compared to traditional animal meat remains a major concern. In order to gain widespread acceptance, cultured meat must closely replicate animal meat in texture, aroma, juiciness, taste, and appearance. One of the main problems in cultured meat is the lack of haemoglobin protein to form the red colour of meat in order to minimise this, companies apply colour agents and animal haemoglobins to overcome this issue in cultured meat[20] . The most crucial sensory deficiency of cultured meat is the lack of because of the lack of fat tissue. Fat content in the meat provides juiciness, tenderness and marble structure, which are desirable for consumer acceptance[21,22] . In general, cellular agriculture and cultured meat could allow humans to produce more food with less land, improve human/animal health, and reduce the environmental effects of consumption. There are still significant challenges to overcome. However, the scalability of cultured meat production remains a major hurdle, as current methods are expensive and require advanced bioreactors, specialised growth media, and high energy inputs. Additionally, consumer acceptance is a key factor as many people may be hesitant to adopt lab-grown meat due to concerns about taste, texture, safety, and the perception of it being "unnatural."[21].

1.3. Edible Insects as an Alternative Food Source

For decades, edible insects have been used as a part of a daily diet in countries in Asia, Africa and America, and now, insects are considered a novel source of protein in Western countries[23–25]. The United Nations has promoted the consumption of insects in the daily diet, especially in Western cultures, as the insect sources are rich in nutrients, such as protein, fat, and minerals[26,27].

Fats are the second major nutrient in edible insects after protein and contain a considerable amount of Omega-3 and Omega-6 that are essential for human body functions[28,29].The fat profile consists of triglycerides, phospholipids, sterols and fat-soluble vitamins as in animal

structure[30]. The triglycerides are the predominant group in insects, and they consist of saturated fatty acids, unsaturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids in their chemical structure[31,32]. In some edible insects, the most dominant saturated fatty acids are palmitic, stearic and arachidonic acids[33–35]. Furthermore, oleic acid and palmitoleic acid are the most common monounsaturated fatty acids in the triglycerides of different insects[33]. Most insect species possess high amounts of unsaturated fatty acids, as the content of polyunsaturated fatty acids of mealworm oil is higher than beef and pork, respectively[36]. Humans or other animals cannot synthesise essential fatty acids, particularly linolenic and linoleic acids, so insect fat could be a functional fat source for food[37].

More than 1000 edible insect species have been discovered to date, and they have different fatty acid content and proportions in dry mass. The fatty acid content and amount in insects are affected by species type, development phase and insect feed source[38].

Although some insect species are rich in omega-3 and omega-6 fatty acids, others are abundant in stearic and oleic acids. The fatty acid profile of insects can be modified by manipulating various factors, allowing them to provide essential fatty acids as an alternative fat source in human diets. This is particularly beneficial when used in cultured meat production, enhancing the nutritional value and sensory qualities of the final product[39].

1.4. Insect cells

Historically, insect cells have been primarily utilised for the production of recombinant proteins. However, over the past two decades, they have garnered attention for their application in large-scale cell culture processes. The primary reasons for their selection include their ability to easily adapt to serum-free media, which lacks animal components and thus reduces costs. Additionally, insect cells can achieve higher cell densities than mammalian cells, have lower

nutrient requirements, grow in ambient conditions without the need for CO2 supplements, and offer cost-effective production[40].

The commonly used insect cell lines are Sf-9, an ovarian tissue from Spodoptera frugiperda and Hi-5 cells, isolated from ovarian tissue of the Trichoplusia ni, cabbage looper[41] .Especially in the last decade, they have been used as commercial products such as flu vaccine (FluBlock, used Sf-9 cells), the cervical cancer vaccine (Cervarix, Hi-5 cells used), and a COVID-19 vaccine (NovaWax, Sf-9 cells were used[42–45]. The study provided a robust perspective that insect cells can be used in food products, as it has been proved that they are also commercially usable.

Furthermore, with the novel technologies, it is possible to develop insect-based food to reduce malnutrition in the future. Firstly, researchers from Wageningen University introduced insect cell-based edible proteins in 2007. The main driving factor for the idea was that insects are nutritionally advantageous as they have high protein levels and can be adapted to different growth conditions[42].

After the term 'cultured meat' gained its reputation, insect cells were considered favourable over traditional cells as they are highly adaptable in ambient growth conditions and provide the most cost-effective scale-up options[1]. The rationale behind the idea of growing insect cells for food (enthomoculture) is not just to provide edible foods, but also to provide insect cells as an ingredient in cultured meat products, to provide sensory and textural features[1,46].

1.4.1. Insect cell metabolism

1.4.1.1. Lipid Biochemistry

Triglycerides (TG) can also be called triacylglycerols (TAG), and they represent the highest energy source as they contribute 9 kcal/g metabolic energy for muscle or molecules in adipocyte[47]. Triglyceride combines glycerol and three molecules of fatty acids with different

chain lengths and structures (**Figure 1.3**). The glycerol molecule has three hydroxyl groups (HO), and fatty acid has a carboxyl group (HOOC-) in its structure. For the triglyceride formation, the hydroxyl groups of the glycerol bind to the carboxyl groups of the fatty acids with ester bonds. Monoacylglycerols (MAG) are formed of glycerol and one acyl group, and those with two acyl groups are diacylglycerols (DAG), and with three acyl glycerol molecules called triacylglycerols or triglyceride molecules (TAG)[47]. TGs can be a form of different-length fatty acids mostly 16, 18, and 20 carbon atoms. Medium and long-chain triglyceride (MLCT) is a vital lipid type that consists of medium-chain triglyceride (MCT) that is prepared by esterification or interesterification and MLCT consists of both medium-chain and long-chain fatty acids that are bound to the same glycerol molecule. Due to its small size and higher solubility, MCT can be quickly transferred to the liver rather than LCT[48,49]. Because of this, LCT has a lower energy and metabolism rate as it needs to join the cycle in the intestinal cells and be transported as chylomicron into the portal circulation before exposure to the beta-oxidation process that would result in ketone production as an energy source. For this reason, it is essential and understandable to use MCT in MLCT to improve its metabolic content and nutritional properties[50].

Fatty acids can be formed into different positions of the glycerol backbone with different or similar chain length fatty acid molecules, so MLCT configurations can be different as MLM, MML, LMM, LLM, LML, MLL (L for long-chain fatty acids and M stands for medium-chain fatty acids). It has been reported that the positions of fatty acids in TAG molecules affect metabolism speed and metabolic digestion; hence, heterogeneous TAG structures and positions must have different digestion and absorption specifications[47]. Each carbon atom in the glycerol molecules is numbered using a "stereospecific numbering (sn) system." As seen in the image below, one fatty acid molecule is attached to the -sn1 position, the second fatty acid can be attached to the -sn2 position and the third group is attached to the -sn3 position[51]

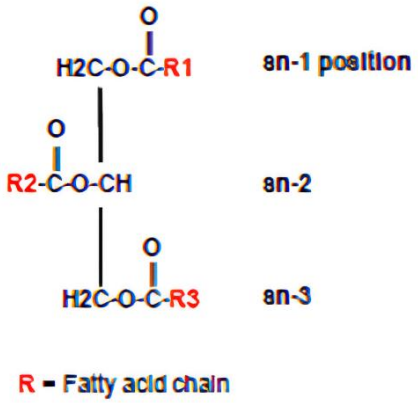

Figure 1. 3. Structure of triglyceride molecule[50]

Saturated fatty acids usually have single bonds with an even number of carbon atoms and are all saturated with hydrogen. They can also be obtained from animal fat and plant oil. They mostly contain 12-22 carbon atoms in their chain. Monounsaturated fatty acids have one carbon-carbon double bond in the chain, and the double bond can be positioned in several positions depending on the fatty acid's nature. They primarily consist of 16-22 carbon atoms and have hydrogen atoms positioned in the same direction of the double bond as the cis configuration. The isomer structures of cis configuration, which are trans forms, may occur during fatty acid saturation processes of oils in industrial production. Those two configurations cause some differences in oil stability in industrial processes and the double bond itself, also restricting the chain's mobility. The cis-formed fatty acids are less stable in the thermodynamic processes and have lower melting points than trans fatty acids[52]. Polyunsaturated fatty acids (PUFA) are classified as simple fatty acids, containing two or more cis-formed double bonds in the carbon chain. They can be categorised as ω-3 and ω-6 fatty acids. In PUFAs, if the first double bond can be placed between the third and fourth carbon atoms, they are called ω-3 fatty acids and alpha-linolenic acids are the leading representative of this group as they are essential for human dietary intake. The other PUFA group is called ω-6 fatty acids and the first double

bond is between the fifth and sixth carbon atoms and linoleic acids are the most common fatty acids in this group[53]. Polyunsaturated fatty acids can be obtained from vegetable oils and fish as they are not synthesised in the human body, so they need to be consumed in the daily diet[52,54].

1.4.1.1.1. Biosynthesis of Triglycerides

Eukaryotic and some prokaryotic organisms can synthesise triglycerides in their cells or organs, and for animals, the liver, intestines, and adipose tissues play significant roles in maintaining this process[55]. Triacylglycerols are stored as lipid droplets that are enclosed by phospholipids and hydrophobic proteins in adipose tissues. These lipid droplets can be identified in different metabolic breakdown pathways characteristic of every droplet. Lipid droplets can be used as a fatty acid store to provide energy when needed, also they can play a role in body structure as they are precursors of some of the vitamins[56]. As stated previously, every human needs to get or synthesise the required number of triglycerides; an excessive dosage of them would be harmful to the body as they can accumulate in adipose tissues in the human body, and it might cause obesity or insulin resistance[57].

1.4.1.1.2. Triglyceride biosynthesis pathways

Triglyceride biosynthesis consists of three main pathways these are, dihydroxyacetone phosphate, sn-glycerol-3-phosphate, which occurs in liver and adipose tissues, and a monoacylglycerol pathway which takes place in the intestines[58]. Several authors have reported that the essential part of triglyceride synthesis is sn-glycerol-3-phosphate or in other terms Kennedy pathway. This synthesis produces most of the liver triglycerides. Triglyceride biosynthesis reactions starting from Acetyl-CoA, with the Kennedy pathway[59], can be seen in **Figure 1.4**. In eukaryotic organisms, triglyceride biosynthesis starts from Acetyl CoA and after seven times of condensation, hydrogenation, dehydration, and hydrogenation, palmitic acid can be synthesised and with further dehydrogenation and chain extension, other unsaturated fatty

acids can be synthesise[53]. The primary source of the fatty acid backbone is produced by the catabolism of glucose through glycolysis.

Several researchers have reported that glycerol is mainly produced by the glycogenesis process with pyruvate[60]. The following reactions take place mainly in the endoplasmic reticulum; firstly, to form lysophosphatidic acid, the precursor sn- glycerol-3-phosphate needs to be esterified by fatty acid coenzyme A with glycerol-3-phosphate acyltransferase (GPAT) enzyme catalysis, followed by the biosynthesis of glycerolipids or phosphatidic acid by acyl glycerophosphate acyltransferase (AGPAT) enzyme. The enzymes in the biosynthesis of triglycerides can be found as specific to different tissues or cells that enable them to express and regulate the reactions in several ways[61].

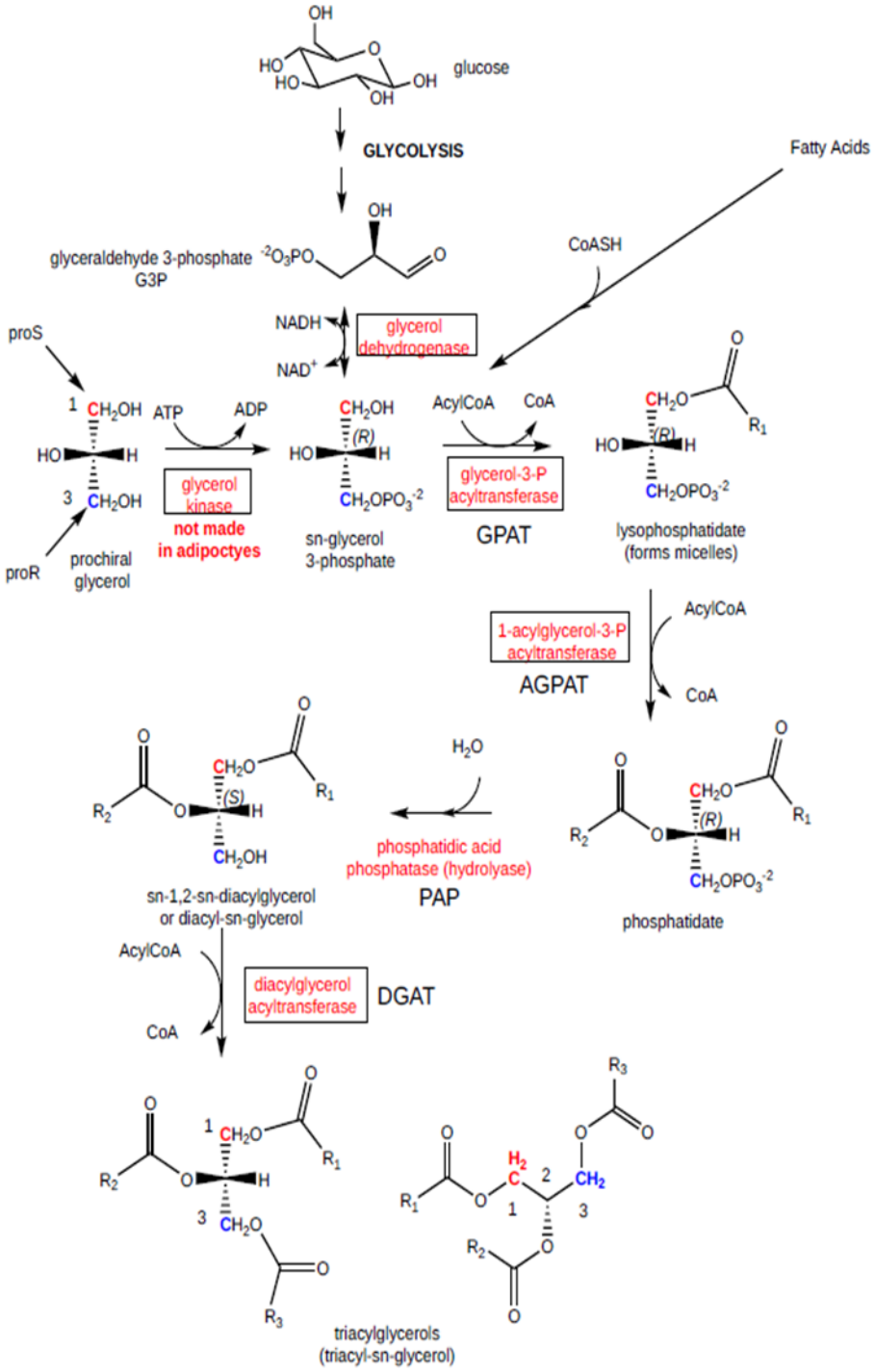

Figure 1. 4. Triglyceride biosynthesis pathway from glycerol-3-phosphate[62]

In most cells, Glycerol-3-phosphate acyltransferase (GPAT) enzymes take place to catalyse the biosynthesis process, and as its activity is slow, it can be described as activity-limiting for the biosynthesis pathway. GPAT enzyme has four different isomers in mammalian cells, and they

can be inhibited by AMP-activated kinase (AMPK). In the cells, over-synthesis of the GPAT enzyme may cause overexpression of triglycerides and reduction of β-oxidation processes[62]

Phosphatidic acid phosphohydrolases (PAPs) are the key enzymes essential to manage lipid biosynthesis for membrane biogenesis or storage purposes. They can be seen in the triglyceride biosynthesis pathways to form triacylglycerols, phosphatidylcholine, and phosphatidylethanolamine. Especially in animal tissues, these PAP activities take place in cytoplasmic proteins which are named Lipins (lipin-1, lipin-2, lipin-3), and they have characteristic activities in different tissues or organs as they can manage the biosynthesis process in adipose tissues, liver, or intestines. Lipins are taking place instead of the endoplasmic reticulum to adjust to the higher number of fatty acids in the cells[63].

As can be seen in **Figure 1.5,** the last step of the biosynthesis of triglyceride is the acylation of 1,2-diacyl-sn-glycerol by diacylglycerol acyltransferases (DGAT) enzymes and which can be classified as DGAT1 and DGAT2. In animal tissues, DGAT1 is mainly located in the endoplasmic reticulum, and it manages the biosynthesis process in the intestine and the liver with a high potential to use a wide range of substrates such as monoacylglycerols and retinol. It has also been reported that it has the potential to reduce the effect of high fat contained in daily diets[64]. DGAT2 enzymes can be found in adipose tissues and some regions of the endoplasmic reticulum or mitochondria[63]. After a meal, in the intestine, most of the triglycerides are synthesised via the monoacylglycerol pathway, and with pancreatic enzymes, dietary triglycerides undergo some reactions to form free fatty acids and can be uptaken by enterocytes. In these tissues, monoacylglycerols can be acetylated by acyl-coenzyme A, resulting in the formation of sn-1,2-diacylglycerols; also, 1-monoacylglycerols formation can be obtained with this reaction[64]. At the last stage, sn-1,2-diacylglycerols react with the DGAT1 enzyme to form triacylglycerols, as seen in **Figure 1.5**.

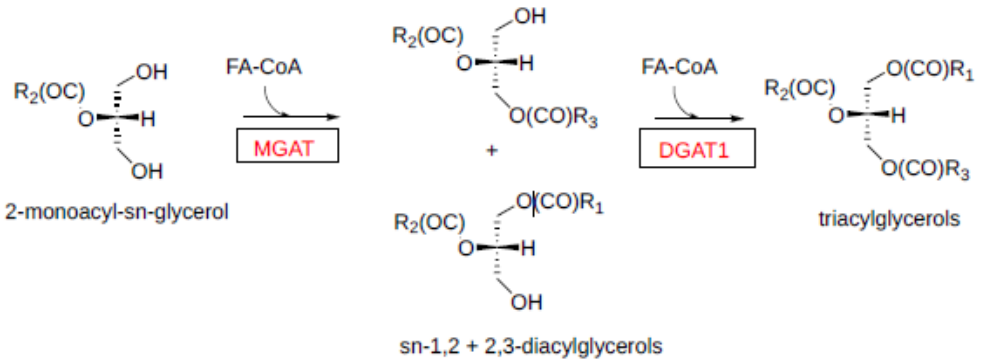

Figure 1. 5. Biosynthesis of triglycerides from monoacylglycerols[63]

1.4.1.2. Insect Lipid Metabolism

Insect cells have limited lipid metabolism, and they need lipids as a supplement inside the medium,but most of them are capable of synthesising essential fatty acids[1]. For instance, they cannot produce cholesterol to form cell membranes or hormones. These cells can use lipids as an energy source for reproduction and development, and lipid deficiency in the growth medium can cause cell degeneration. Hi-5 cells use the fat body as a coordination centre, similar to mammalian adipose tissue and the fat body is composed of stored glycogen and lipids to maintain body functions and synthesise hormones, enzymes and antimicrobial peptides[65]. Lipids can be served with serum albumin for in vivo analysis, but it is not a fatty acid-specific binding protein[66].

1.4.1.3. Carbohydrate metabolism

Knowing insect cell metabolism is crucial for preparing and supplying enough nutrition in a medium. It has been reported that cell type affects metabolic efficiency, and Hi-5 cells have more intense metabolic activity than Sf-9 cells[41,67,68]. Like mammalian cells, insect cells can

be grown with a medium that contains glucose as a primary carbohydrate source[41]. Glucose is the most crucial carbon and energy source for insect cell growth[69]. Several authors have reported that glucose is a preferred energy source compared to fructose and sucrose[70]. On the other hand, with the lack of glucose, cell growth decreases but cell types can remain viable if there are enough amino acid compounds. On the contrary, an excessive amount of glucose in the medium can cause the accumulation of lactate and alanine. When sucrose is added to the medium, it has been reported that Sf-9 and Hi-5 cells did not consume the sugar during the growth phase [71].

1.4.1.4. Amino acid metabolism

Insect cells can use amino acids as energy and biosynthesis sources, although most amino acids cannot be synthesised by cells. Cells can utilise glutamine, aspartate, serine, arginine, and glutamate[72,73]. In vitro studies showed that methionine addition in the medium could postpone cell death for Sf-9 cell lines. Methionine and cysteine were crucial for cell growth, and it was found that cystine was the only amino acid that killed the Sf-9 cells[73,74]. Furthermore, researchers recently revealed that insect cells can grow in a cysteine-free medium if the cell inoculum can be taken early as they metabolise methionine to produce cysteine[71,75]. It has been reported that glutamine deficiency affects cell growth rate and needs to be supplied in the media[71].

Hi-5 and Tn5 cells differ from Sf-9 cells in terms of amino acid consumption, as it has been found that Hi-5 cells consume asparagine very fast, and they require a significant amount of glutamine, cysteine, and tyrosine inside the medium. In addition, asparagine is used quickly by Hi-5 cells, and its deficiency inside the medium can cause the starting of the stationary phase of the cells; however the increased amount of asparagine does not affect their growth rate but also increases the consumption rate[71].

1.4.1.5. Cellular metabolites

Insect cells accumulate low lactate in the medium after glucose consumption. Contrary to mammalian cells. It has been reported that Sf-9 cells are not able to produce lactate in a medium with a glucose supply. Hi-5 cells accumulate higher amounts of lactate, usually between 10-20 mM, and Hi-5 and Sf-9 cells can consume the produced lactate with glucose deficiency in the medium[76]. On the other hand, it has been reported that lactate might be toxic to the insect cells as it can increase the osmolarity and change the pH of the medium. As a result of the lactate accumulation, clump formation can be observed in the medium even if the cells have already adapted to the environment[73].

Ammonia is another metabolic by-product produced from amino acid catabolism. Insect cells are not sensitive to ammonia like mammalian cells. It has been reported that adding ten mM ammonia to the medium does not affect the growth of Sf-9 cells, but adding 30 mM ammonia slightly affects Hi-5 cell growth. Also, Hi-5 cells can accumulate up to 20 mM ammonia during the growth phase, related to initial concentrations of asparagine and glutamine[73,77].

1.5. The Role of Fat in Sensory and Textural Properties in Meat

1.5.1. The effect of fat on food taste

The assessment of fat inside meat and meat food products highly depends on the consumer's sensory capacities, genetic and cultural backgrounds, the quality of the fat itself, and environmental considerations about the impact of the food. Consumer acceptance of meat products is highly related to their meat role in texture, flavour, and tenderness[78].

Tenderness and flavour in meat occur with the cooking, which involves different chemical reactions between fatty tissues during the cooking. Around 1000 volatile compounds are released during the cooking process. These volatile compounds are released from lipid degradation, and differences in volatile compound composition in different species are related

to the differences in lipid-sourced volatile compounds[79]. These compounds can be aldehydes, ketones, alcohols, hydrocarbons, carboxylic acids, and esters. Hydrocarbons have been reported to interact in lipid oxidation reactions[80]. These reactions occur with exposure to air and heating of the food product, and stale and rancid flavours might develop after the oxidation[80].

Oxidation of lipids in meats and meat products might be affected by several factors such as meat handling and processing, animal treatments before slaughtering, and meat cooking[81] . The first point of the lipid oxidation of the meat is the postmortem phase of the freshly slaughtered animals. At this stage, due to the initiation of the peroxidation, lipid oxidation can start, and the shelf life of the meat might be decreased as a result of the development of rancid flavours. In terms of chemical reaction, oxidation is a complex reaction series between fatty acid and free oxygen, starting the synthesis of hydroperoxides, which include aldehydes, ketones, alcohols, and acids that are highly responsible for the unwanted taste and flavours in the meat[82]. As a result, lipid oxidation in meats might have a negative effect on extended storage periods on meat flavour and customer acceptance, as the reaction increases the sulfur and rancid taste in the products[83,84].

1.5.2. Mechanism of fat taste

The term 'fat' refers to natural triglycerides, and it is a very important major component of the human daily diet with carbohydrates, proteins, and vitamins. Every diet must contain the required amount of fats to prevent the health effects of deficiencies or overconsumption of the component[85]. Overconsumption of fats might affect health, with an increased risk of diabetes and obesity. Also, insufficient dietary fat intake can cause skin lesions, premature ageing, and loss of healthy vision[86]. People must consume essential fatty acids in their daily diet to maintain body activities and further synthesise important bioactive cell compounds[87] .

People can associate fatty foods with the level of texture, oral irritation, creaminess, viscosity, and mouth fullness, as fat taste cannot be sensed by humans, as most triglycerides are insoluble in human saliva. Several researchers have reported that triglycerides are broken down to free fatty acids by the chemical reaction of lingual lipase, but fat does not have a clear perceptual taste as sweet, umami, bitter, or sour tastes[88,89]. On the other hand, it has been recommended that triglycerides only have a detection threshold in the human mouth, but there are some differences in terms of sense quality related to fatty acid types[90]. Short-chain fatty acids might have a sour taste, while long-chain fatty acids (>16 C) can have an unpleasant taste in foods[91]. The desired final profile of food and food taste could be prepared and designed by using the different contributions of long and short-chain fatty acid concentrations and besides this, the taste could be also improved with the consideration of saturated fatty acid (e.g., stearic acid), monounsaturated fatty acid (e.g., oleic acid) and polyunsaturated fatty acids (e.g., linoleic acid) combinations[92].

1.6. Targeted Approach: Modifying Insect Cell Fat Composition

There is a large, unbridged gap between cells grown in culture and structured tissues such as muscle, and the most significant missing factor is the lack of the fat component that is essential for the textural and sensory properties of meat, such as mouth feel and flavour. The co-culture of adipocytes and muscle cells is complex, and no one has achieved the formation of a properly structured extracellular matrix and distributed fat tissue. Using mammalian cells for cultured meat production has been defined with broad difficulties, and mammalian muscle cells do not entirely contribute to cultured meat's sensory and textural propertie[78].

There is still a lack of cell differentiation techniques and the creation of a defined medium for optimal cell growth. The muscle cells also do not have significant macro components such as glycogen and fat, which are vital to the food's taste and texture. Insect cells can easily grow in

ambient conditions without CO_2 gas, have higher adaptation skills to environmental fluctuations, and reach higher density than mammalian cells[1]. Modifying insect cell fat composition through a targeted approach involves manipulating the metabolic pathways and genes responsible for lipid synthesis and regulation in these cells. This is an interesting and growing area of research, especially in biotechnology, where insect cells are used for bioproduction, such as recombinant protein expression, and the production of high-value compounds, including lipids[41,93] .

This research aimed to modify insect cell fat composition by enriching it with free fatty acids to increase fat composition in insect cells.

Chapter 2. Materials and Methods

2.1. Insect cell culture

Trichoplusia ni (2×10^4 viable cells/cm^2) was seeded into T-75 (TPP® tissue culture flasks, Z707546, Merck) flasks under a laminar hood (MSC- Advantage™ Class II Biological Safety Cabinets, 51025411, Thermo Fischer). Insect cells were stored at 24-28 °C using a non-humidified incubator (MyTemp™ mini digital incubator, Z763357, Merck). Samples were monitored until they reached 90% confluency, corresponding to an observation of 9×10^6 viable cells/cm². Once this density was achieved, the cells were subcultured every three days at a 1:5 ratio of viable cells to fresh medium.(Express Five™ SFM, 10486025, Thermo Fischer Australia). This procedure was repeated consistently throughout the study, resulting in a total of 42 passages being performed.

Cell stock was prepared using cryopreservation media (45 % conditioned media+ 45 % fresh media+ 10 % DMSO, Recovery™ Cell Culture Freezing Medium, 12648010, Thermo Fischer) was added and centrifuged (Heraeus Biofuge Stratos High-Speed Benchtop Centrifuges) at 100–200 × g for 5 min to obtain a cell pellet. The pellet was resuspended with a cold freezing medium, dispensing the content as aliquots into cryovials. Cells were transferred to a nitrogen tank and stored at -80 ° C until analysis.

2.2. Cellular quantification

Cell number was performed using a microscope (model) at 20X magnification coupled with a Nikon Eclipse TS100 Inverted Routine Microscope. The cell number was calculated as follows (Equation 1):

$$\text{Total}\left(\frac{\text{cells}}{\text{mL}}\right) = \frac{\text{Total cells counted x Dilution factor x 1000 cells/mL}}{\text{The number of squares counted}} \quad \text{Equation 1}$$

Cell counting was performed with a hemocytometer (Bright-Line™ Hemacytometer, Z359629, Sigma-Aldrich.

2.3. Fatty acid loading of insect cells

A stock solution of 150 mM Oleic acid (OA) and ethanol (Oleic acid, O1383 , ethanol (Ethyl alcohol, Pure, 459836). Fatty acid loading in cells was performed with bovine serum (Bovine Serum Albumin solution (BSA), A7284) and fatty acid solution at a molar ratio of 5:1, respectively, where 0.4 mM BSA and 2.0 mM fatty acid molar ratios were added to 267 µl of 150 mM OA stock solution.

Figure 2.1 summarises the fatty acid loading procedure. Bovine serum albumin was weighed in duplicates in 50 mL Falcon tubes and cell culture medium was added. Samples were handshake for 1 min and vortexed for 1 min, and control samples were carried out using a cell culture medium. Then, a stock solution of 150mM was added to the fatty acid samples and ethanol was added to the control sample (320 µl) and then vortexed for 1 min. Tubes were placed in a shaker for 24 h at 37 ^{0}C and sterile filtered (pore size 0.22 µm, Millex™ PVDF syringe filter, SLGVR33RB, Merck**)** before the analysis. Oleic acid loading in insect cells was performed at different concentrations: 0.1, 0.2, and 0.3 mM oleic acid (OA). Vehicle control solutions (VC) were performed at the same concentrations to investigate the effect of the carrier(ethanol) on insect cells. The final ethanol concentration in the medium was 1.6% (v/v) in the fatty acid tubes and in the control tube. Samples were analysed each day for 3 days.

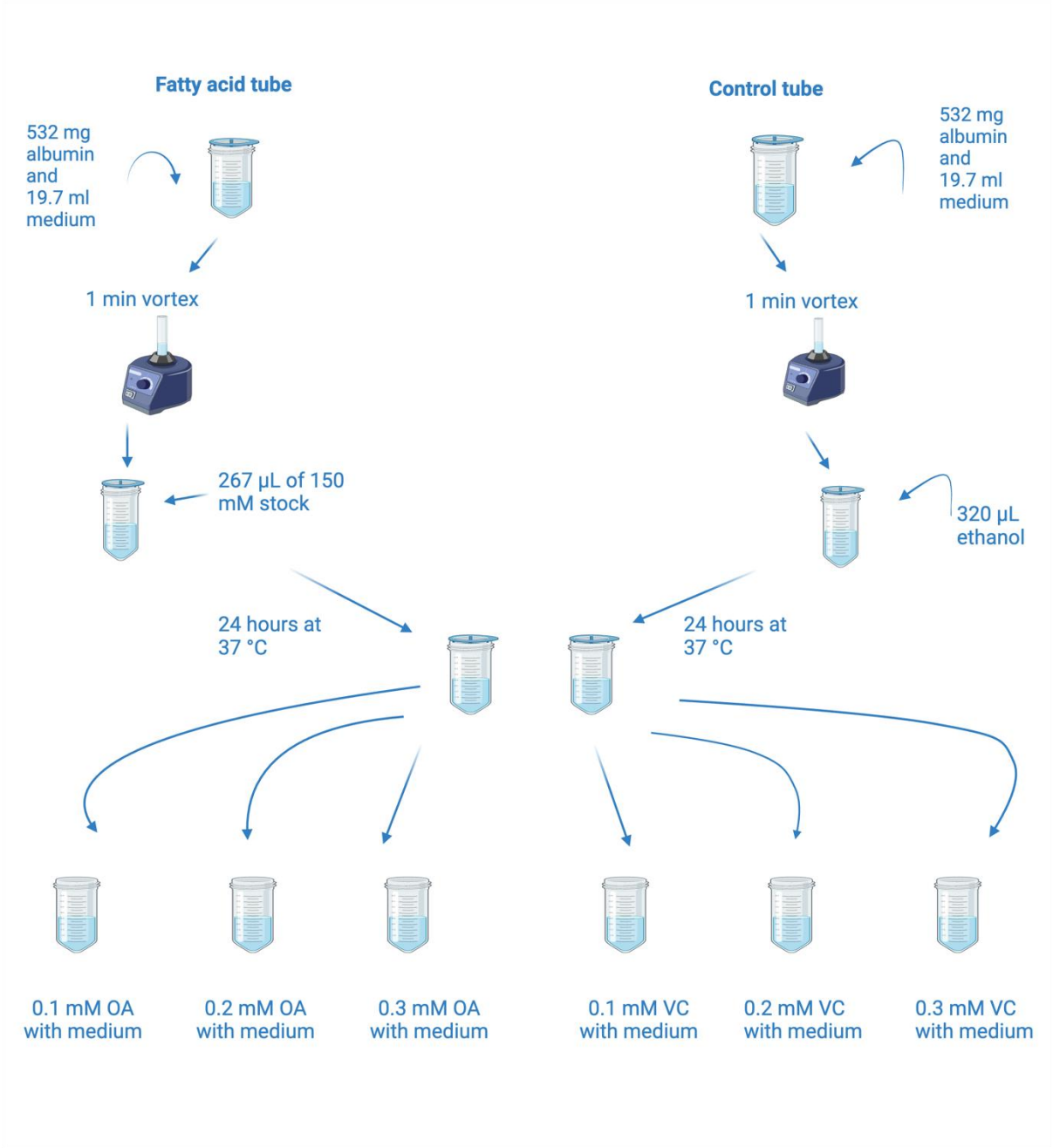

Figure 2. 1. Fatty acid loading procedure summary (Created in https://BioRender.com)

2.4. Flow-cytometry Analysis

Samples (control, OA and VC) were incubated for 15 min in the dark to improve cell proliferation and approximately 3 mL of cell suspension was collected. Then, samples were vortexed (Personal Vortex Mixer - VM1, IC-VM1) for 1 min and stained with Nile red solution. Samples were analysed using a flow cytometer to acquire a total number of 10000

events in the FacsCanto II Flow Cytometer (BD Bioscience). The flow cytometry was used at Ex 515nm / Em 585nm. Phospholipid-associated fluorescence was collected in the Ex 550 nm/ Em 660 nm channels. Forward scatter (low angle scatter, related to size) (FSC) and Side scatter (90-degree scatter, related to granularity (SSC) were analysed using BD FACSDiva 8.0 software.

Median fluorescence intensity was quantified from histogram plots that were gated to exclude autofluorescence, using unstained cells as the control. Cell viability was carefully monitored throughout the experiments and was precisely estimated using trypan blue during cell counting, ensuring that the majority of cells were alive prior to staining. Lipid accumulation in both triglyceride and phospholipid pools was quantified rigorously using flow cytometry with single-cell gating, combined with FSC/SSC-based exclusion of cell fragments and apoptotic bodies, minimizing the contribution of non-viable cellular material to the measured signal. Common viability dyes were not employed in parallel with Nile Red staining because their fluorescence spectra interfere with Nile Red's signal, complicating accurate lipid quantification. While the absence of a viability dye represents a potential limitation, the combination of precise viability assessment and careful flow cytometry gating provides confidence that the measured fluorescence predominantly reflects lipid content in viable cells.

2.5. GC-MS Analysis

Samples were centrifuged for 4 min at 5000 rpm and the pellet was transferred to an Eppendorf tube. Then, samples were submitted 5 min ultrasonicator for cell disruption, vortexed for 10 sec and then centrifuged for 20 sec to obtain cell pellets. This process was repeated three times, followed by freeze-drying (Epsilon 2-4 LSCplus). Dry cell pellets were treated with 500 µL of methanol (Sigma-Aldrich) and acetyl chloride (Acetyl chloride, 00990, Sigma-Aldrich), mixed and sonicated for 5 min. 330 µL hexane (Sigma-Aldrich) was added to the samples and heated at 60 ^{o} C for 1 h with a hot plate. Then, samples were cooled in an

ice bath for 10 min and washed with 330 µL MilliQ water.

To remove the organic layer, samples were vortexed (Personal Vortex Mixer - VM1, IC-VM1) for 10 seconds and centrifuged (LabCo Compact Micro Centrifuge, 400.003.005) for 20 sec and the supernatant was removed under a fume hood and dried using liquid nitrogen to remove the organic layer, then 100 µL of hexane was added to dissolve the lipids. The tube content was transferred to vials for GC-MS analysis. For this purpose, the PerkinElmer Gas Chromatograph Clarus 680 device was used, operating at an oven temperature of 300°C. The carrier gas flow rate was set to 1.30 mL/min, with a pressure of 10.5 psi and the injection volume of the sample was 1 µL. The initial temperature was set at 60°C and increased gradually to 300 °C, and the split ratio was 15 mL/m. A standard curve was performed 0 to 1% with Lauric acid (C:12) as internal standard. For each calibration point 50 µL of Lauric acid from a 1 mg/mL stock solution was incorporated to ensure precision. The relative peak area was calculated using the oleic acid ratio to the C:12 peak area (**Appendix Figure A.85**). Fatty acid content is reported as ratios normalized to oleic acid, as the purpose of the experiment was to assess overall changes in fatty acid composition rather than to obtain absolute quantitative measurements.

2.6. Statistical Analysis

Data collected were analysed with GraphPad Prism™ software (version 10.0 for Microsoft). Students' two-tailed test was used to compare two groups, and ANOVA analysis was used with Tukey's Test. p-values < 0.05 were considered statistically significant.

Chapter 3. Results and Discussion

3.1 Cell growth and morphology of Hi-5 cells without treatment

The cell growth of the Hi-5 cells was studied for six consecutive days to evaluate cell growth. Results are shown in **Figure 3.1**. the cell number increased from D1 (3×10^6) to D3 (9×10^6) and then decreased at D4 (8.4×10^6) to D6 (4×10^6), showing that the stationary phase started. Results showed that over incubation time, the media composition and quality had an important impact on cell growth, proliferation and product formation[94].

Morphology of Hi-5 control cells stained with Nile Red Hi-5 using Bright-field and fluorescent microscopy images (100X) for D1, D2 and D3 are shown in **Figure 3.2**. Results showed that cell confluency on Hi-5 control cells increased from D1 (30%) to D3 (60%), decreasing from D4 (30%) to D6 (10%).

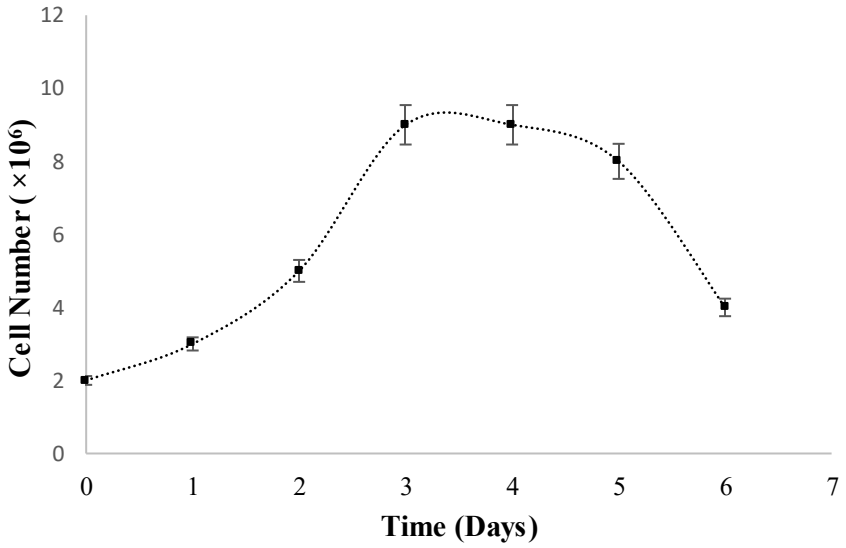

Figure 3. 1. Insect cell growth of Hi-5 control cells during six consecutive days

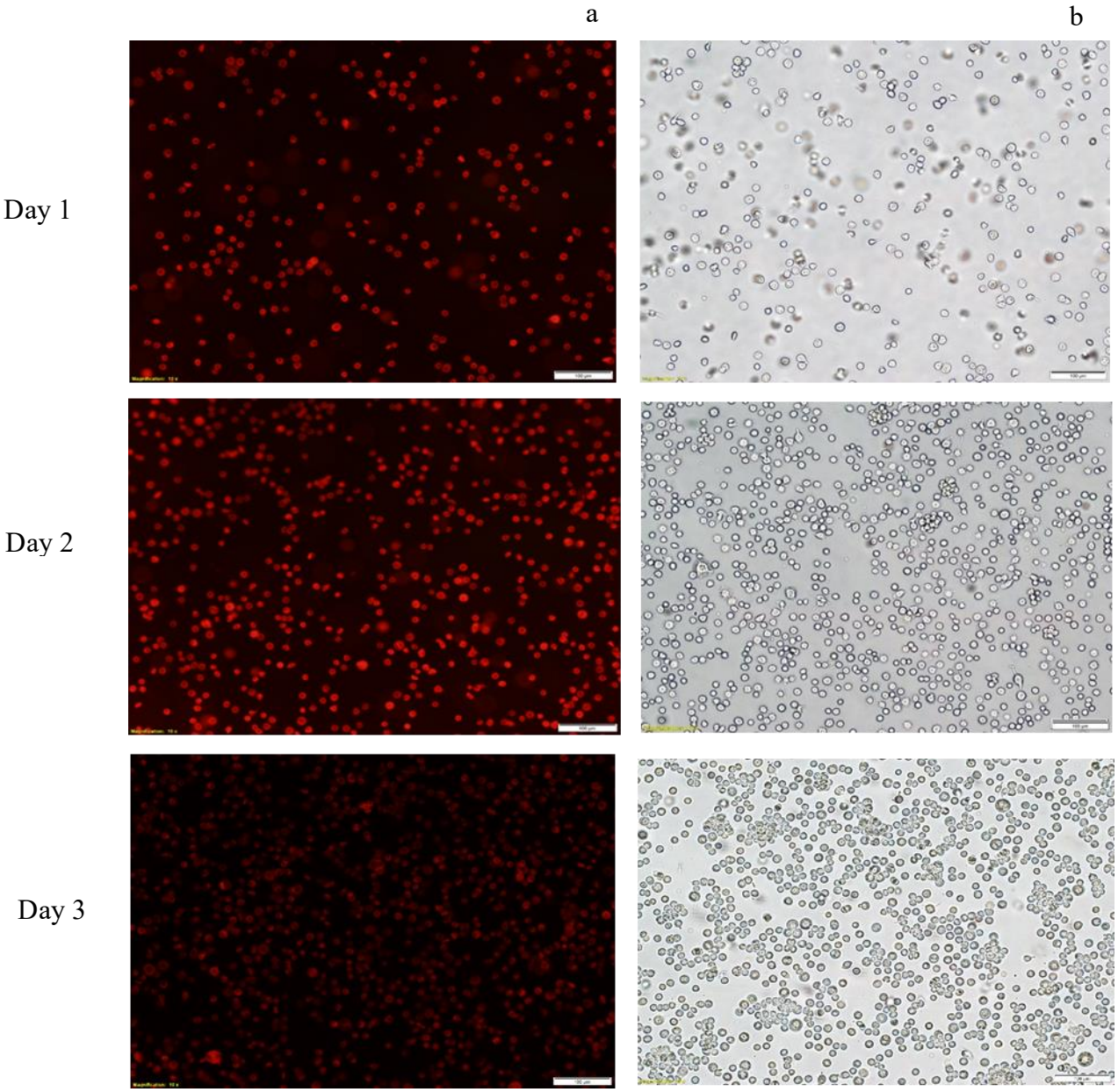

Figure 3. 2. Fluorescent microscopy (a) and Bright-field (b) images of Hi-5 control cells stained with Nile Red at 100X for Day 1 (D1), Day 2 (D2) and Day 3 (D3)

3.2. Cell growth, morphology, and fatty acid composition of Hi-5 cells with fatty acid

3.2.1 Cell growth of Hi-5 with fatty acid

Statistical differences (p<0.05) were observed for cell growth for Hi-5 cells with treatment (0.1, 0.2 - and 0.3-mM OA), vehicle control (0.1, 0.2- and 0.3-mM ethanol) and control during 3 consecutive days(**Figure 3.3**). Control samples showed an increase in cell growth until day 3, while Hi-5 with 0.1, 0.2- and 0.3-mM OA and vehicle control with 0.1, 0.2- and 0.3-mM ethanol had a similar trend; cell growth decreased from Day 1 to Day 3. The lowest Hi-5 cell growth was observed for 0.3 mM vehicle control, and the highest cell growth of Hi-5 cells was for control samples.

The growth dynamics of Hi 5 cells under oleic acid (OA) treatment and vehicle control(VC) showed a clear difference from the untreated control group. Whereas control samples displayed a steady increase in proliferation over the three-day period, both OA-treated and vehicle control groups exhibited a progressive decline in cell growth from Day 1 to Day 3. This pattern indicates that the inhibitory effect was not specific to OA but instead mostly linked to the ethanol solvent used for its preparation. The most pronounced reduction occurred in the 0.3 mM vehicle control, highlighting a dose-dependent cytotoxic effect of ethanol on Hi 5 cells. In contrast, untreated controls maintained the highest proliferation rates, underscoring the detrimental impact of solvent exposure on cell viability. These results emphasize the importance of evaluating vehicle effects in experimental design, as solvent toxicity can obscure or confound treatment-specific outcomes. Moreover, the similar decline observed in both OA-treated and vehicle groups, indicates that OA at the tested concentrations did not produce inhibitory effects beyond those caused by ethanol. Therefore, the results reinforcing the need

for optimized delivery strategies to minimize solvent interference and accurately assess OA biological activity.

A study on BEAS-2B cells revealed that, ethanol is responsible for biological effects on complicate interpretation, when used as carrier for water soluble pollutants[95]. At concentrations above 0.05–1.0% (v/v), ethanol induces dose-dependent reductions in cell viability and activates oxidative stress and apoptotic pathways, responses that overlap with pollutant-induced mechanisms. This overlap underscores the necessity of vehicle controls to distinguish solvent toxicity from pollutant-specific effects. Optimized delivery strategies or alternative carriers are therefore essential to minimize solvent interference and ensure accurate assessment of pollutant impacts[95]. Furthermore, another study also showed that common solvents like ethanol, DMSO, and acetone are not biologically neutral in cell-based assays; even at low levels they reduce viability and trigger stress responses that overlap with pollutant effects[96]. The study also found that dosing methods alter bioavailability and toxicity, creating variability across labs, and recommends vehicle-only controls, optimized solvent use, and safer alternatives such as cyclodextrins or nanoparticles[96].

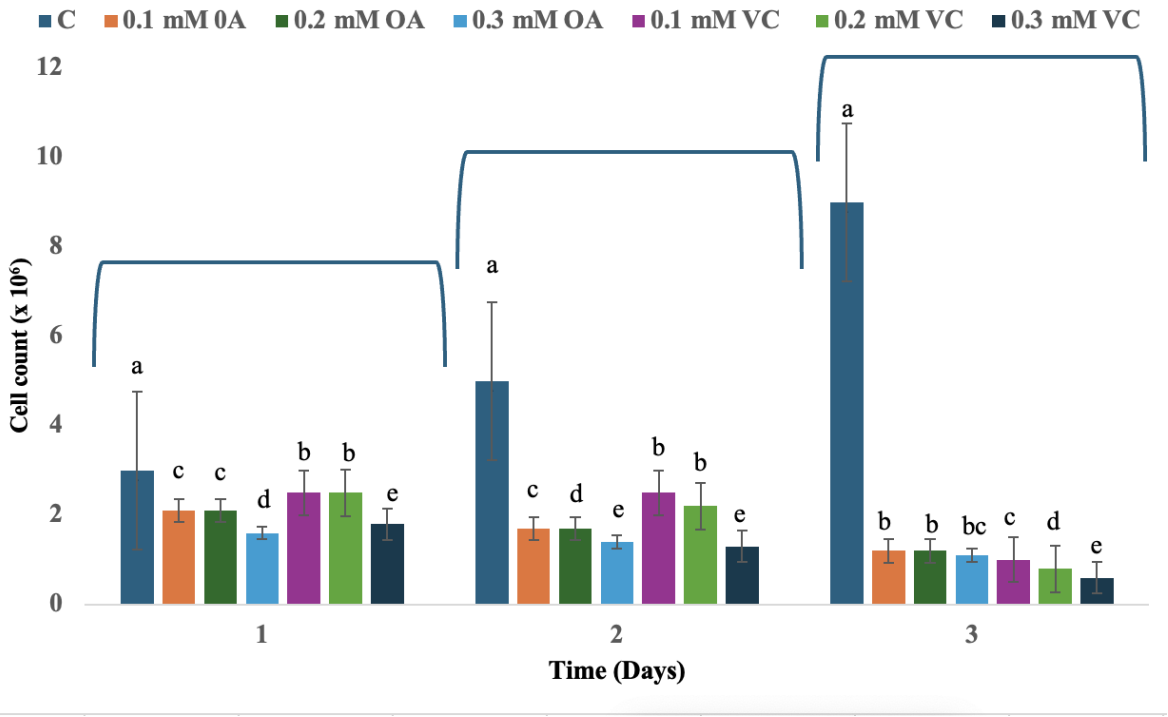

Figure 3. 3. Cell counts of Hi-5 cells with 0.1 mM, 0.2-, and 0.3-mM oleic acid (OA), vehicle control 0.1 mM, 0.2-, and 0.3-mM ethanol (VC) and control (C) during 3 consecutive days; Mean values ± SD followed by different letters indicates statistical differences ($p < 0.05$, Tukey's test).

Morphology of Hi-5 cells with treatment 0.1, 0.2- and 0.3-mM OA, stained with Nile Red using fluorescent microscopy images (100 X) for Days 1,2 and 3 are shown in **Figure 3.4** and Bright microscopy images are shown in **Appendix Figure A.76-Figure A.86**. Results from fluorescence microscopy showed that cell confluency on Hi-5 cells with 0.1, 0.2- and 0.3-mM OA decreased from Day 2(70%) to Day 3(30%). A similar trend was observed for the vehicle control as in **Figure 3.5**, where Hi-5 cell confluency decreased from Day 2(60%) to Day 3(20%). Furthermore, a similar trend was observed for OA and vehicle control samples, where confluency increased from Day 1(30%) to Day 2(60-70%). Control samples were stated in **Figure 3.2**, where an increase in cell confluency was observed until day 3 (**Appendix Figure A.67- Figure A.75**).

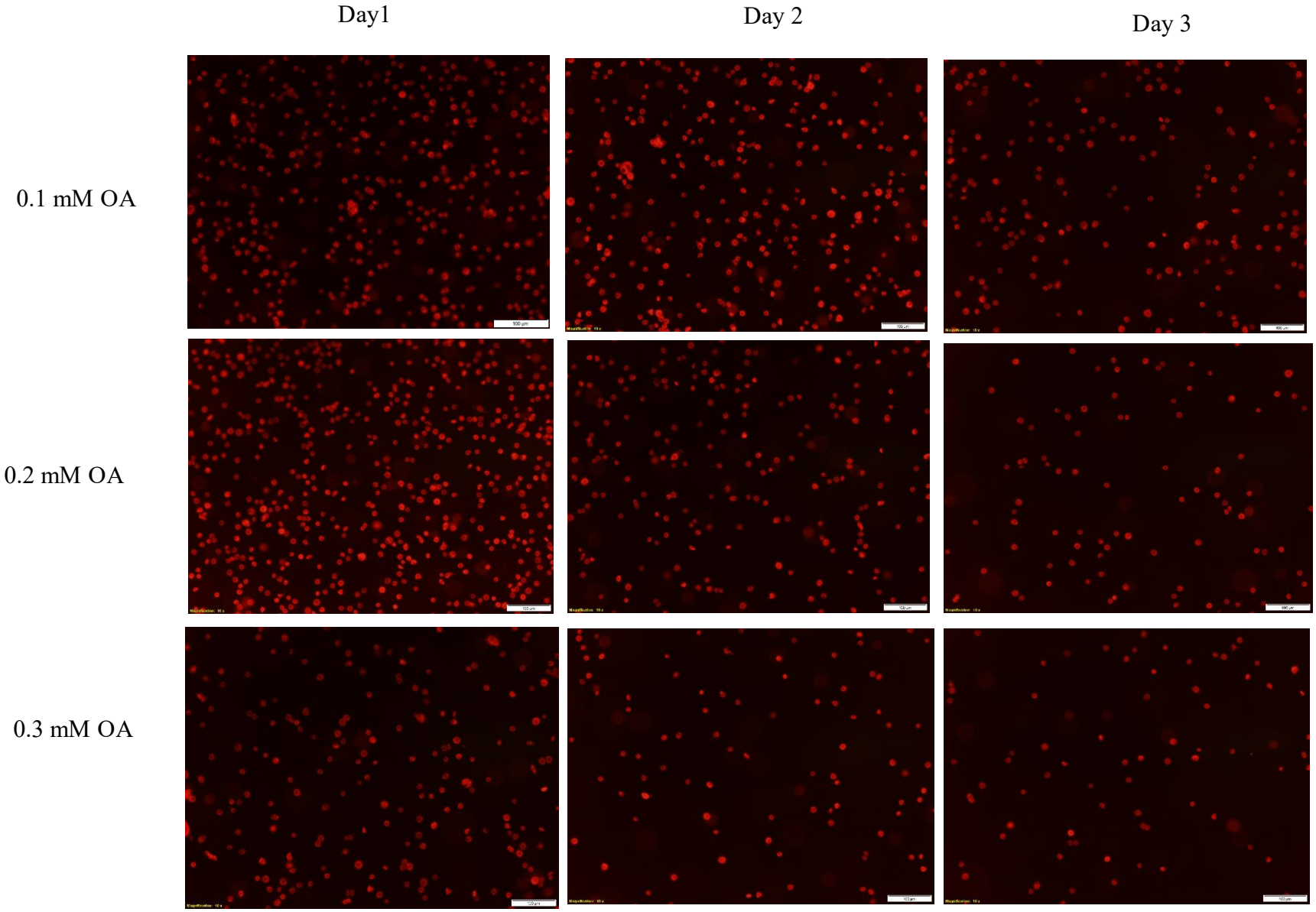

Figure 3. 4. Fluorescence image of Hi-5 cells treated with oleic acid (OA) 0.1, 0.2, and 0.3 mM for Day 1, Day 2 and Day 3

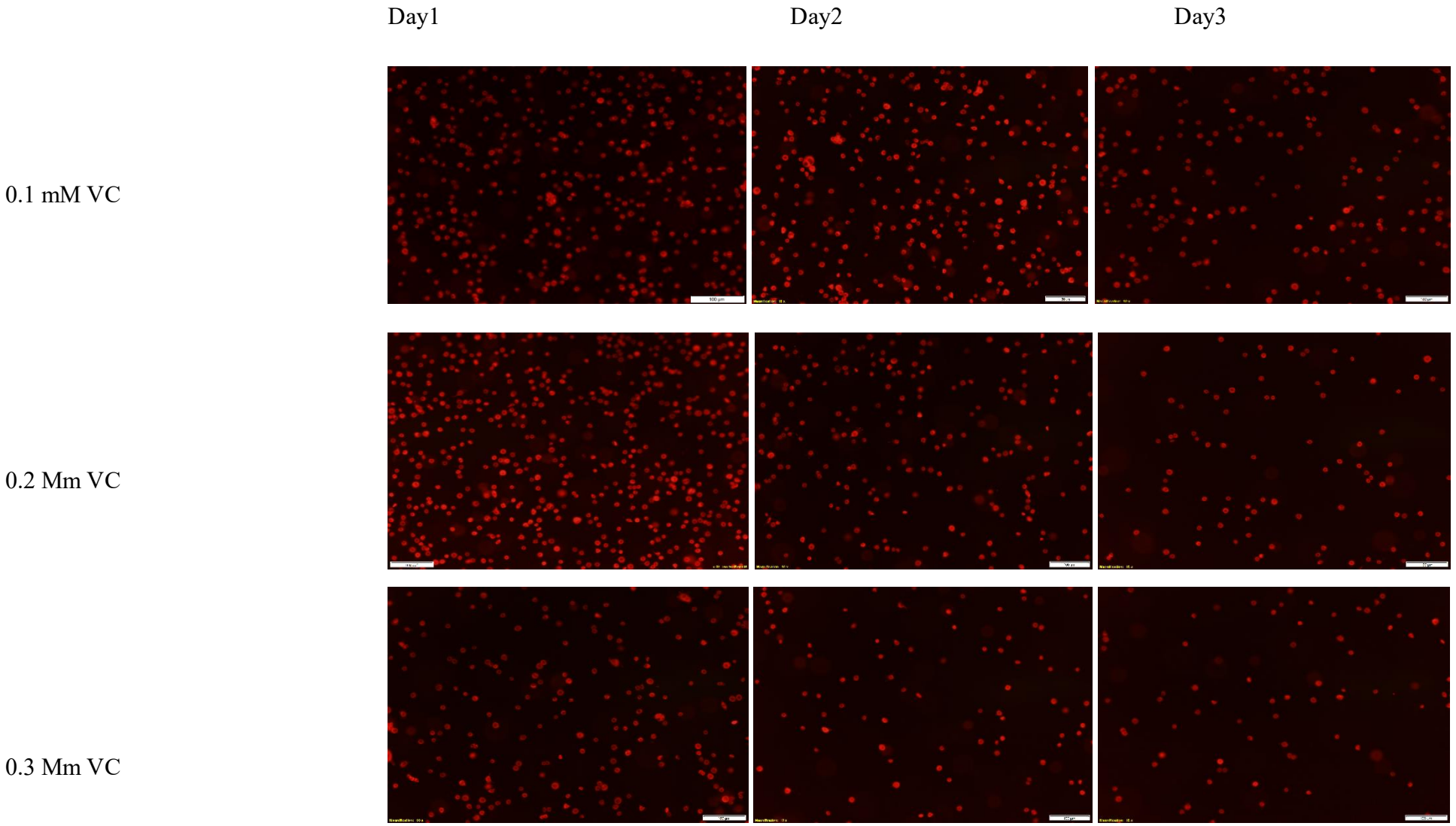

Figure 3. 5. Fluorescence image of Hi-5 vehicle control cells (VC) 0.1, 0.2- and 0.3-mM for Day 1, Day 2 and Day 3

Changes in cell granularity of Hi-5 cells as vehicle control with 0.1, 0.2-, and 0.3-mM ethanol and control during 3 consecutive days are shown in **Figure 3.6.** Statistical differences (p<0.05) were observed among the samples. A lower cell granularity was observed for the control samples when compared to OA and vehicle control. OA samples showed higher cell granularity for 0.3 mM OA when compared to 0.2- and 0.1-mM OA. A similar trend was observed with 0.3 mM OA samples, as the cell granularity increased from Day 1(96.822) to Day 3(118.347). Also, cell granularity data representations are seen in **Figure 3.7** and **Figure 3.8** with coloured histograms. Observations in 3T3-L1 cells revealed, a positive correlation between cell granularity and lipid storage capacity. Because the granules failed to enlarge, the cells could not accumulate lipids efficiently and reduced the lipid content. Cells containing smaller granules exhibited impaired fat storage, resulting in reduced lipid accumulation.[97] On the other hand, a study on human T-cell leukaemia cell lines reported similar results between cell viability and cell granularity. The increased number of dead cells was considered a cause of increased cell granularity and decreased cell size[98].

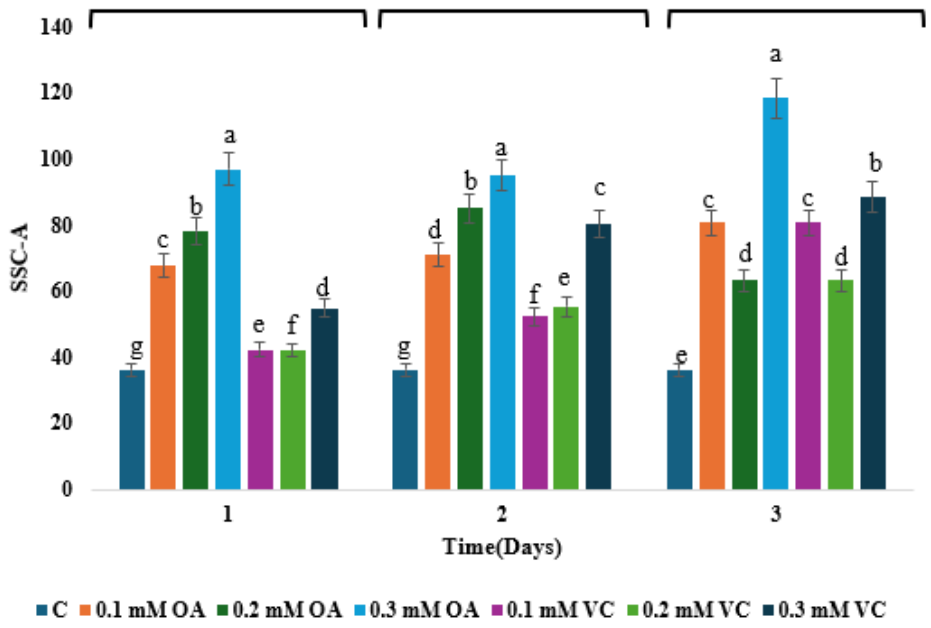

Figure 3. 6. Changes in cell granularity of Hi-5 cells with 0.1 mM, 0.2-, and 0.3-mM oleic acid (OA), vehicle control 0.1 mM, 0.2-, and 0.3-mM ethanol (VC) and control (C) during 3 consecutive days; Mean values ± SD followed by different letters indicates statistical differences ($p < 0.05$, Tukey's test).

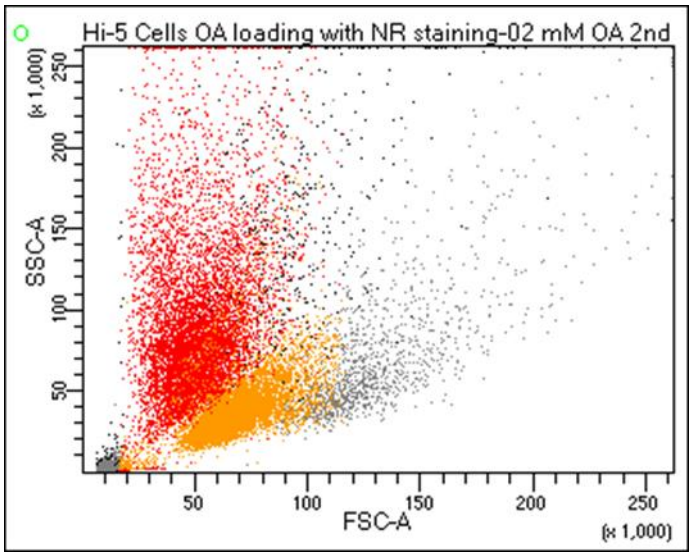

Figure 3. 7. Representative flow cytometry FSC/SCC dot plot illustrating cell size and morphology changes after treatment with OA(0.2 mM OA-treated samples on the 2nd day(red colour), and nontreated cells(yellow colour))

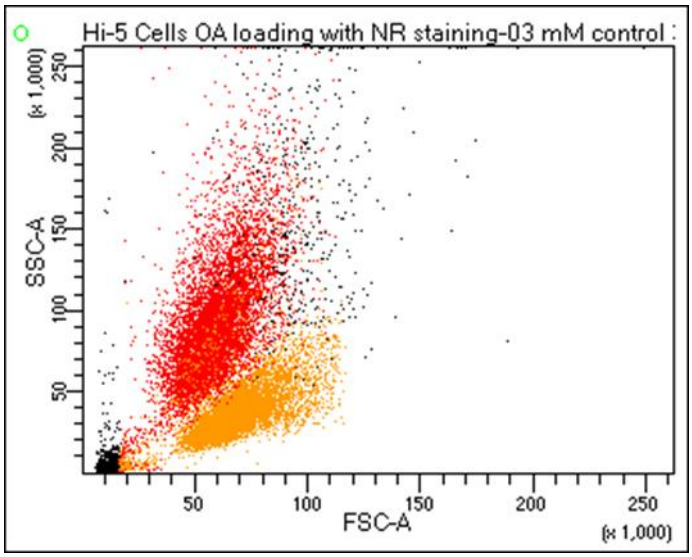

Figure 3. 8. Representative flow cytometry FSC/SCC dot plot illustrating cell size and morphology changes after treatment with OA(0.3 mM vehicle control(VC) samples on the 3rd day(red colour), and nontreated cells(yellow colour))

Changes in cell size of Hi-5 cells with 0.1, 0.2-, and 0.3-mM OA, vehicle control and control for 3 days are shown in **Figure 3.9.** Statistical differences (p<0.05) were observed among samples. The highest cell sizes were observed for control samples at Day 2 (67.14%) and Day 3(67.12%). A similar trend was observed for OA samples among the 3 days, where the cell granularity decreased from 0.1 mM to 0.3 mM. Vehicle control samples showed higher cell size for 0.1 mM samples. A study on *E. coli* reported that cell size depends on incubation time, growth phase and cell numbers[99], where higher dead cell amounts contributed to lower cell size[98].

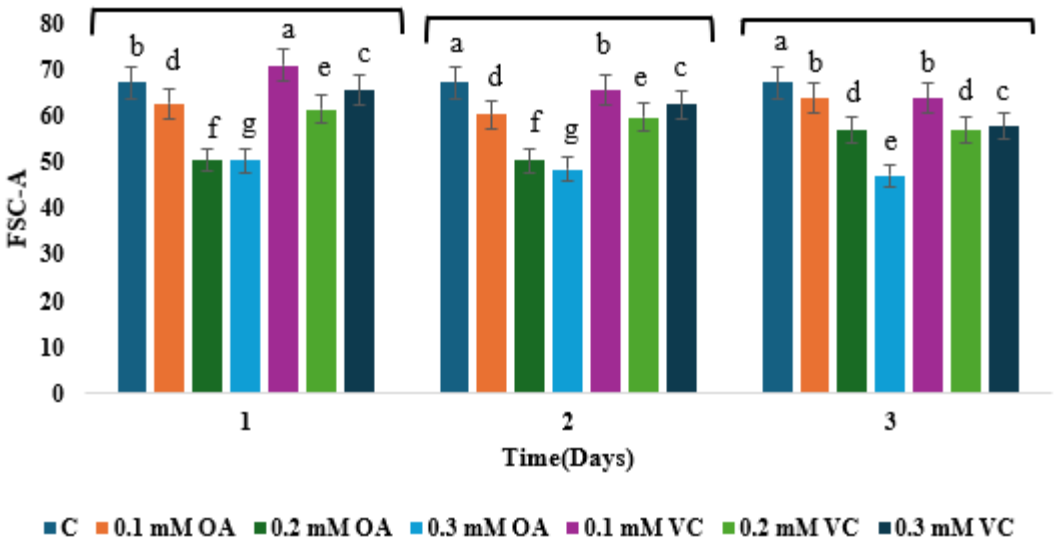

Figure 3. 9. Changes in cell size of Hi-5 cells with 0.1 mM, 0.2-, and 0.3-mM oleic acid (OA), vehicle control 0.1 mM, 0.2-, and 0.3-mM ethanol (VC) and control (C) during 3 consecutive days; Mean values ± SD followed by different letters indicates statistical differences ($p < 0.05$, Tukey's test).

Figure 3.10 shows the relative Triglyceride content in Hi-5 cells loaded with 0.1 mM, 0.2 mM, and 0.3 mM vehicle control, Hi-5 cells treated with oleic acid (OA) and control samples for 3 days. Significant difference ($p<0.05$) was observed among the samples. Qualitative data were also represented in **Figure 3.11** and **Figure 3.12**. Due to oleic acid metabolism, the highest fluorescent intensity was obtained with 0.3 mM oleic acid-treated samples, followed by 0.2- and 0.1-mM oleic acid, and the lowest values were for control samples. A lower relative triglyceride intensity result was observed for vehicle control samples when compared to OA samples. A study on marine dinoflagellate cells reported that there was a non-linear correlation between lipid content and carbon content, suggesting that after a carbon saturation point, cells might not be able to take more lipids into their system[100].

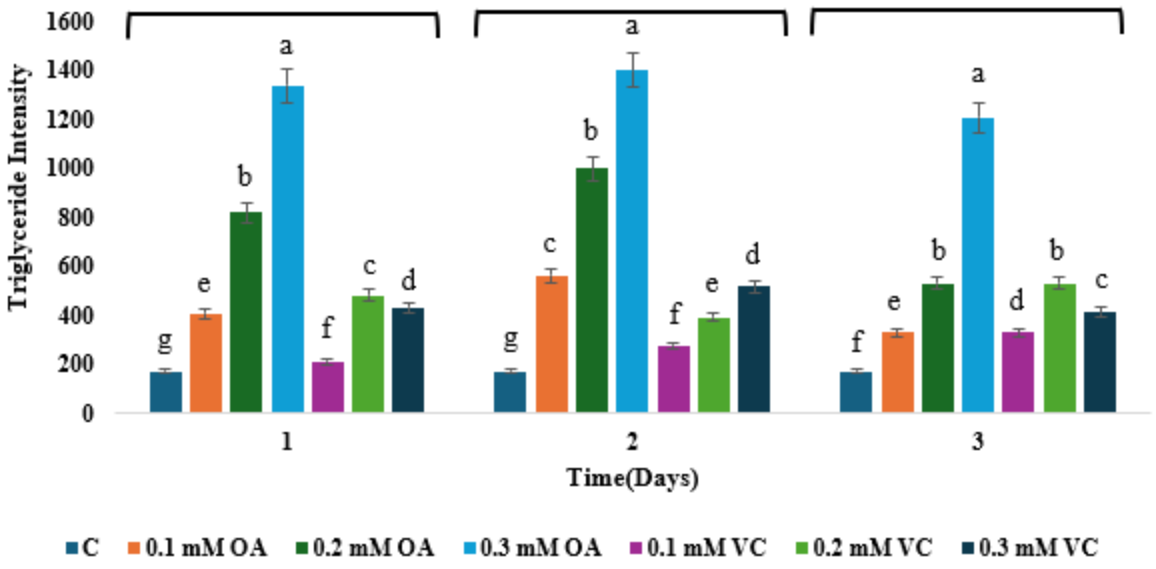

Figure 3. 10. Relative Triglyceride content of Hi-5 cells with 0.1 mM, 0.2-, and 0.3-mM oleic acid (OA), vehicle control 0.1 mM, 0.2-, and 0.3-mM ethanol (VC) and control (C) during 3 consecutive days; Mean values ± SD followed by different letters indicates statistical differences ($p < 0.05$, Tukey's test).

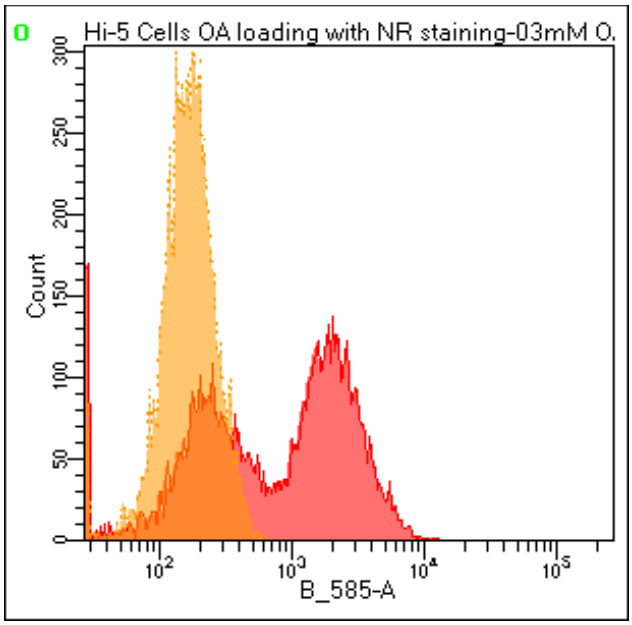

Figure 3. 11. Relative triglyceride content effect on 0.3 mM OA-treated samples on the 1st day(red colour) and nontreated samples (yellow colour)

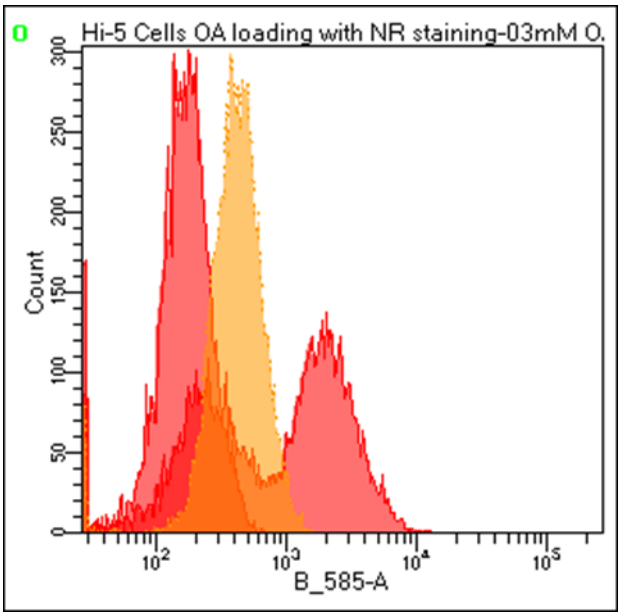

Figure 3. 12. Relative triglyceride content effect on 0.3 mM OA-treated samples on the 1st day(red colour) and 0.3 mM vehicle control samples on the 1st day (red colour)

Results reported in **Figure 3.10** suggest that OA-treated Hi-5 cells could have reached the carbon saturation point on day 2 and decreased on day 3. It has also been reported that an increased lipid content was observed in microalgae species with a reduction in cell growth[101]. In general, results showed that Hi-5 cells treated with OA and control samples showed a negative correlation between cell count results and triglyceride intensity results.

According to the phospholipid intensity results of flow cytometry analysis shown in **Figure 3.13**, a statistical difference ($p<0.05$) was observed among samples during 3 consecutive days. Furthermore, phospholipid intensity data representations are shown in **Figure 3.14** and **Figure 3.15**. The highest values were observed for vehicle control samples. A similar trend was observed for OA and vehicle control samples on the third day, as the phospholipid intensity values decreased from 0.1 mM to 0.3 mM. For 0.1- and 0.2 mM OA samples, an increase was observed from Day 1 to Day 3; however, 0.3 mM OA samples showed the highest value on Day 1.

The primary change observed following OA loading was an increase in fluorescence associated with the triglyceride pool, indicating that OA was preferentially incorporated into neutral lipid stores. In contrast, fluorescence associated with the phospholipid pool did not show a meaningful change. This outcome is biologically expected, as cellular membranes tightly regulate phospholipid composition to preserve membrane fluidity, structural integrity, and functionality. Phospholipid acyl chain length, saturation, and conformation play a critical role in these properties, and substantial incorporation of OA into membrane phospholipids would be expected to disrupt membrane dynamics. The absence of significant changes in phospholipid-associated fluorescence therefore suggests that OA loading predominantly promoted lipid storage rather than structural membrane remodeling.

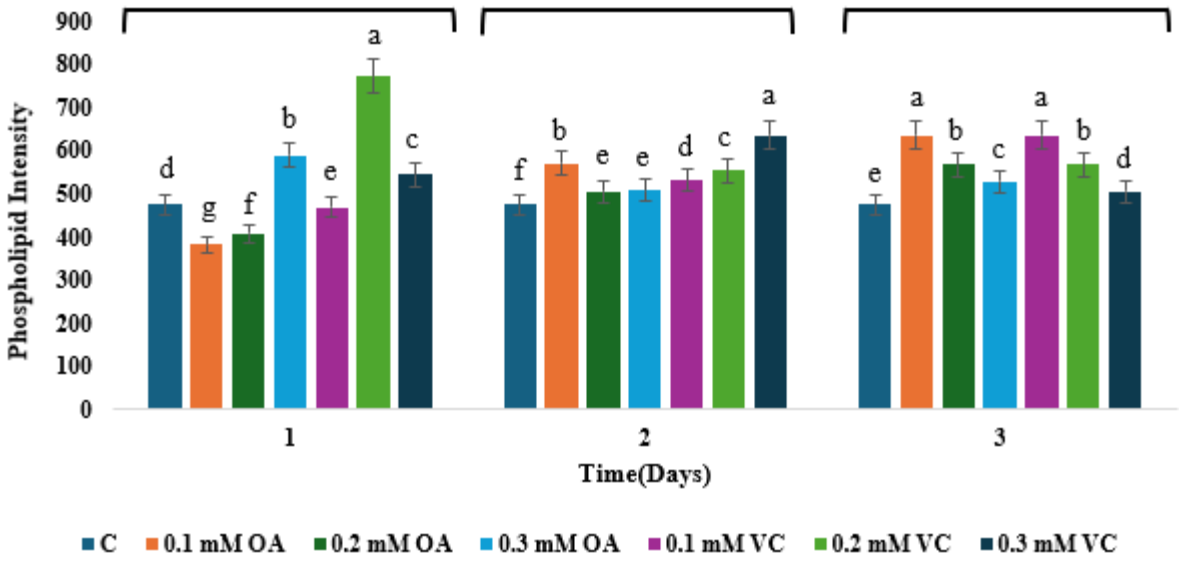

Figure 3. 13. Changes in Phospholipid fluorescence intensity of Hi-5 cells with 0.1 mM, 0.2, and 0.3-mM oleic acid (OA), vehicle control 0.1 mM, 0.2-, and 0.3-mM ethanol (VC) and control (C) during 3 consecutive days; Mean values ± SD followed by different letters indicates statistical differences ($p < 0.05$, Tukey's test).

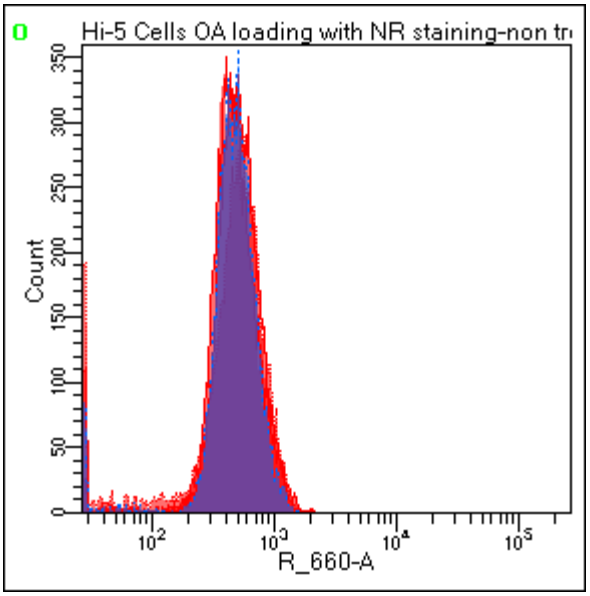

Figure 3. 14. Phospholipid fluorescence intensity plot of 0.1 mM oleic acid (OA) samples on the 3rd day(blue colour) and non-treated samples(red colour)

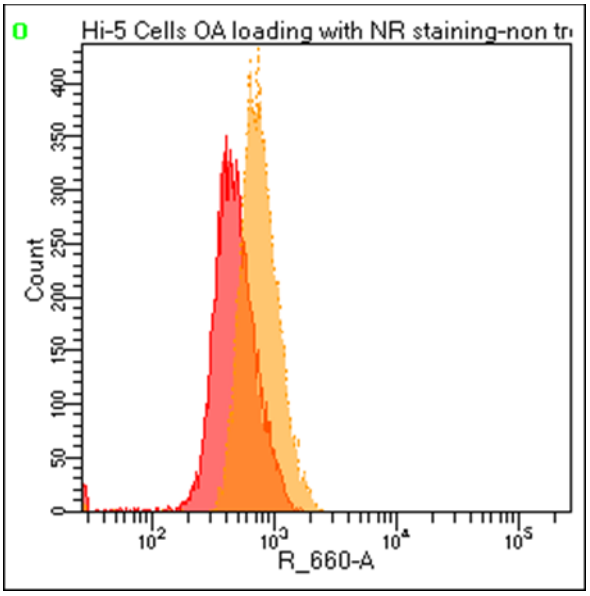

Figure 3. 15. Phospholipid fluorescence intensity plot of 0.2 mM vehicle control (VC) samples on the 1st day(yellow colour) and non-treated samples(red colour)

3.3. GC-MS Analysis

Changes in Fatty acid composition of Hi-5 cells with 0.1 mM, 0.2, and 0.3 mM oleic acid (OA), 0.1 mM, 0.2-, and 0.3-mM vehicle control (VC) and control (C) for samples at Day 2 were chosen for GC-MS analysis due to optimal cell morphology, cell growth and relative triglyceride analysis previously reported (**Figure 3.10).** As can be seen in **Figure 3.16**, higher content of oleic acid was seen for Hi-5 control samples, followed by 0.1 mM OA, 0.2 mM OA and 0.1 mM VC. Changes in the fatty acid profile of Hi-5 cells treated with oleic acid can be related to insect cells' production of other metabolites, and could also be a result of the cell death that we monitored after a high amount of oleic acid and/or ethanol-albumin as carriers.

Higher levels of stearic acid were observed for Hi-5 control samples, and a trend was seen in 0.1 mM, 0.2, and 0.3 mM oleic acid(OA). The fatty acid composition of Hi-5 cells showed concentration-dependent changes in response to oleic acid (OA) supplementation. Stearic acid (18:0), the precursor of oleic acid via stearoyl-CoA desaturase, was elevated in control cells but declined across OA and VC treatments, reflecting its utilization in desaturation pathways[102].

On the other hand, higher palmitic acid was observed for 0.1 mM OA and lower levels for 0.2 and 0.3 mM oleic acid (OA) concentrations, suggesting a compensatory mechanism to limit lipotoxicity. Taken together, these results highlight the dynamic interplay between saturated and monounsaturated fatty acids. Furthermore, OA appears to regulate palmitic acid levels in a concentration-dependent manner, underscoring its potential role in limiting lipotoxic stress[103] .This observation aligns with findings from a previous study which demonstrated that OA protects hepatocytes against the cytotoxic effects of palmitic acid by promoting its esterification into triglycerides and subsequent storage in lipid granules[104].A study on pancreatic cells found that palmitic acid triggers apoptosis via endoplasmic reticulum stress, mitochondrial dysfunction, leading to protease activation. In contrast, oleic acid counteracts

these effects by promoting triglyceride synthesis and lipid droplet formation, diverting palmitic acid from toxic pathways depending on concentration[105].

A different trend was observed for palmitoleic acid; higher levels were seen for 0.1 mM VC and 0.1 mM OA, and the lowest levels for 0.3 mM oleic acid (OA) and the highest level for non-treated samples. The metabolic response to oleic acid (OA) supplementation revealed a dose-dependent regulation of fatty acid synthesis and desaturation. The highest levels were detected in non-treated samples, suggesting that baseline cellular metabolism favors palmitoleic acid accumulation when no external compounds are introduced. At 0.1 mM OA, palmitoleic acid levels were elevated, suggesting that moderate OA concentrations stimulate lipogenesis and desaturase activity, likely through enhanced substrate availability and upregulation of stearoyl-CoA desaturase (SCD)[106]. However, as OA concentration increased, palmitoleic acid reached its lowest point at 0.3 mM. This indicates that excessive OA may trigger feedback inhibition of lipogenic enzymes, downregulate SCD activity, or shift metabolism toward storage and elongation pathways rather than desaturation[107]. Overall, the results suggest that low OA promotes fatty acid synthesis and desaturation, while high OA suppresses these processes, leading to reduced palmitic and palmitoleic acid accumulation.

The use of ethanol as a carrier introduces an additional layer of complexity. Ethanol can undergo non-oxidative metabolism to form fatty acid ethyl esters (FAEEs), which are lipophilic metabolites that integrate into membranes and organelles, where they interfere with mitochondrial activity and reduce ATP availability[108,109]. Their formation from ethanol and fatty acids may alter lipid incorporation, signaling, and membrane dynamics[110]. In cell culture systems, this means that FAEE generation could confound the interpretation of fatty acid metabolism, as observed differences between OA-treated and VC groups may reflect not only OA supplementation but also ethanol-derived FAEE effects. This highlights the importance of considering carrier-derived metabolites when evaluating lipidomic data in cell systems.

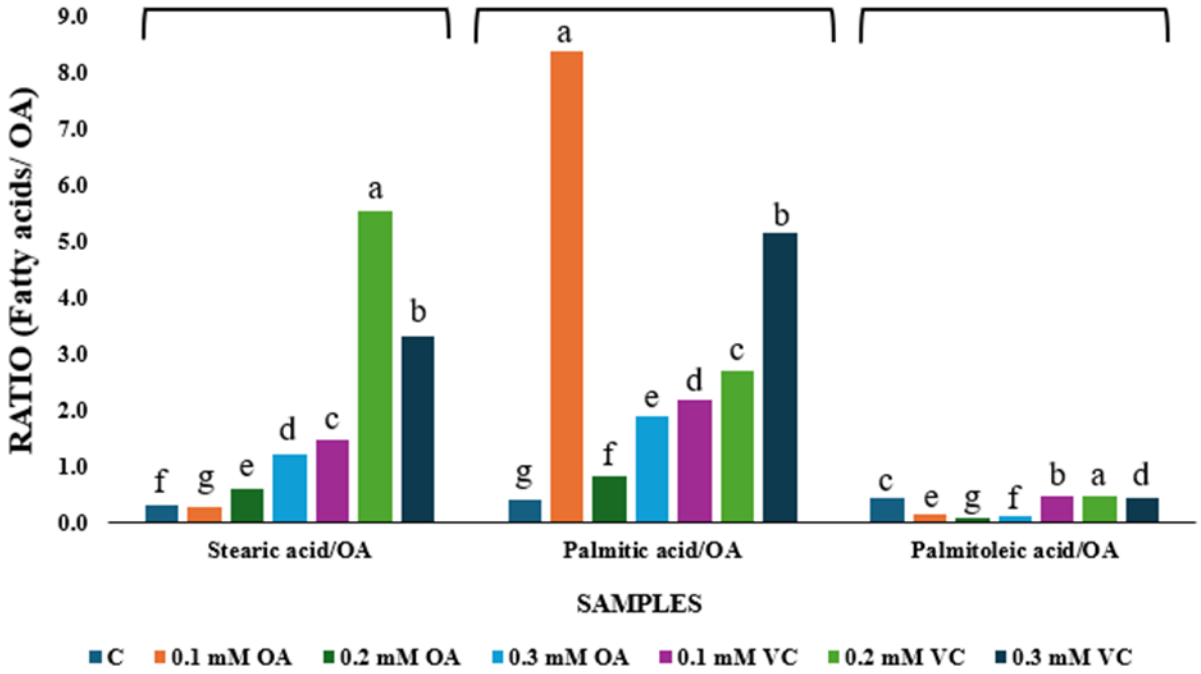

Figure 3. 16. Changes in Fatty acid composition(fatty acids/OA) of Hi-5 cells with 0.1 mM, 0.2, and 0.3-mM oleic acid (OA), vehicle control 0.1 mM, 0.2-, and 0.3-mM ethanol (VC) and control (C); Mean values ± SD followed by different letters indicates statistical differences ($p < 0.05$, Tukey's test).

Chapter 4. Conclusion

This study explored a novel approach to modifying the lipid profile of Hi-5 insect cells through enrichment with exogenous oleic acid (OA), aiming to emulate fat characteristics relevant to cultured meat applications. The experimental findings provide detailed insights into the morphological, metabolic, and lipid compositional changes that occurred in insect cells subjected to OA loading.

Firstly, the vehicle control (VC), often considered an inert baseline, was shown to induce morphological alterations, such as reduced cell size and elevated granularity, alongside a high level of cytotoxicity. These changes suggest that even the carrier medium can influence cellular physiology and must be carefully accounted for in experimental designs. In addition to morphological shifts, the VC treatment alone was sufficient to cause detectable alterations in the cellular lipid profile, highlighting its potential confounding effects.

OA loading led to a more pronounced reduction in cell size and increased cell granularity compared to VC. These changes likely reflect stress responses or alterations in cell membrane dynamics due to lipid accumulation. Notably, intracellular lipid content increased significantly following OA treatment, with even the lowest concentration tested (0.1 mM) nearly doubling lipid levels within the cells. This suggests high bioavailability and uptake efficiency of OA by Hi-5 insect cells.

GC-MS analysis of the lipid profile indicated that OA was not merely sequestered but actively metabolised and incorporated into endogenous lipid pathways. Specifically, cells treated with 0.1 mM OA exhibited the most pronounced increase in palmitic acid content, a saturated fatty acid relevant for mimicking the lipid composition of animal fat. This finding underscores the potential of metabolic alteration in insect cells to yield tailored fatty acid profiles.

Flow cytometry analysis provided further granularity to the uptake dynamics. Cells treated with 0.1 mM OA displayed a single peak in fluorescence intensity, indicating a uniform uptake

across the cell population. In contrast, the 0.2 mM and 0.3 mM OA treatments resulted in bimodal histograms, suggesting heterogeneous uptake and possibly early onset of cytotoxicity or stress-induced variation in cellular responses.

In conclusion, this research demonstrated the feasibility of modifying the lipid content and composition of insect cells using targeted fatty acid supplementation. The study demonstrated that even low concentrations of OA can achieve significant enrichment of intracellular lipids with controlled distribution and modest cytotoxicity. These findings support the use of insect cells as a customizable and sustainable lipid source for cultured meat applications, particularly to replicate the sensory and functional roles of animal fat. Future research should delve deeper into the enzymatic and regulatory mechanisms governing fatty acid metabolism in insect cells and explore scale-up strategies to transition from bench-scale studies to industrial applications.

Chapter 5. Future work

This study provides foundational insights into the feasibility of modifying insect cell lipid profiles for potential use in cultured meat systems. Building on these findings, future work should pursue the following directions:

1. Mechanistic Understanding of Lipid Metabolism in Insect Cells

Further investigation is needed to elucidate the enzymatic and regulatory pathways governing fatty acid uptake, esterification, and transformation in insect cells. Omics-based approaches such as transcriptomics, proteomics and lipidomics could identify key regulators and bottlenecks in lipid biosynthesis and remodelling.

2. Optimisation of Fatty Acid Delivery Vehicles

To reduce cytotoxicity and enhance uptake, formulation strategies using food-grade carriers such as alcohol-free albumin loading, phospholipids, liposomes, surfactants, polymeric vesicles or tailored emulsions should be explored. These delivery systems could improve bioavailability and support scale-up by offering more consistent and biocompatible loading conditions.

3. Broader Fatty Acid Supplementation Studies

Expanding beyond oleic acid, future studies should assess the metabolic fate of various saturated and polyunsaturated fatty acids. This includes stearic, linoleic, and alpha-linolenic acids to establish a more comprehensive understanding of how chain length and saturation influence incorporation, toxicity, and lipid droplet formation.

4. Tailoring Triglyceride Profiles for Functionality

Work is needed to define the optimal triglyceride profiles that best mimic animal fat in terms of melting point, mouthfeel, and oxidative stability. The ability to selectively enrich insect cells with targeted fatty acid combinations may allow fine-tuning of sensory properties in cultured meat prototypes.

5. Scale-Up and Bioprocess Integration

Translating these findings into industrial applications will require the development of scalable culture systems. This includes adapting protocols for stirred-tank bioreactors and optimising nutrient delivery and harvest strategies for lipid-enriched insect cells.

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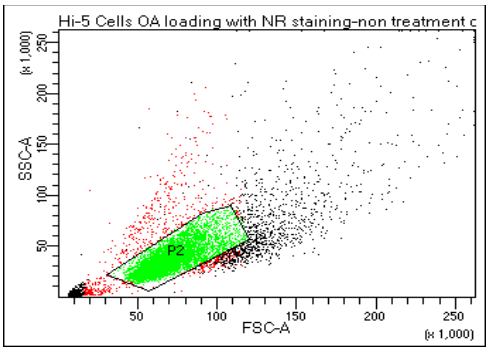
APPENDIX

Flow Cytometry Analysis

FSC-A Vehicle control results



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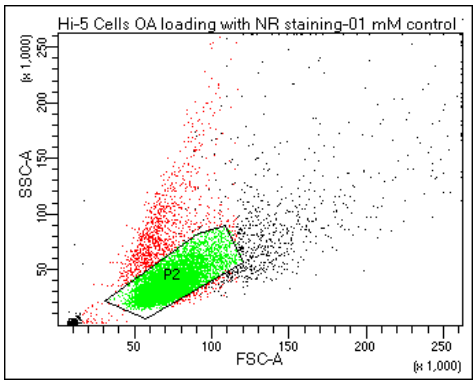
Tube Name:	non treatment cells(no control n...
Record Date:	Jun 14, 2023 2:52:56 PM
$OP:	Researchers
GUID:	42421543-408e-4964-944b-09...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	73,507	40,369
P1	8,273	82.7	67,145	36,227
P2	7,793	77.9	68,300	34,943

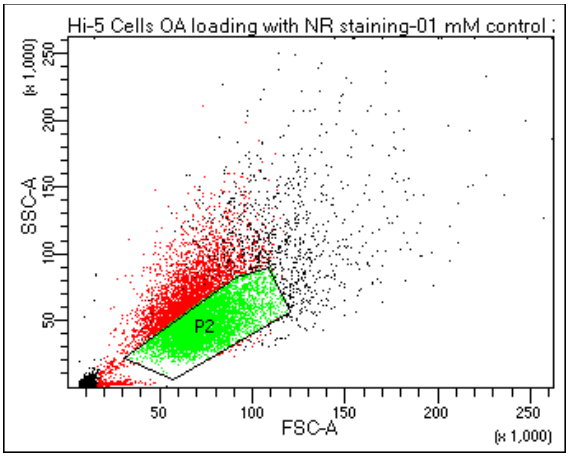
Figure A. 1. FSC-A results of non-treated cell samples



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM control 1st day
Record Date:	Jun 7, 2023 2:36:51 PM
$OP:	Researchers
GUID:	9901c4f8-4265-495f-95d6-f48c...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	76,851	46,867
P1	8,952	89.5	70,896	42,167
P2	7,961	79.6	71,809	37,091

Figure A. 2. FSC-A results of 0.1 mM vehicle control cell samples on the 1st day



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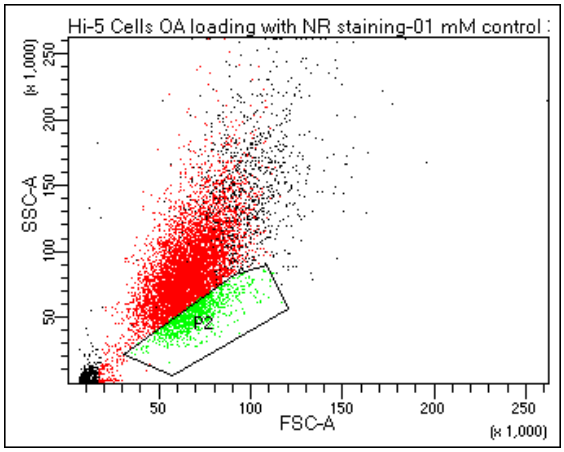
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM control 2nd day
Record Date:	Jun 8, 2023 3:03:22 PM
$OP:	Researchers
GUID:	285a6fc3-a825-42be-b51c-f4cb...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	12,461	####	60,226	47,980
P1	8,610	69.1	65,461	52,233
P2	5,523	44.3	72,906	46,523

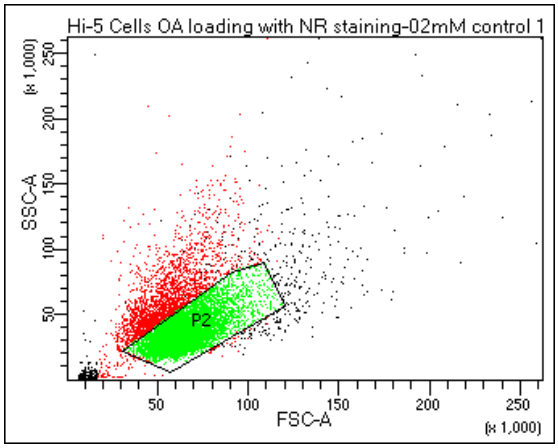
Figure A. 3. FSC-A results of 0.1 mM vehicle control cell samples on the 2nd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM control 3rd day
Record Date:	Jun 9, 2023 2:15:20 PM
$OP:	Researchers
GUID:	a996d91d-f082-49ab-bb29-b72...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	61,590	79,031
P1	7,701	77.0	63,705	80,598
P2	1,084	10.8	69,748	52,153

Figure A. 4. FSC-A results of 0.1 mM vehicle control cell samples on the 3rd day



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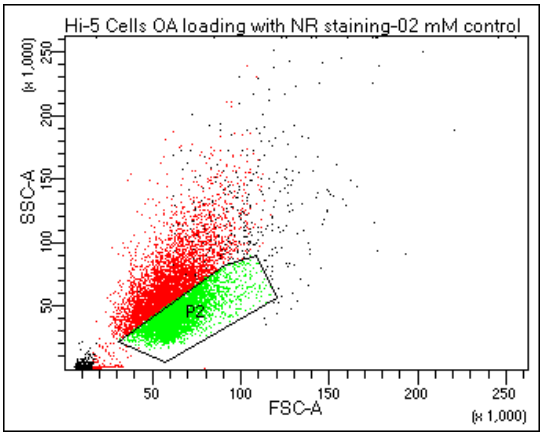
GUID:	35802879-6fe6-48cd-b13f-28c3...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	62,863	42,954
P1	9,160	91.6	61,350	42,007
P2	7,159	71.6	64,520	35,954

Figure A. 5. FSC-A results of 0.2 mM vehicle control cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02 mM control 2nd day_002
Record Date:	Jun 8, 2023 3:12:26 PM
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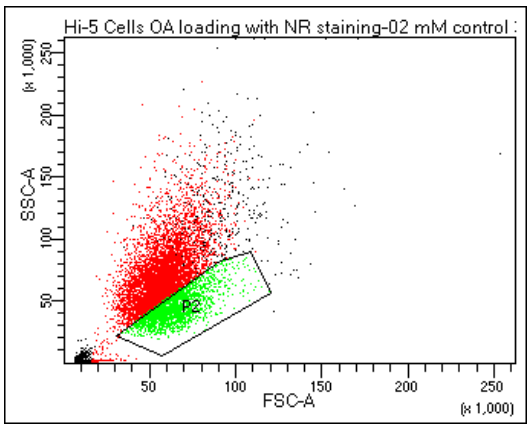
GUID:	74c5af4b-5527-40eb-ad35-58b...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	55,560	51,134
P1	8,254	82.5	59,648	55,191
P2	4,015	40.2	64,268	42,585

Figure A. 6. FSC-A results of 0.2 mM vehicle control cell samples on the 2nd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02 mM control 3rd day_002
Record Date:	Jun 9, 2023 2:46:52 PM
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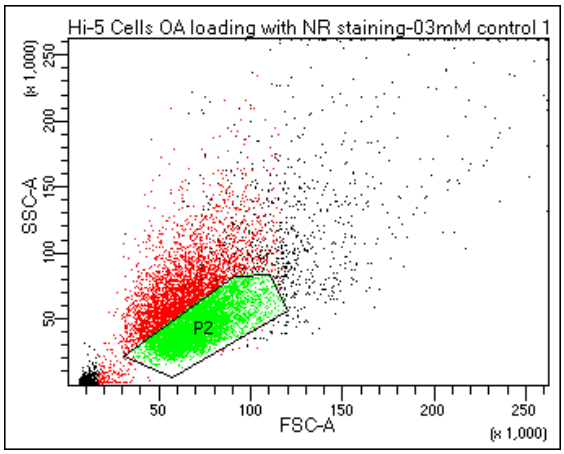
GUID:	9fa09247-2b74-4e4a-adb8-67fb...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	53,354	58,909
P1	8,347	83.5	56,769	63,211
P2	2,289	22.9	63,704	44,075

Figure A. 7. FSC-A results of 0.2 mM vehicle control cell samples on the 3rd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03mM control 1st day_003
Record Date:	Jun 7, 2023 3:26:14 PM
$OP:	Researchers
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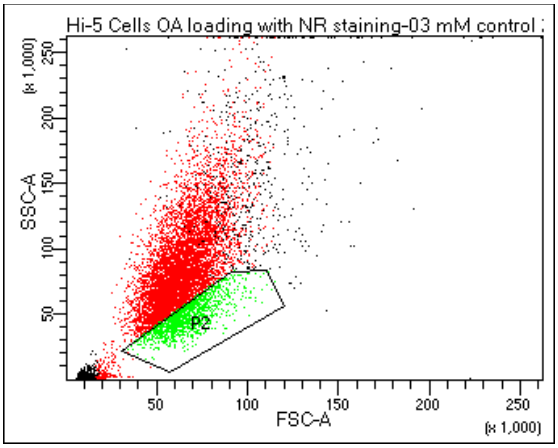
Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	69,748	59,401
P1	8,355	83.6	65,466	54,756
P2	5,398	54.0	69,875	44,170

Figure A. 8. FSC-A results of 0.3 mM vehicle control cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03 mM control 2nd day_004
Record Date:	Jun 8, 2023 3:33:13 PM
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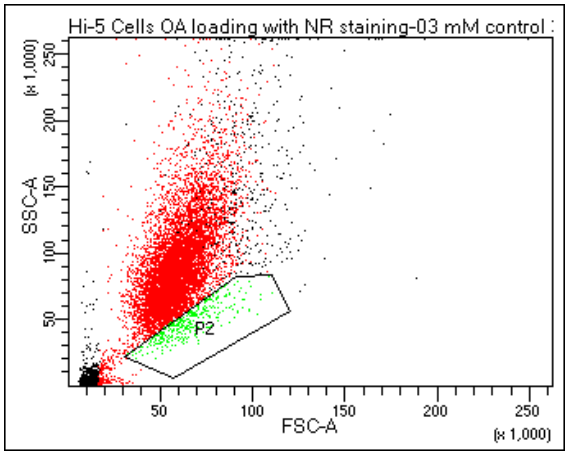
Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	57,857	74,034
P1	8,021	80.2	62,404	80,263
P2	1,398	14.0	66,001	47,554

Figure A. 9. FSC-A results of 0.3 mM vehicle control cell samples on the 2nd day



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Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03 mM control 3rd day_004
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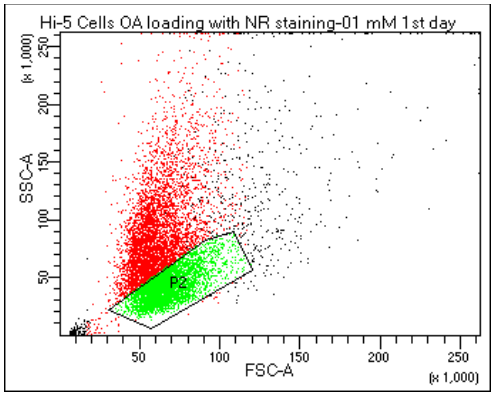
Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,069	####	53,526	81,175
P1	8,305	82.5	57,799	88,447
P2	361	3.6	64,178	46,821

Figure A. 10. FSC-A results of 0.3 mM vehicle control cell samples on the 3rd day

FSC-A oleic acid treatment results



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM 1st day
Record Date:	Jun 7, 2023 2:55:52 PM
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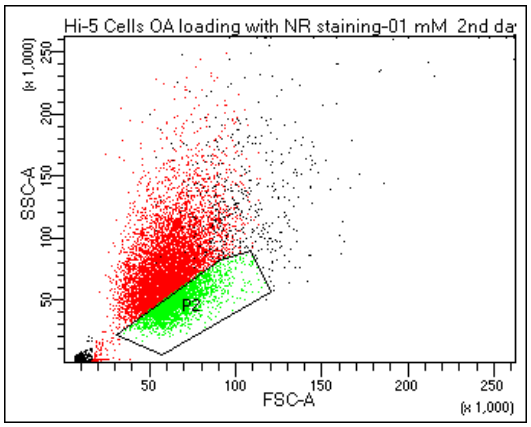
GUID:	8b0eccf8-7733-4491-b64c-dbbf...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	65,910	70,546
P1	9,261	92.6	62,674	67,723
P2	4,344	43.4	66,729	41,263

Figure A. 11. FSC-A results of 0.1 mM oleic acid-treated cell samples on the 1st day



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Tube Name:	01 mM 2nd day_001
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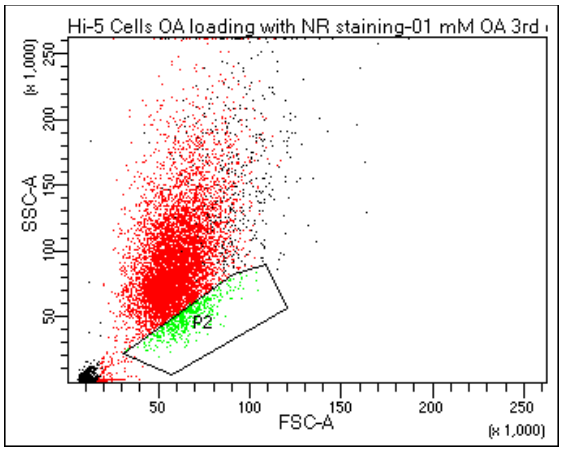
GUID:	189af78a-65cf-4333-82a0-da80...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	59,512	69,347
P1	8,632	86.3	60,325	70,758
P2	2,399	24.0	66,375	47,275

Figure A. 12. FSC-A results of 0.1 mM oleic acid-treated cell samples on the 2nd day



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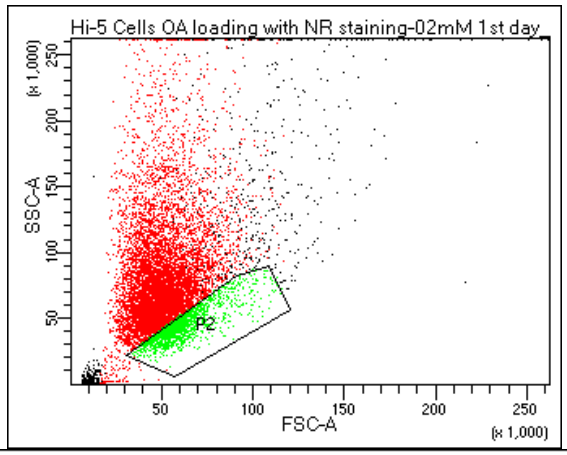
Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	50,756	76,839
P1	7,722	77.2	57,428	88,994
P2	529	5.3	64,713	48,439

Figure A. 13. FSC-A results of 0.1 mM oleic acid-treated cell samples on the 3rd day



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Tube Name:	02mM 1st day_002
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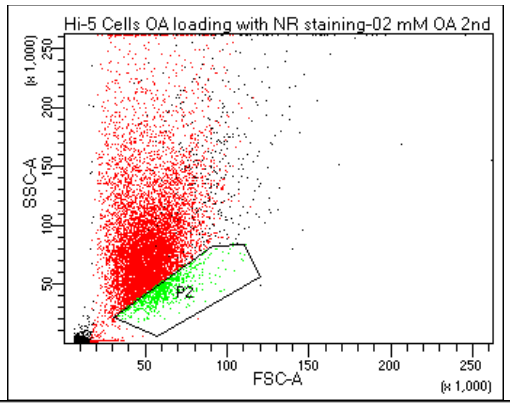
GUID:	c8c7c65e-d7a5-4051-b37f-7cd0...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	52,678	80,123
P1	9,156	91.6	50,398	78,152
P2	1,474	14.7	61,174	43,891

Figure A. 14. FSC-A results of 0.2 mM oleic acid-treated cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02 mM OA 2nd day_003
Record Date:	Jun 8, 2023 3:27:52 PM
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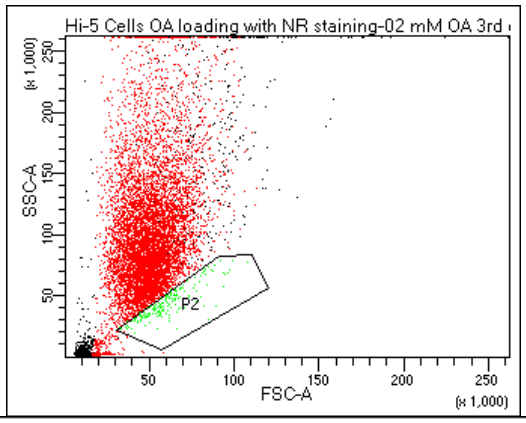
Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	48,038	79,705
P1	8,514	85.1	50,295	85,079
P2	727	7.3	60,448	43,751

Figure A. 15. FSC-A results of 0.2 mM oleic acid-treated cell samples on the 2nd day



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Tube Name:	02 mM OA 3rd day_003
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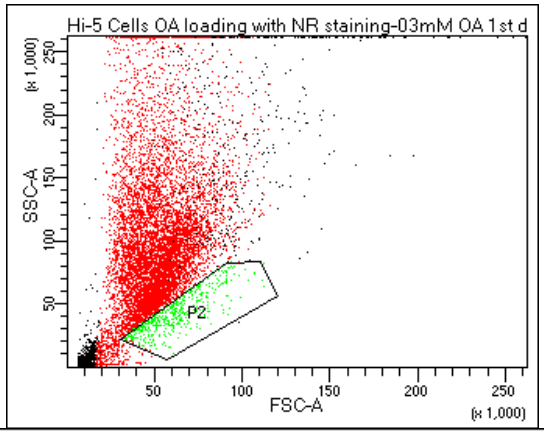
Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	45,145	88,740
P1	8,349	83.5	48,880	98,371
P2	221	2.2	59,412	44,215

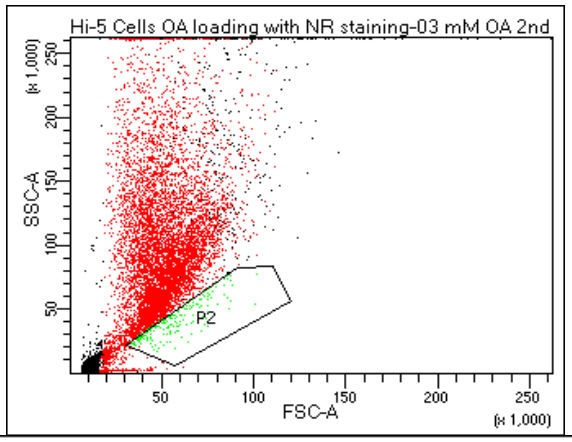
Figure A. 16. FSC-A results of 0.2 mM oleic acid-treated cell samples on the 3rd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03mM OA 1st day_004
Record Date:	Jun 7, 2023 3:42:57 PM
$OP:	Researchers
GUID:	4e0b5405-fe92-4c9d-b2c6-5c9...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	48,723	92,005
P1	8,368	83.7	50,328	96,822
P2	509	5.1	60,196	41,724

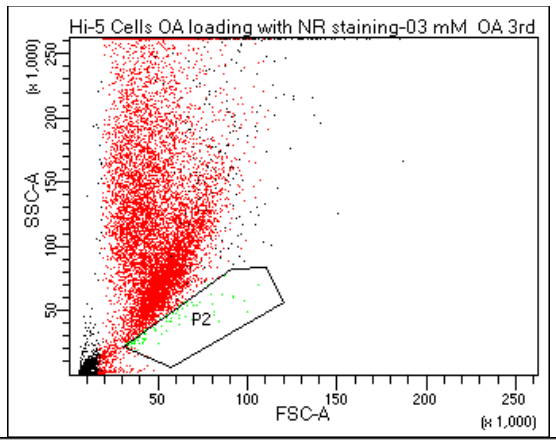
Figure A. 17. FSC-A results of 0.3 mM oleic acid-treated cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03 mM OA 2nd day_005
Record Date:	Jun 8, 2023 3:52:07 PM
$OP:	Researchers
GUID:	4d887a00-a31b-4b7f-910b-8e4...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,138	####	43,489	82,976
P1	7,983	78.7	48,481	94,936
P2	227	2.2	54,450	39,611

Figure A. 18. FSC-A results of 0.3 mM oleic acid-treated cell samples on the 2nd day

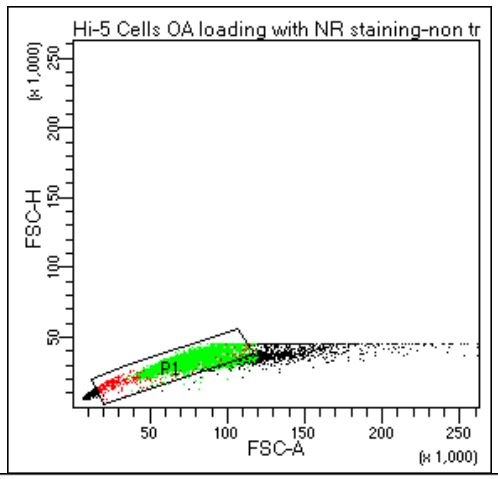


Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03 mM OA 3rd day_005
Record Date:	Jun 9, 2023 3:22:07 PM
$OP:	Researchers
GUID:	ef1225fd-de85-42d9-91dd-5426...

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean
All Events	10,000	####	43,508	106,218
P1	8,362	83.6	47,131	118,347
P2	99	1.0	53,189	38,684

Figure A. 19. FSC-A results of 0.3 mM oleic acid-treated cell samples on the 3rd day

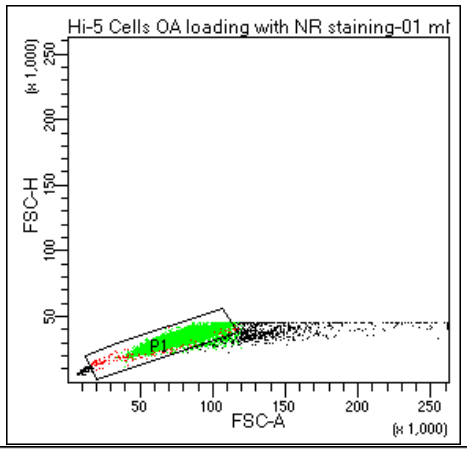
FSC-A vehicle control results



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	non treatment cells(no control n...
Record Date:	Jun 14, 2023 2:52:56 PM
$OP:	Researchers
GUID:	42421543-408e-4964-944b-09...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	73,507	30,861
P1	8,273	82.7	67,145	31,113
P2	7,793	77.9	68,300	31,481

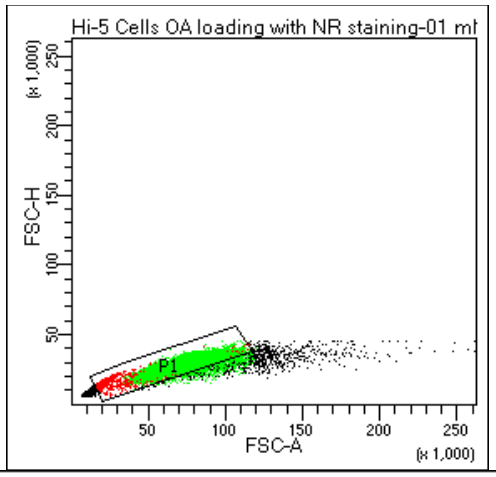
Figure A. 20. FSC-A results of non-treated cell samples



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM control 1st day
Record Date:	Jun 7, 2023 2:36:51 PM
$OP:	Researchers
GUID:	9901c4f8-4265-495f-95d6-f48c...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	76,851	31,809
P1	8,952	89.5	70,896	31,576
P2	7,961	79.6	71,809	32,060

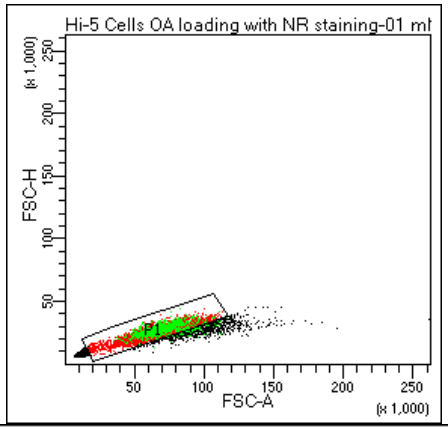
Figure A. 21. FSC-A results of 0.1 mM vehicle control cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM control 2nd day
Record Date:	Jun 8, 2023 3:03:22 PM
$OP:	Researchers
GUID:	285a6fc3-a825-42be-b51c-f4cb...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	12,461	####	60,226	22,973
P1	8,610	69.1	65,461	26,056
P2	5,523	44.3	72,906	27,963

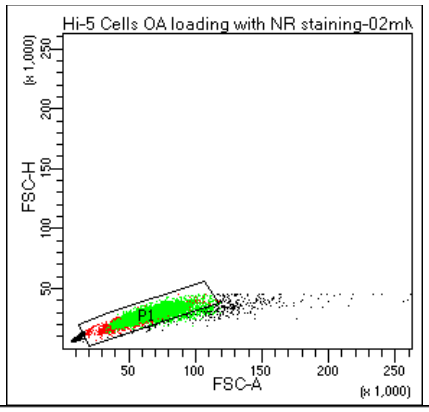
Figure A. 22. FSC-A results of 0.1 mM vehicle control cell samples on the 2nd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM control 3rd day
Record Date:	Jun 9, 2023 2:15:20 PM
$OP:	Researchers
GUID:	a996d91d-f082-49ab-bb29-b72...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	61,590	22,945
P1	7,701	77.0	63,705	24,703
P2	1,084	10.8	69,748	27,119

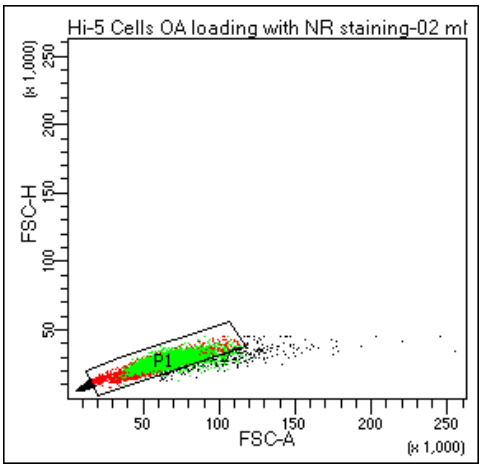
Figure A. 23. FSC-A results of 0.1 mM vehicle control cell samples on the 3rd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02mM control 1st day_001
Record Date:	Jun 7, 2023 3:04:04 PM
$OP:	Researchers
GUID:	35802879-6fe6-48cd-b13f-28c3...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	62,863	26,662
P1	9,160	91.6	61,350	26,980
P2	7,159	71.6	64,520	28,170

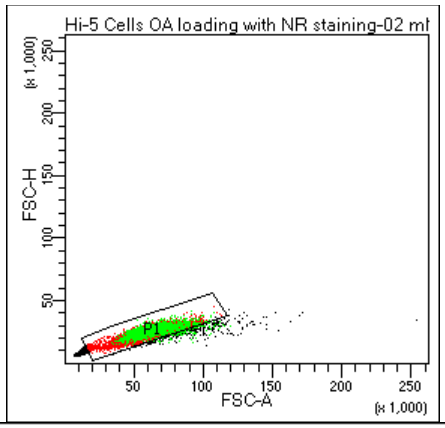
Figure A. 24. FSC-A results of 0.2 mM vehicle control cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02 mM control 2nd day_002
Record Date:	Jun 8, 2023 3:12:26 PM
$OP:	Researchers
GUID:	74c5af4b-5527-40eb-ad35-58b...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	55,560	22,903
P1	8,254	82.5	59,648	24,876
P2	4,015	40.2	64,268	26,531

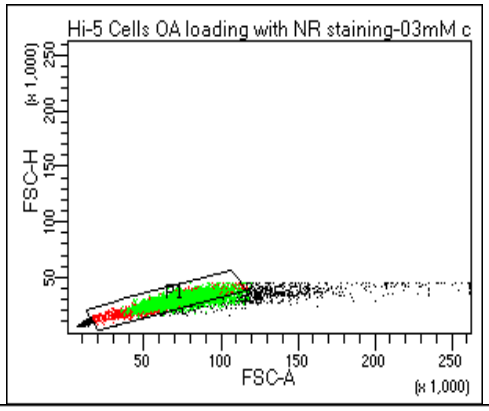
Figure A. 25. FSC-A results of 0.2 mM vehicle control cell samples on the 2nd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02 mM control 3rd day_002
Record Date:	Jun 9, 2023 2:46:52 PM
$OP:	Researchers
GUID:	9fa09247-2b74-4e4a-adb8-67fb...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	53,354	21,134
P1	8,347	83.5	56,769	22,725
P2	2,289	22.9	63,704	25,394

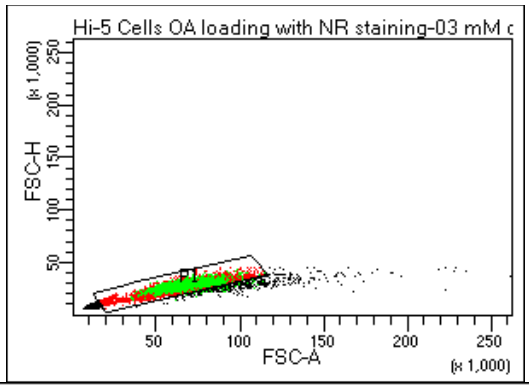
Figure A. 26. FSC-A results of 0.2 mM vehicle control cell samples on the 3rd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03mM control 1st day_003
Record Date:	Jun 7, 2023 3:26:14 PM
$OP:	Researchers
GUID:	15c8c25e-1e2c-4ce6-b6d2-e25...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	69,748	26,857
P1	8,355	83.6	65,466	27,238
P2	5,398	54.0	69,875	28,755

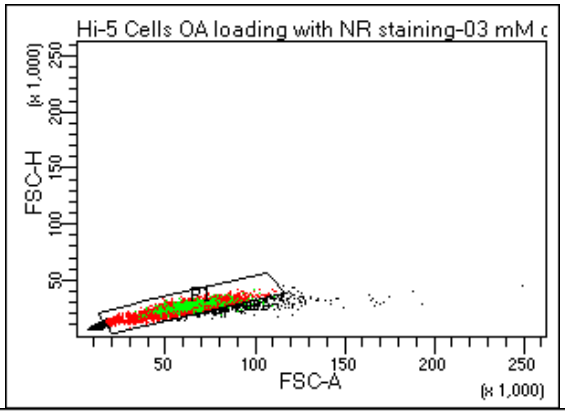
Figure A. 27. FSC-A results of 0.3 mM vehicle control cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03 mM control 2nd day_004
Record Date:	Jun 8, 2023 3:33:13 PM
$OP:	Researchers
GUID:	b3b35504-e99a-42cd-8a86-898...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	57,857	23,222
P1	8,021	80.2	62,404	25,566
P2	1,398	14.0	66,001	27,422

Figure A. 28. FSC-A results of 0.3 mM vehicle control cell samples on the 2nd day

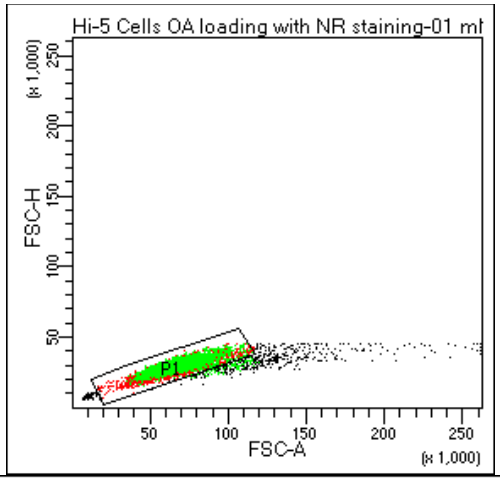


Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03 mM control 3rd day_004
Record Date:	Jun 9, 2023 3:15:17 PM
$OP:	Researchers
GUID:	6f75dd9c-862c-4bfc-b5cc-dc6af...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,069	####	53,526	22,049
P1	8,305	82.5	57,799	24,083
P2	361	3.6	64,178	26,383

Figure A. 29. FSC-A results of 0.3 mM vehicle control cell samples on the 3rd day

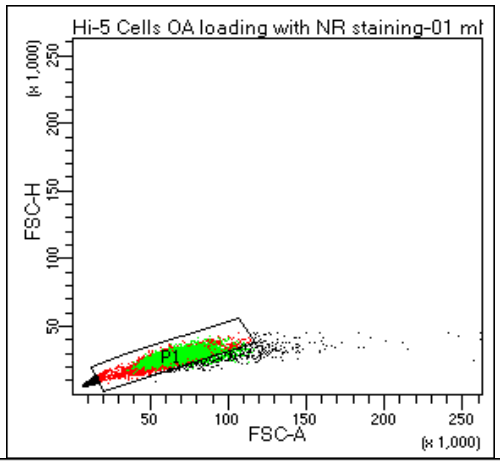
FSC-A oleic acid treatment results



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM 1st day
Record Date:	Jun 7, 2023 2:55:52 PM
$OP:	Researchers
GUID:	8b0eccf8-7733-4491-b64c-dbbf...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	65,910	27,365
P1	9,261	92.6	62,674	27,287
P2	4,344	43.4	66,729	29,343

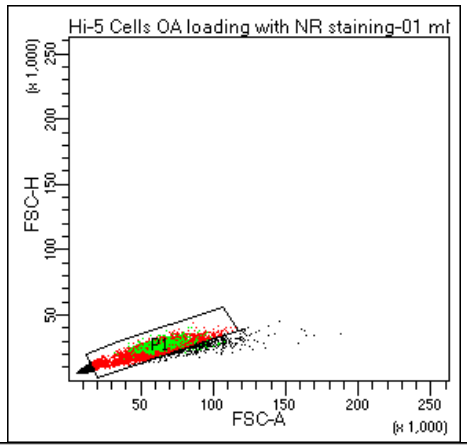
Figure A. 30. FSC-A results of 0.1 mM oleic acid-treated cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM 2nd day_001
Record Date:	Jun 8, 2023 3:05:29 PM
$OP:	Researchers
GUID:	189af78a-65cf-4333-82a0-da80...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	59,512	23,906
P1	8,632	86.3	60,325	24,946
P2	2,399	24.0	66,375	27,345

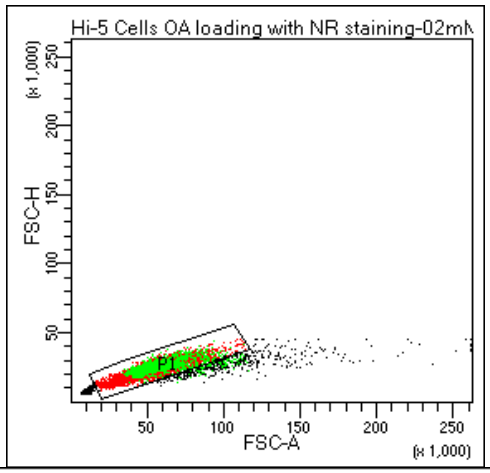
Figure A. 31. FSC-A results of 0.1 mM oleic acid-treated cell samples on the 2nd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM OA 3rd day_001
Record Date:	Jun 9, 2023 2:28:41 PM
$OP:	Researchers
GUID:	0fb602ff-7735-4b55-879a-cf3c8...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	50,756	20,453
P1	7,722	77.2	57,428	23,161
P2	529	5.3	64,713	26,682

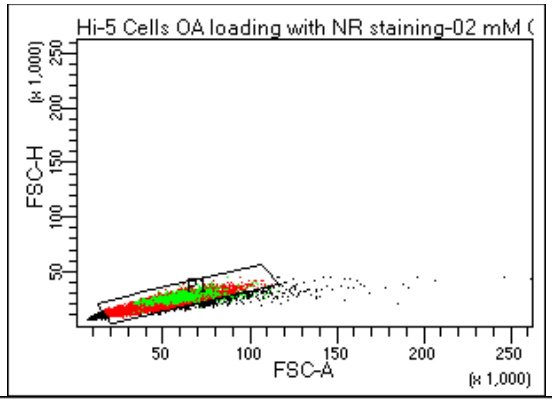
Figure A. 32. FSC-A results of 0.1 mM oleic acid-treated cell samples on the 3rd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02mM 1st day_002
Record Date:	Jun 7, 2023 3:17:45 PM
$OP:	Researchers
GUID:	c8c7c65e-d7a5-4051-b37f-7cd0...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	52,678	22,317
P1	9,156	91.6	50,398	22,417
P2	1,474	14.7	61,174	26,047

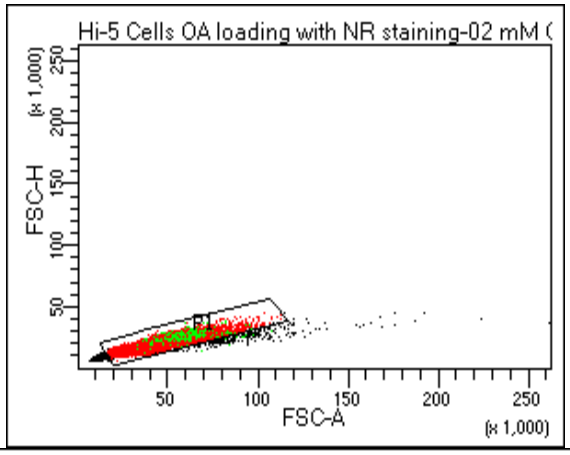
Figure A. 33. FSC-A results of 0.2 mM oleic acid-treated cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02 mM OA 2nd day_003
Record Date:	Jun 8, 2023 3:27:52 PM
$OP:	Researchers
GUID:	c5d8e2b7-9aa5-41e6-b348-59e...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	48,038	20,480
P1	8,514	85.1	50,295	21,779
P2	727	7.3	60,448	25,777

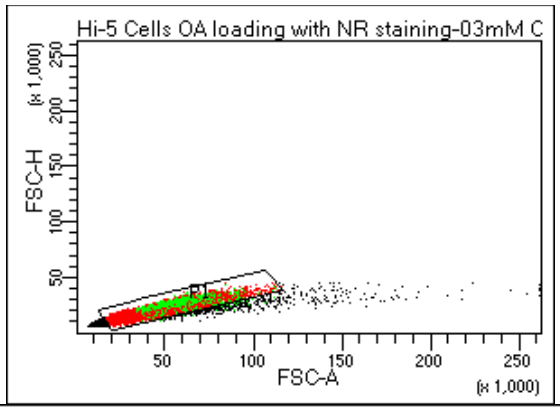
Figure A. 34. FSC-A results of 0.2 mM oleic acid-treated cell samples on the 2nd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02 mM OA 3rd day_003
Record Date:	Jun 9, 2023 2:53:45 PM
$OP:	Researchers
GUID:	8c2b1e1d-9ee0-4081-a0d4-eab...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	45,145	19,553
P1	8,349	83.5	48,880	21,265
P2	221	2.2	59,412	25,269

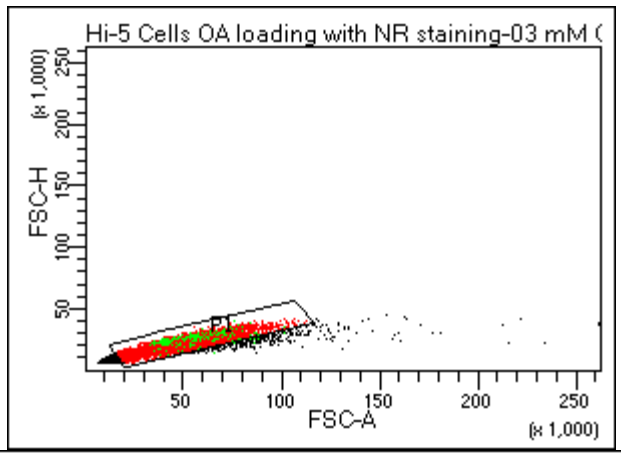
Figure A. 35. FSC-A results of 0.2 mM oleic acid-treated cell samples on the 3rd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03mM OA 1st day_004
Record Date:	Jun 7, 2023 3:42:57 PM
$OP:	Researchers
GUID:	4e0b5405-fe92-4c9d-b2c6-5c9...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	48,723	20,381
P1	8,368	83.7	50,328	21,741
P2	509	5.1	60,196	26,149

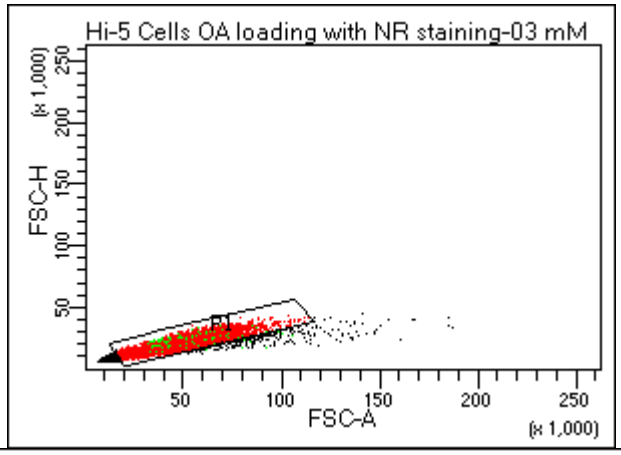
Figure A. 36. FSC-A results of 0.3 mM oleic acid-treated cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03 mM OA 2nd day_005
Record Date:	Jun 8, 2023 3:52:07 PM
$OP:	Researchers
GUID:	4d887a00-a31b-4b7f-910b-8e4...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,138	####	43,489	19,019
P1	7,983	78.7	48,481	21,311
P2	227	2.2	54,450	24,675

Figure A. 37. FSC-A results of 0.3 mM oleic acid-treated cell samples on the 2nd day

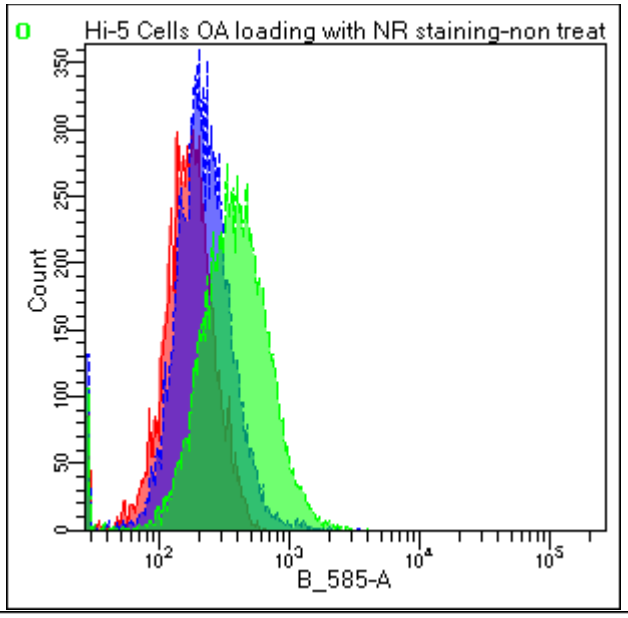


Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03 mM OA 3rd day_005
Record Date:	Jun 9, 2023 3:22:07 PM
$OP:	Researchers
GUID:	ef1225fd-de85-42d9-91dd-5426...

Population	#Events	%Parent	FSC-A Mean	FSC-H Mean
All Events	10,000	####	43,508	19,136
P1	8,362	83.6	47,131	20,849
P2	99	1.0	53,189	23,022

Figure A. 38. FSC-A results of 0.3 mM oleic acid-treated cell samples on the 3rd day

Intracellular lipid fluorescence intensity results for vehicle control and oleic acid treated cell samples

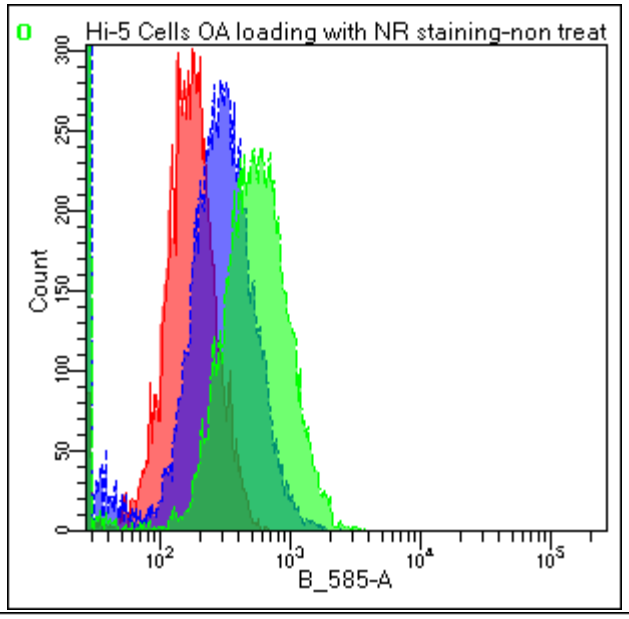


Tube: non treatment cells(no control no OA)

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P3	4,395	44.0	44.0
P1	8,273	82.7	82.7
Hi-5 Cells OA loading with NR staining/01 mM control 1st da	10,000	####	100.0
Hi-5 Cells OA loading with NR staining/01 mM control 1s	8,952	89.5	89.5
Hi-5 Cells OA loading with NR staining/01 mM control 1s	3,819	38.2	38.2
Hi-5 Cells OA loading with NR staining/01 mM 1st day/All Ev	10,000	####	100.0
Hi-5 Cells OA loading with NR staining/01 mM 1st day/P	9,261	92.6	92.6
Hi-5 Cells OA loading with NR staining/01 mM 1st day/P	5,559	55.6	55.6

Non-treated cell samples
Vehicle control samples (VC)
Oleic acid treated samples (OA)

Figure A. 39. Intracellular lipid fluorescence intensity results for non-treated and oleic-acid-treated cell samples for 0.1 mM on the 1st day

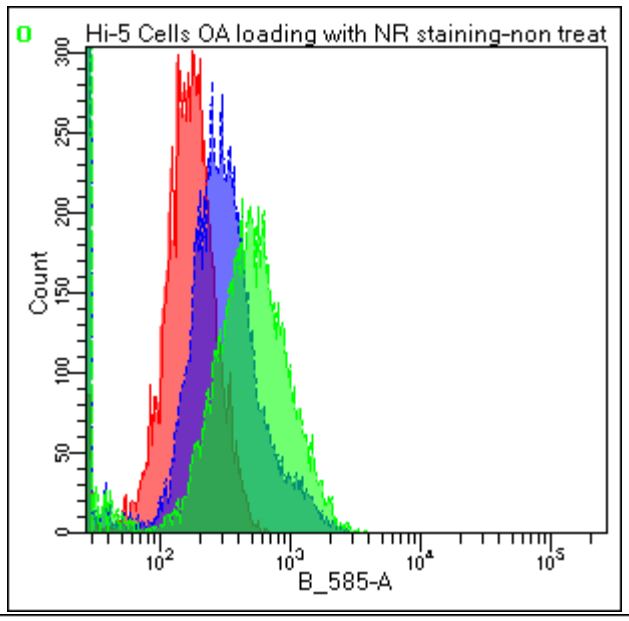


Tube: non treatment cells(no control no OA)

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P3	4,395	44.0	44.0
P1	8,273	82.7	82.7
Hi-5 Cells OA loading with NR staining/01 mM	12,461	####	100.0
Hi-5 Cells OA loading with NR staining/01 ı	8,610	69.1	69.1
Hi-5 Cells OA loading with NR staining/01 ı	5,635	45.2	45.2
Hi-5 Cells OA loading with NR staining/01 mM	10,000	####	100.0
Hi-5 Cells OA loading with NR staining/01 ı	8,632	86.3	86.3
Hi-5 Cells OA loading with NR staining/01 ı	5,821	58.2	58.2

Non-treated cell samples
Vehicle control samples (VC)
Oleic acid treated samples (OA)

Figure A. 40. Intracellular lipid fluorescence intensity results for 0.1 mM vehicle control and oleic-acid treated cell samples on the 2nd day

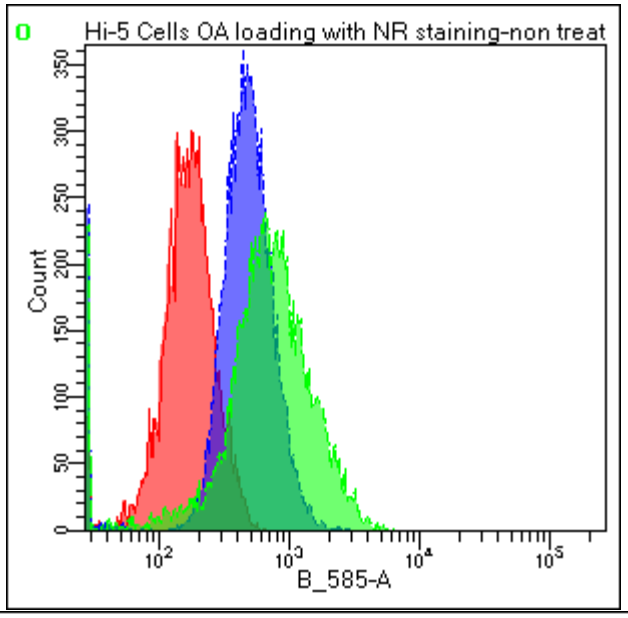


Tube: non treatment cells(no control no OA)

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P3	4,395	44.0	44.0
P1	8,273	82.7	82.7
Hi-5 Cells OA loading with NR staining/01 ml	10,000	####	100.0
Hi-5 Cells OA loading with NR staining/01	7,701	77.0	77.0
Hi-5 Cells OA loading with NR staining/01	4,628	46.3	46.3
Hi-5 Cells OA loading with NR staining/01 ml	10,000	####	100.0
Hi-5 Cells OA loading with NR staining/01	7,722	77.2	77.2
Hi-5 Cells OA loading with NR staining/01	3,367	33.7	33.7

Non-treated cell samples
Vehicle control samples (VC)
Oleic acid treated samples (OA)

Figure A. 41. Intracellular lipid fluorescence intensity results for 0.1 mM vehicle control and oleic-acid treated cell samples on the 3rd day

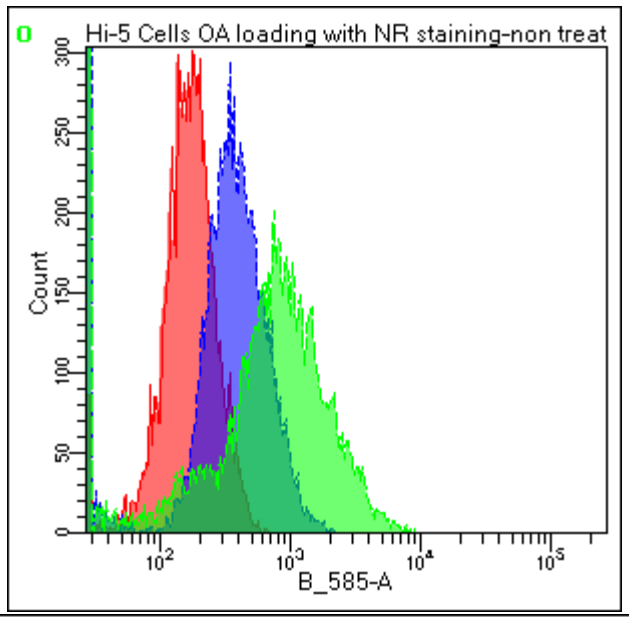


Tube: non treatment cells(no control no OA)

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P3	4,395	44.0	44.0
P1	8,273	82.7	82.7
Hi-5 Cells OA loading with NR staining/02mM cc	10,000	####	100.0
Hi-5 Cells OA loading with NR staining/02ml	9,160	91.6	91.6
Hi-5 Cells OA loading with NR staining/02ml	6,473	64.7	64.7
Hi-5 Cells OA loading with NR staining/02mM 1s	10,000	####	100.0
Hi-5 Cells OA loading with NR staining/02ml	9,156	91.6	91.6
Hi-5 Cells OA loading with NR staining/02ml	4,931	49.3	49.3

Non-treated cell samples
Vehicle control samples (VC)
Oleic acid treated samples (OA)

Figure A. 42. Intracellular lipid fluorescence intensity results for 0.2 mM vehicle control and oleic-acid treated cell samples on the 1st day

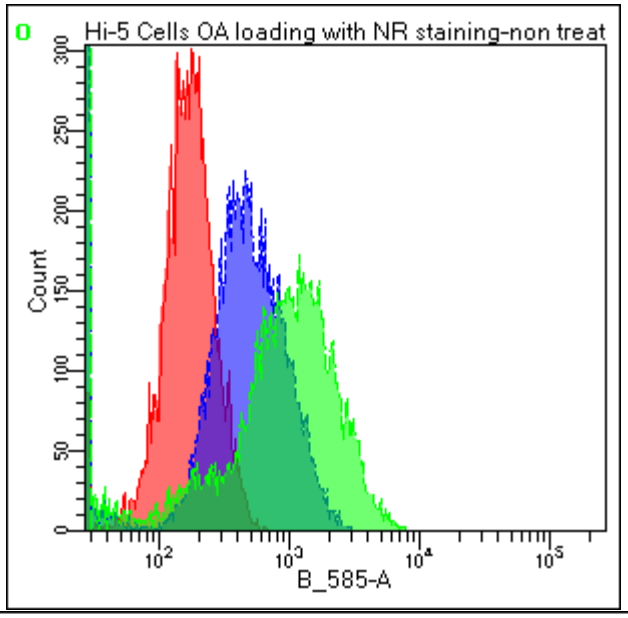


Tube: non treatment cells(no control no OA)

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P3	4,395	44.0	44.0
P1	8,273	82.7	82.7
Hi-5 Cells OA loading with NR stain	10,000	####	100.0
Hi-5 Cells OA loading with NR st	8,254	82.5	82.5
Hi-5 Cells OA loading with NR st	6,484	64.8	64.8
Hi-5 Cells OA loading with NR stain	10,000	####	100.0
Hi-5 Cells OA loading with NR st	8,514	85.1	85.1
Hi-5 Cells OA loading with NR st	3,931	39.3	39.3

Non-treated cell samples
Vehicle control samples (VC)
Oleic acid treated samples (OA)

Figure A. 43. Intracellular lipid fluorescence intensity results for 0.2 mM vehicle control and oleic-acid treated cell samples on the 2nd day



Tube: non treatment cells(no control no OA)

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P3	4,395	44.0	44.0
P1	8,273	82.7	82.7
Hi-5 Cells OA loading with NR stain	10,000	####	100.0
Hi-5 Cells OA loading with NR st	8,347	83.5	83.5
Hi-5 Cells OA loading with NR st	6,311	63.1	63.1
Hi-5 Cells OA loading with NR stain	10,000	####	100.0
Hi-5 Cells OA loading with NR st	8,349	83.5	83.5
Hi-5 Cells OA loading with NR st	2,548	25.5	25.5

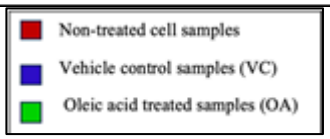
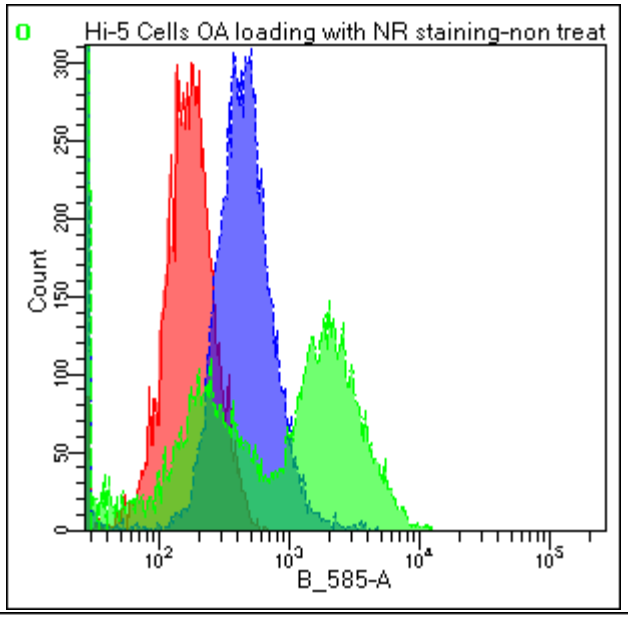


Figure A. 44. Intracellular lipid fluorescence intensity results for 0.2 mM vehicle control and oleic-acid treated cell samples on the 3rd day

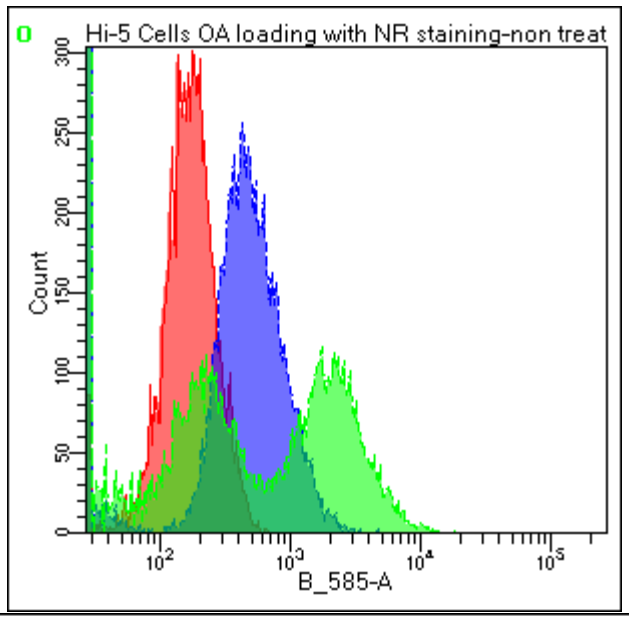


Tube: non treatment cells(no control no OA)

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P3	4,395	44.0	44.0
P1	8,273	82.7	82.7
Hi-5 Cells OA loading with NR staining/03mM cor	10,000	####	100.0
Hi-5 Cells OA loading with NR staining/03mM	8,355	83.6	83.6
Hi-5 Cells OA loading with NR staining/03mM	5,721	57.2	57.2
Hi-5 Cells OA loading with NR staining/03mM OA	10,000	####	100.0
Hi-5 Cells OA loading with NR staining/03mM	8,368	83.7	83.7

Non-treated cell samples
Vehicle control samples (VC)
Oleic acid treated samples (OA)

Figure A. 45. Intracellular lipid fluorescence intensity results for 0.3 mM vehicle control and oleic-acid treated cell samples on the 1st day



Tube: non treatment cells(no control no OA)

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P3	4,395	44.0	44.0
P1	8,273	82.7	82.7
Hi-5 Cells OA loading with NR stain	10,000	####	100.0
Hi-5 Cells OA loading with NR st	8,021	80.2	80.2
Hi-5 Cells OA loading with NR st	4,638	46.4	46.4
Hi-5 Cells OA loading with NR stain	10,138	####	100.0
Hi-5 Cells OA loading with NR st	7,983	78.7	78.7
Hi-5 Cells OA loading with NR st	2,773	27.4	27.4

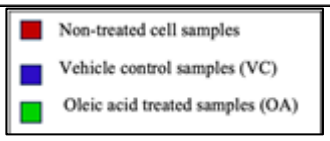
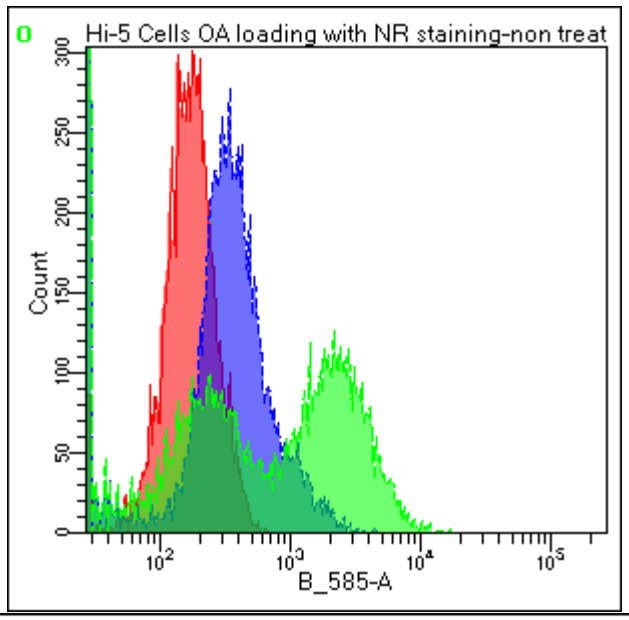


Figure A. 46. Intracellular lipid fluorescence intensity results for 0.3 mM vehicle control and oleic-acid treated cell samples on the 2nd day



Tube: non treatment cells(no control no OA)

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P3	4,395	44.0	44.0
P1	8,273	82.7	82.7
Hi-5 Cells OA loading with NR stain	10,069	####	100.0
Hi-5 Cells OA loading with NR st	8,305	82.5	82.5
Hi-5 Cells OA loading with NR st	3,763	37.4	37.4
Hi-5 Cells OA loading with NR stain	10,000	####	100.0
Hi-5 Cells OA loading with NR st	8,362	83.6	83.6
Hi-5 Cells OA loading with NR st	2,145	21.4	21.4

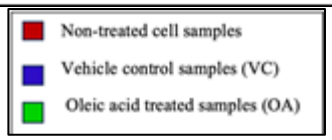


Figure A. 47. Intracellular lipid fluorescence intensity results for 0.3 mM vehicle control and oleic-acid treated cell samples on the 3rd day

Phospholipid fluorescence intensity results of non-treated and vehicle control insect cell samples

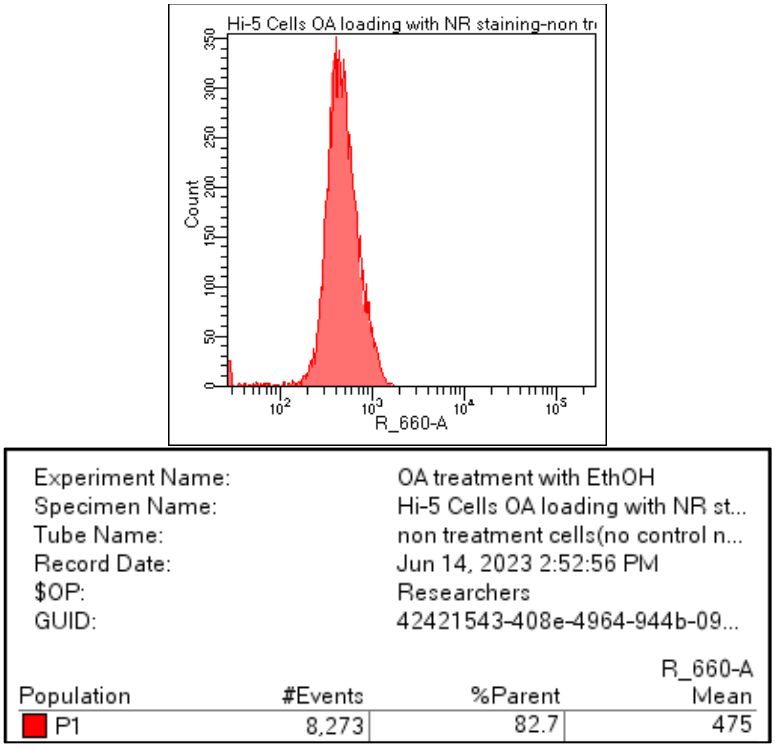
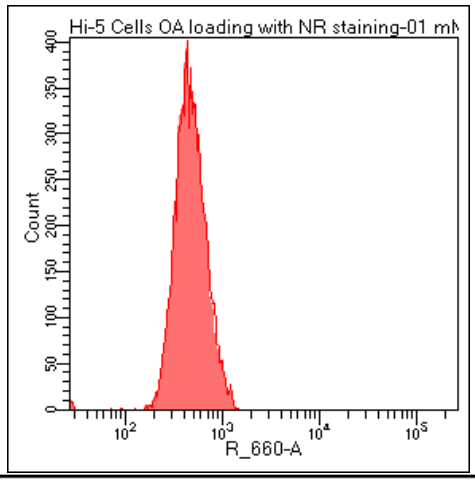


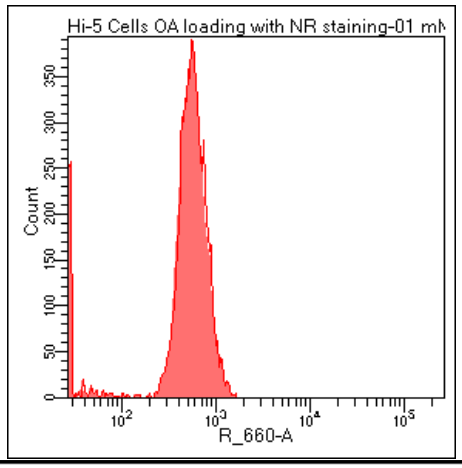
Figure A. 48. Phospholipid fluorescence intensity results of non-treated cell samples



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM control 1st day
Record Date:	Jun 7, 2023 2:36:51 PM
$OP:	Researchers
GUID:	9901c4f8-4265-495f-95d6-f48c...

Population	#Events	%Parent	R_660-A Mean
P1	8,952	89.5	469

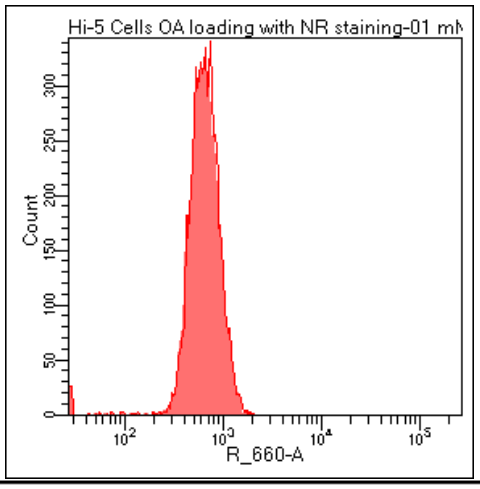
Figure A. 49. Phospholipid fluorescence intensity results of 0.1 mM vehicle control cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM control 2nd day
Record Date:	Jun 8, 2023 3:03:22 PM
$OP:	Researchers
GUID:	285a6fc3-a825-42be-b51c-f4cb...

Population	#Events	%Parent	R_660-A Mean
P1	8,610	69.1	531

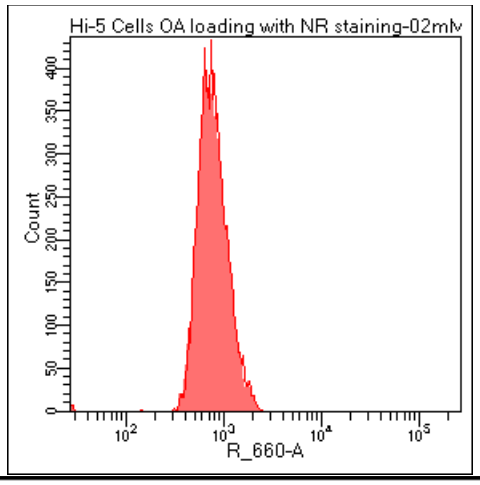
Figure A. 50. Phospholipid fluorescence intensity results of 0.1 mM vehicle control cell samples on the 2nd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM control 3rd day
Record Date:	Jun 9, 2023 2:15:20 PM
$OP:	Researchers
GUID:	a996d91d-f082-49ab-bb29-b72...

Population	#Events	%Parent	R_660-A Mean
P1	7,701	77.0	635

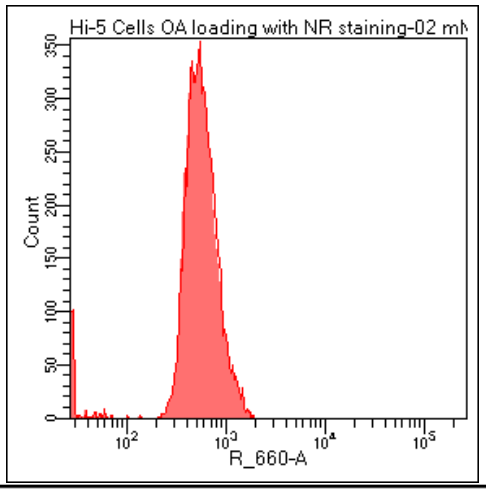
Figure A. 51. Phospholipid fluorescence intensity results of 0.1 mM vehicle control cell samples on the 3rd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02mM control 1st day_001
Record Date:	Jun 7, 2023 3:04:04 PM
$OP:	Researchers
GUID:	35802879-6fe6-48cd-b13f-28c3...

Population	#Events	%Parent	R_660-A Mean
P1	9,160	91.6	773

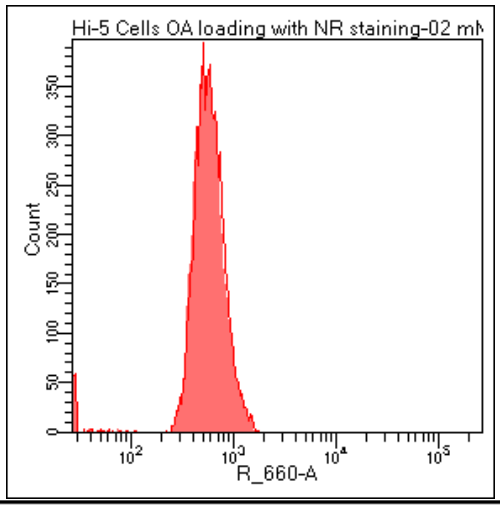
Figure A. 52. Phospholipid fluorescence intensity results of 0.2 mM vehicle control cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02 mM control 2nd day_002
Record Date:	Jun 8, 2023 3:12:26 PM
$OP:	Researchers
GUID:	74c5af4b-5527-40eb-ad35-58b...

Population	#Events	%Parent	R_660-A Mean
P1	8,254	82.5	555

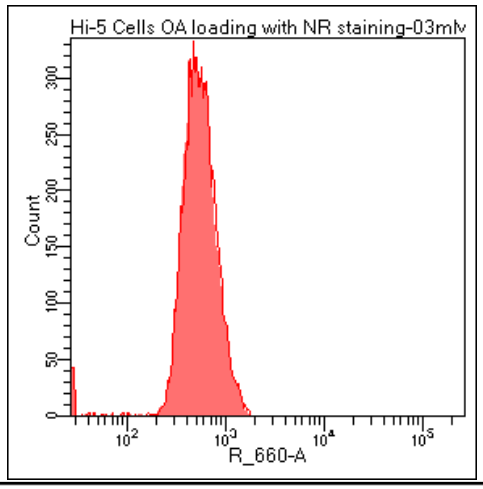
Figure A. 53. Phospholipid fluorescence intensity results of 0.2 mM vehicle control cell samples on the 2nd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02 mM control 3rd day_002
Record Date:	Jun 9, 2023 2:46:52 PM
$OP:	Researchers
GUID:	9fa09247-2b74-4e4a-adb8-67fb...

Population	#Events	%Parent	R_660-A Mean
P1	8,347	83.5	567

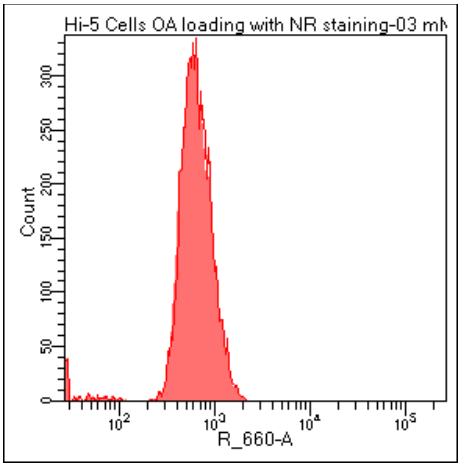
Figure A. 54. Phospholipid fluorescence intensity results of 0.2 mM vehicle control cell samples on the 3rd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03mM control 1st day_003
Record Date:	Jun 7, 2023 3:26:14 PM
$OP:	Researchers
GUID:	15c8c25e-1e2c-4ce6-b6d2-e25...

Population	#Events	%Parent	R_660-A Mean
P1	8,355	83.6	544

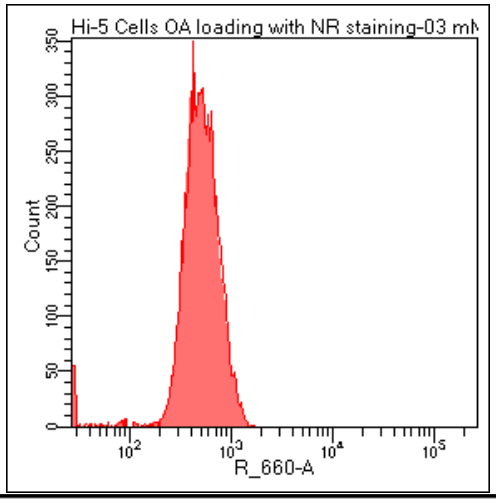
Figure A. 55. Phospholipid fluorescence intensity results of 0.3 mM vehicle control cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03 mM control 2nd day_004
Record Date:	Jun 8, 2023 3:33:13 PM
$OP:	Researchers
GUID:	b3b35504-e99a-42cd-8a86-898...

Population	#Events	%Parent	R_660-A Mean
P1	8,021	80.2	635

Figure A. 56. Phospholipid fluorescence intensity results of 0.3 mM vehicle control cell samples on the 2nd day

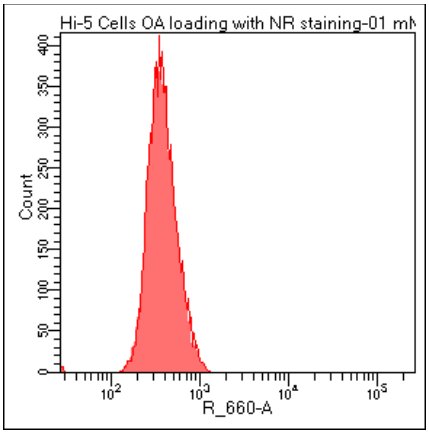


Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03 mM control 3rd day_004
Record Date:	Jun 9, 2023 3:15:17 PM
$OP:	Researchers
GUID:	6f75dd9c-862c-4bfc-b5cc-dc6af...

Population	#Events	%Parent	R_660-A Mean
P1	8,305	82.5	506

Figure A. 57. Phospholipid fluorescence intensity results of 0.3 mM vehicle control cell samples on the 3rd day

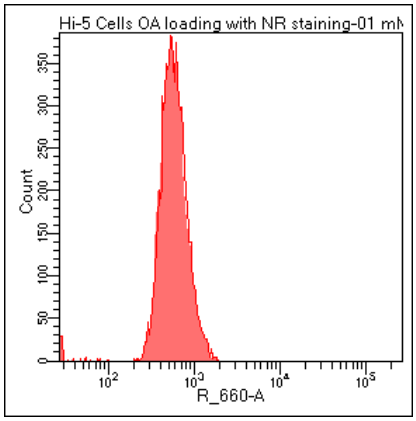
Phospholipid fluorescence intensity results for oleic acid-treated cell samples



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM 1st day
Record Date:	Jun 7, 2023 2:55:52 PM
$OP:	Researchers
GUID:	8b0eccf8-7733-4491-b64c-dbbf...

Population	#Events	%Parent	R_660-A Mean
P1	9,261	92.6	382

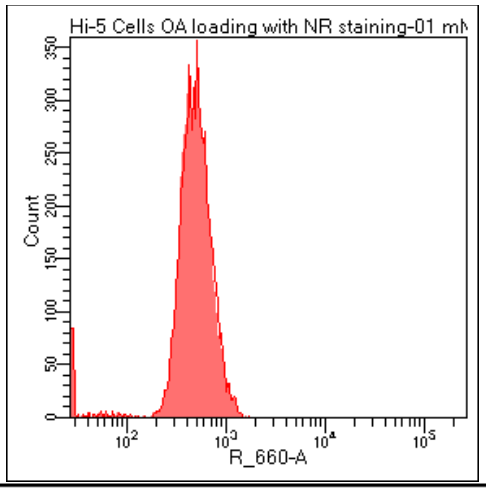
Figure A. 58. Phospholipid fluorescence intensity results for 0.1 mM oleic acid-treated cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM 2nd day_001
Record Date:	Jun 8, 2023 3:05:29 PM
$OP:	Researchers
GUID:	189af78a-65cf-4333-82a0-da80...

Population	#Events	%Parent	R_660-A Mean
P1	8,632	86.3	571

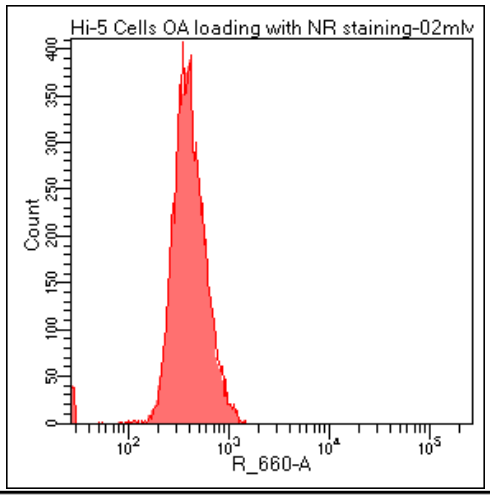
Figure A. 59. Phospholipid fluorescence intensity results for 0.1 mM oleic acid-treated cell samples on the 2nd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	01 mM OA 3rd day_001
Record Date:	Jun 9, 2023 2:28:41 PM
$OP:	Researchers
GUID:	0fb602ff-7735-4b55-879a-cf3c8...

Population	#Events	%Parent	R_660-A Mean
P1	7,722	77.2	483

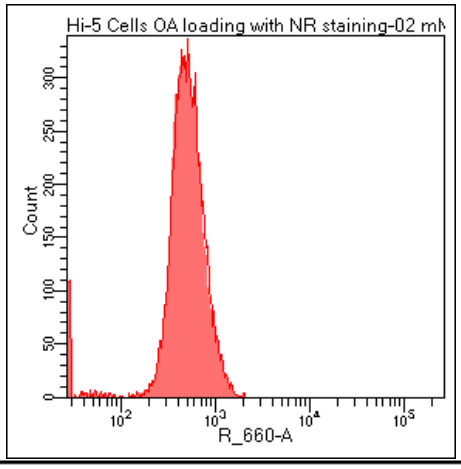
Figure A. 60. Phospholipid fluorescence intensity results for 0.1 mM oleic acid-treated cell samples on the 3rd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02mM 1st day_002
Record Date:	Jun 7, 2023 3:17:45 PM
$OP:	Researchers
GUID:	c8c7c65e-d7a5-4051-b37f-7cd0...

Population	#Events	%Parent	R_660-A Mean
P1	9,156	91.6	408

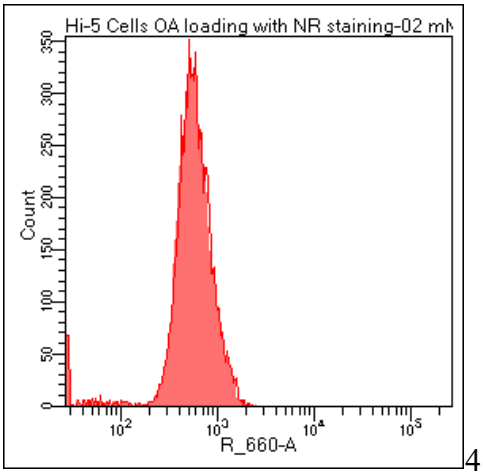
Figure A. 61. Phospholipid fluorescence intensity results for 0.2 mM oleic acid-treated cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02 mM OA 2nd day_003
Record Date:	Jun 8, 2023 3:27:52 PM
$OP:	Researchers
GUID:	c5d8e2b7-9aa5-41e6-b348-59e...

Population	#Events	%Parent	R_660-A Mean
P1	8,514	85.1	506

Figure A. 62. Phospholipid fluorescence intensity results for 0.2 mM oleic acid-treated cell samples on the 2nd day

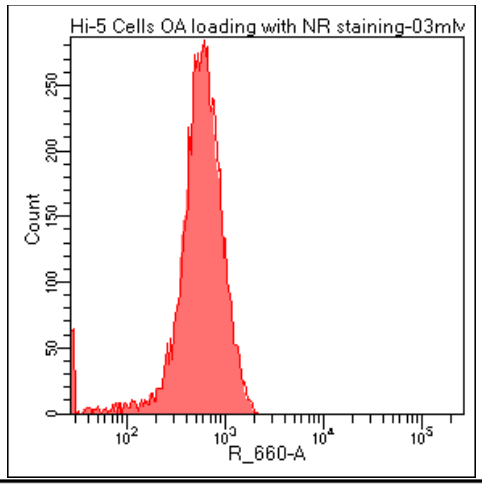


4

Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	02 mM OA 3rd day_003
Record Date:	Jun 9, 2023 2:53:45 PM
$OP:	Researchers
GUID:	8c2b1e1d-9ee0-4081-a0d4-eab...

Population	#Events	%Parent	R_660-A Mean
P1	8,349	83.5	578

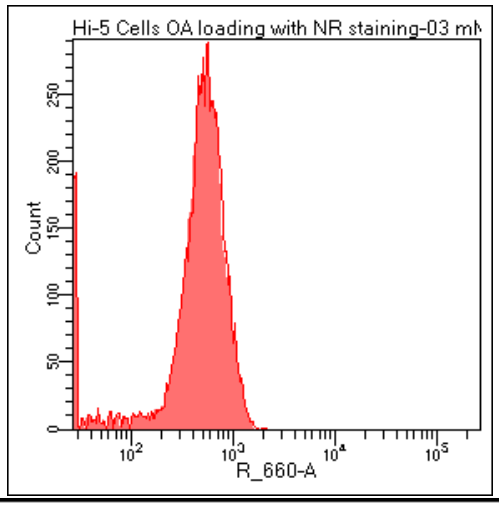
Figure A. 63. Phospholipid fluorescence intensity results for 0.2 mM oleic acid-treated cell samples on the 3rd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03mM OA 1st day_004
Record Date:	Jun 7, 2023 3:42:57 PM
$OP:	Researchers
GUID:	4e0b5405-fe92-4c9d-b2c6-5c9...

Population	#Events	%Parent	R_660-A Mean
P1	8,368	83.7	590

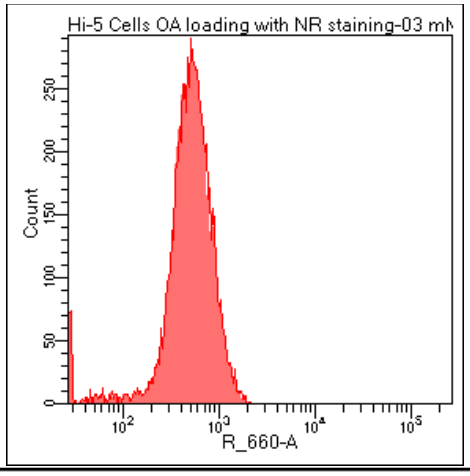
Figure A. 64. Phospholipid fluorescence intensity results for 0.3 mM oleic acid-treated cell samples on the 1st day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03 mM OA 2nd day_005
Record Date:	Jun 8, 2023 3:52:07 PM
$OP:	Researchers
GUID:	4d887a00-a31b-4b7f-910b-8e4...

Population	#Events	%Parent	R_660-A Mean
P1	7,983	78.7	507

Figure A. 65. Phospholipid fluorescence intensity results for 0.3 mM oleic acid-treated cell samples on the 2nd day



Experiment Name:	OA treatment with EthOH
Specimen Name:	Hi-5 Cells OA loading with NR st...
Tube Name:	03 mM OA 3rd day_005
Record Date:	Jun 9, 2023 3:22:07 PM
$OP:	Researchers
GUID:	ef1225fd-de85-42d9-91dd-5426...

Population	#Events	%Parent	R_660-A Mean
P1	8,362	83.6	528

Figure A. 66. Phospholipid fluorescence intensity results for 0.3 mM oleic acid-treated cell samples on the 3rd day

Microscopic images of vehicle control and oleic acid-treated cell samples

Fluorescence images of vehicle control cell samples

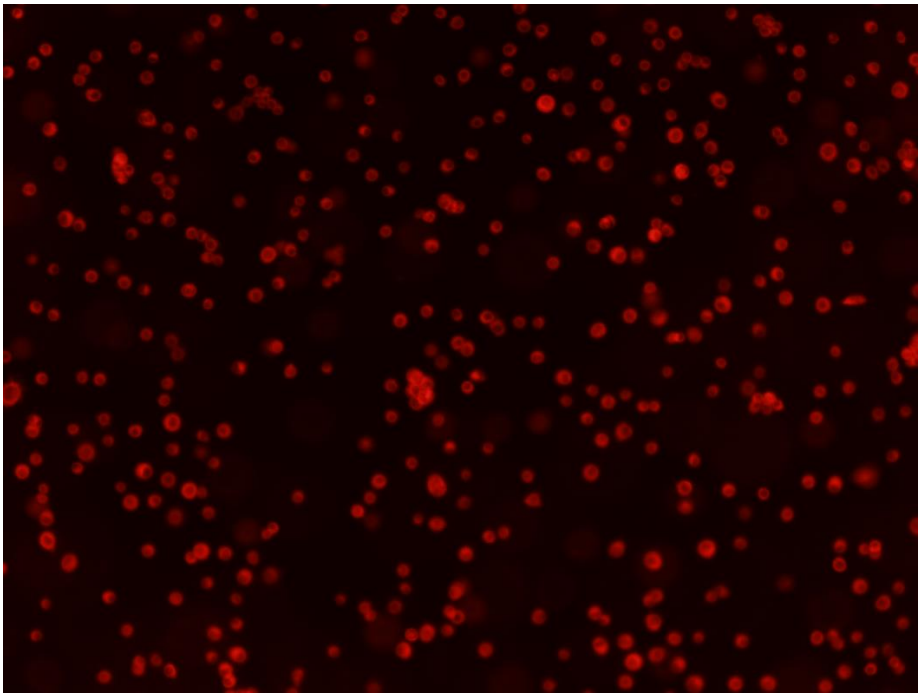


Figure A. 67. Fluorescence image of 0.1 mM vehicle control cell samples on the 1st day

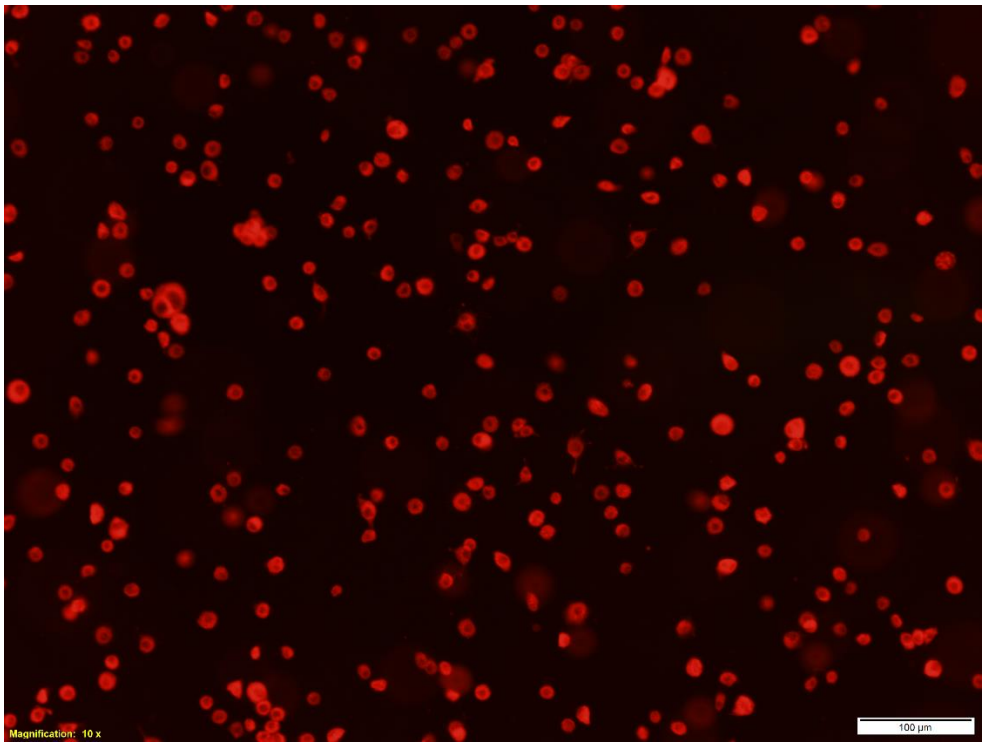


Figure A. 68. Fluorescence image of 0.1 mM vehicle control cell samples on the 2nd day

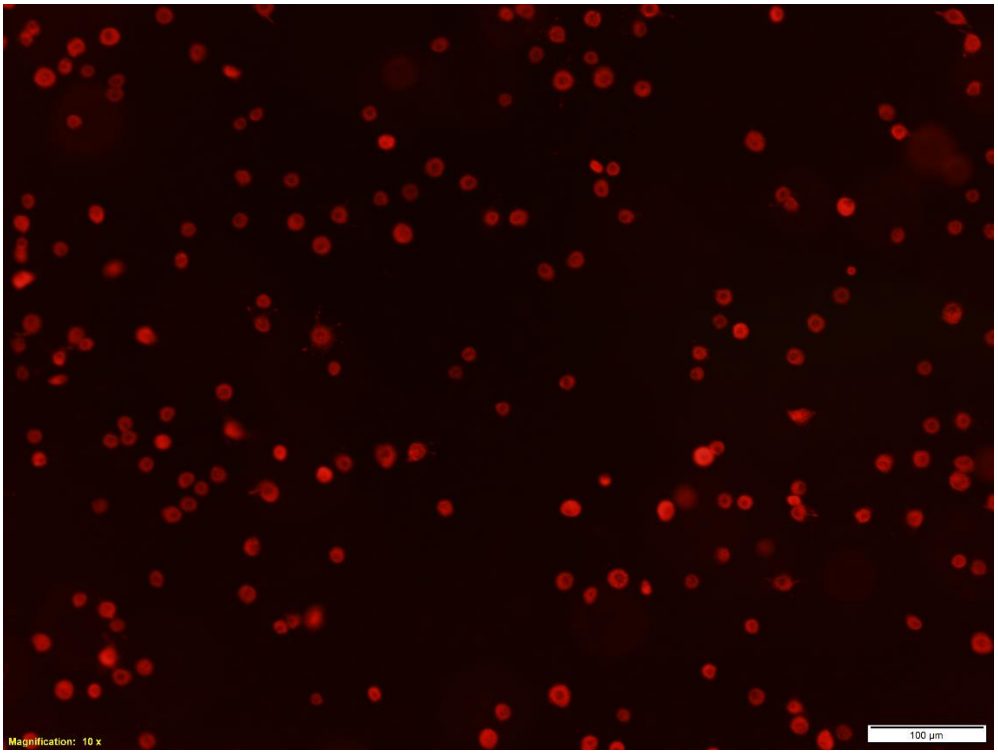


Figure A. 69. Fluorescence image of 0.1 mM vehicle control cell samples on the 3rd day

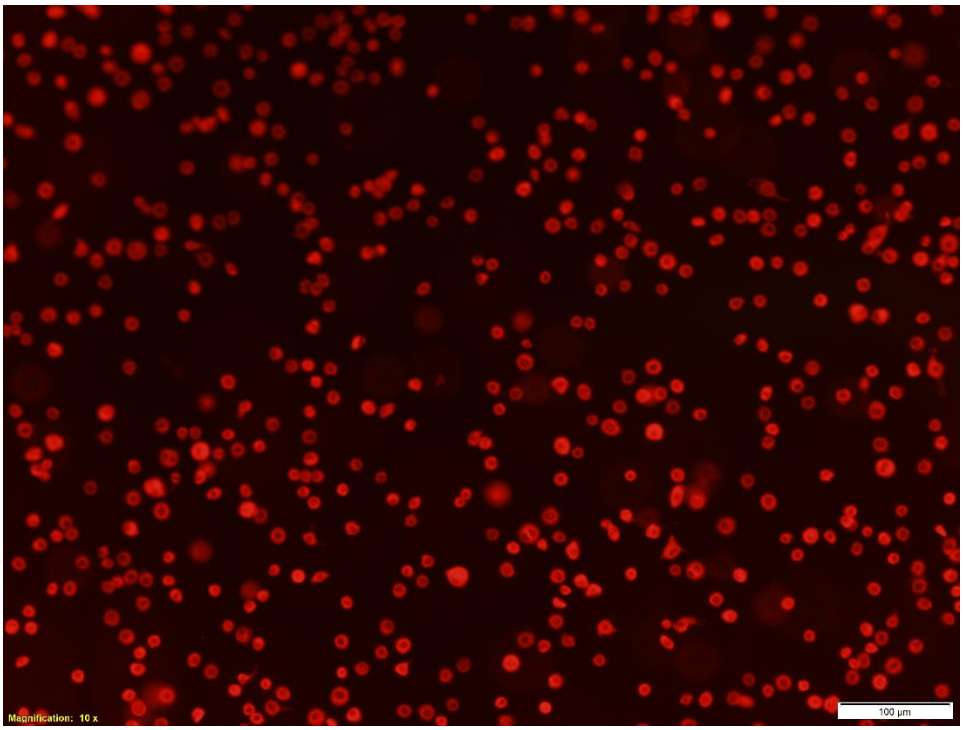


Figure A. 70. Fluorescence image of 0.2 mM vehicle control cell samples on the 1st day

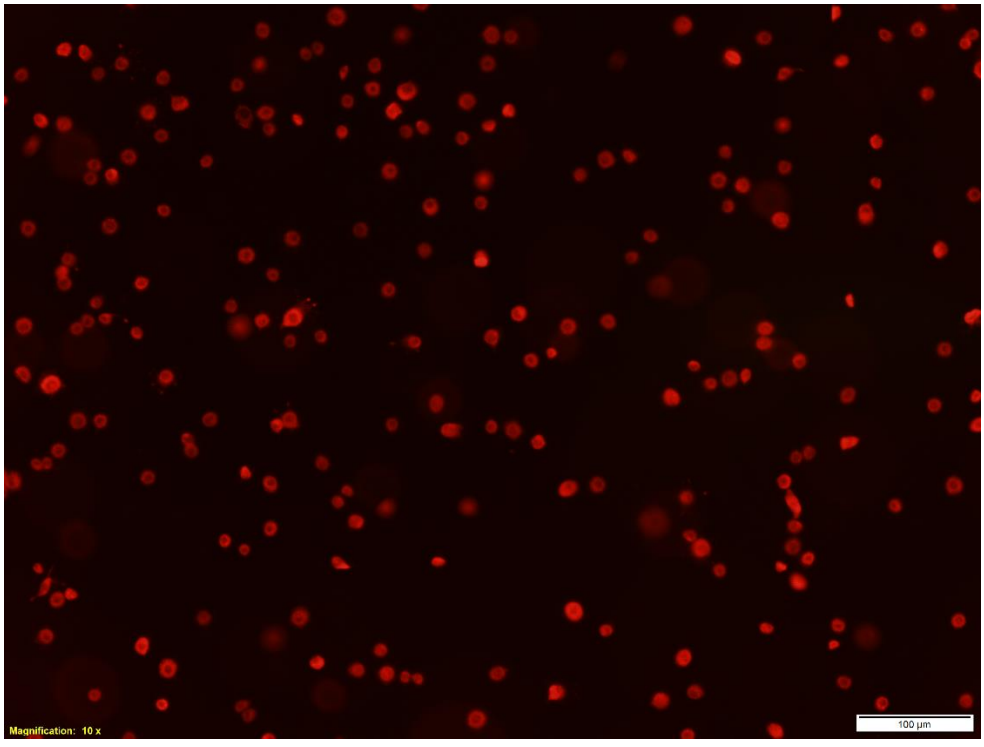


Figure A. 71. Fluorescence image of 0.2 mM vehicle control cell samples on the 2nd day

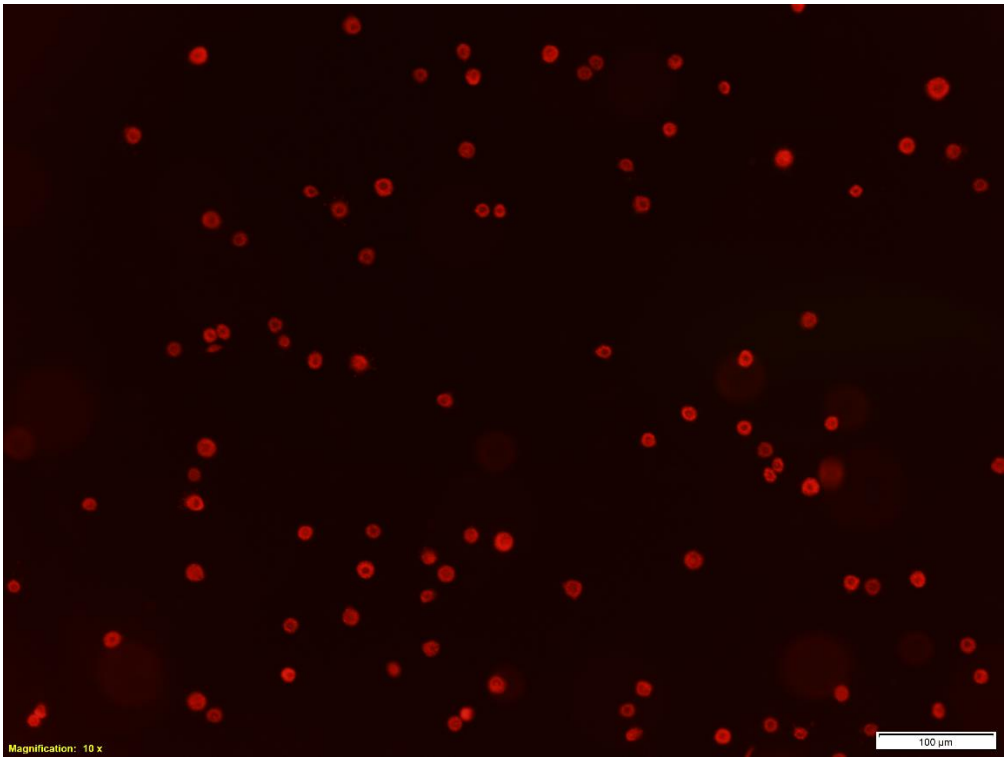


Figure A. 72. Fluorescence image of 0.2 mM vehicle control cell samples on the 3rd day

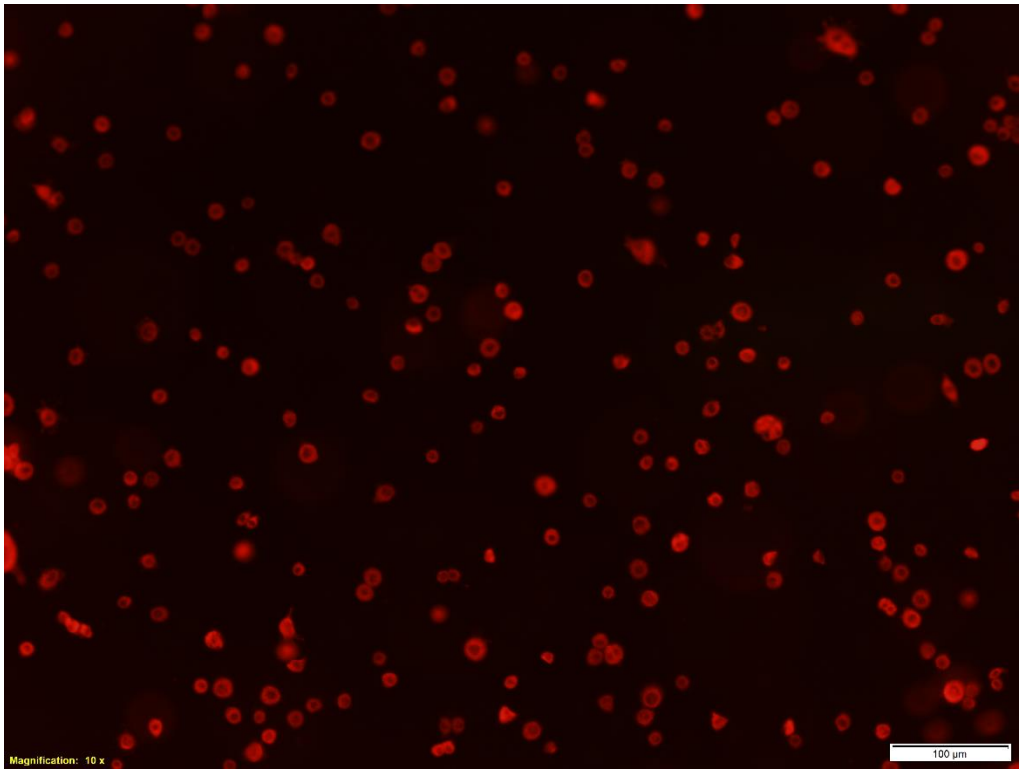


Figure A. 73. Fluorescence image of 0.3 mM vehicle control cell samples on the 1st day

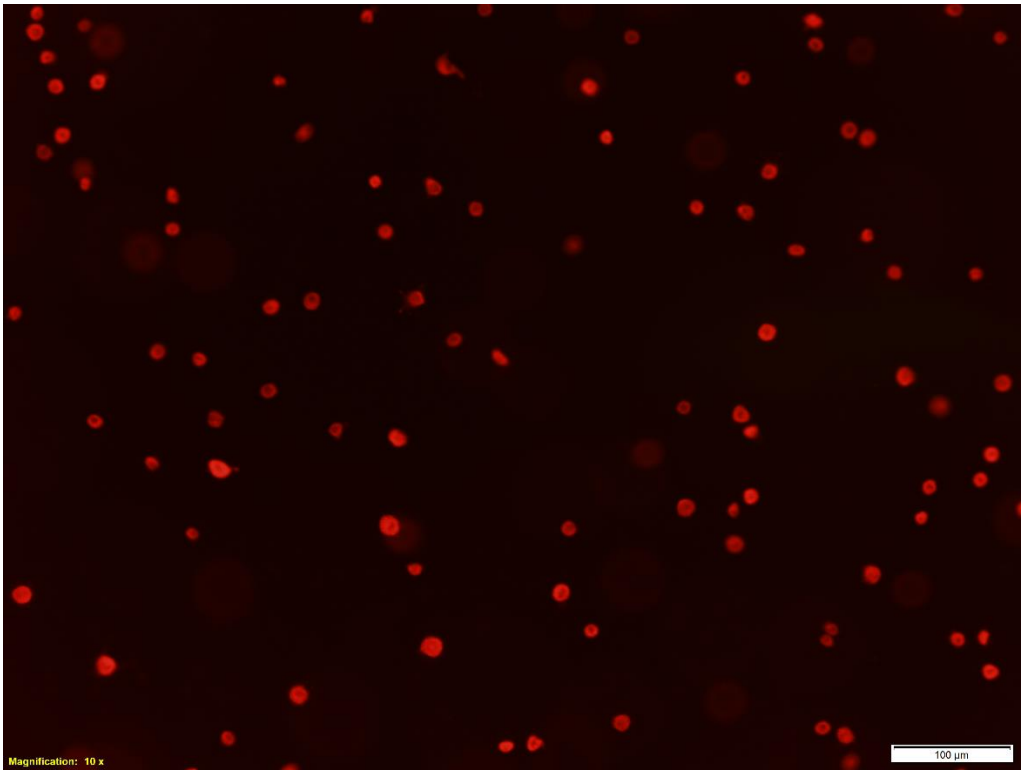


Figure A. 74. Fluorescence image of 0.3 mM vehicle control cell samples on the 2nd day

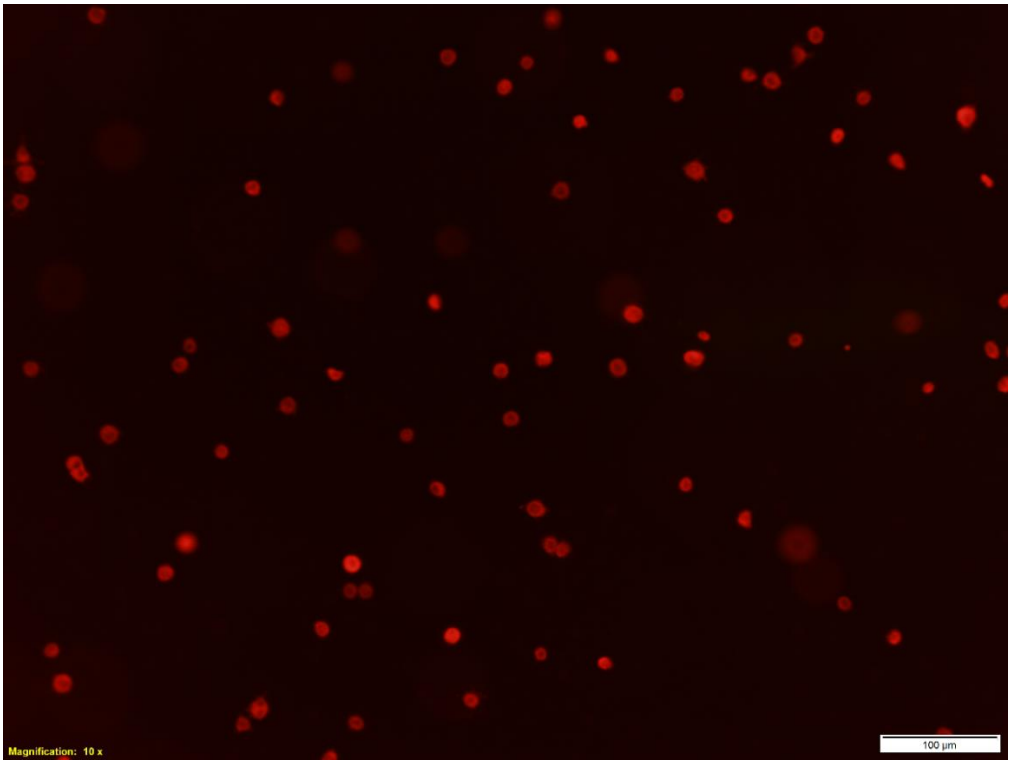


Figure A. 75. Fluorescence image of 0.3 mM vehicle control cell samples on the 3rd day

Fluorescence images of oleic acid-treated cell samples

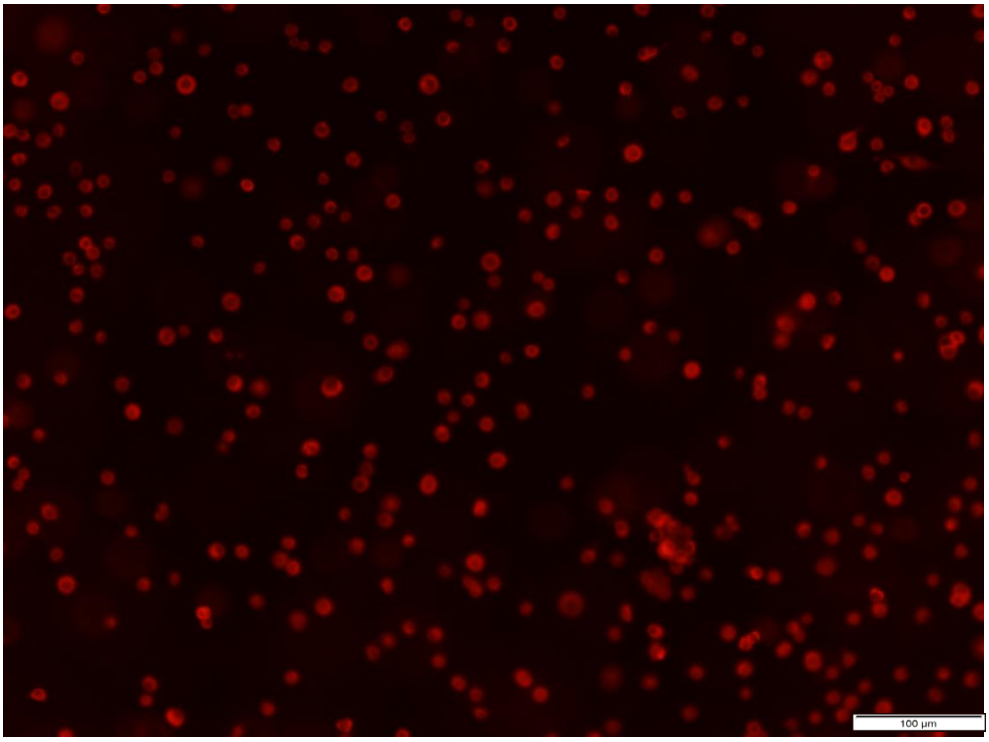


Figure A. 76. Fluorescence image of 0.1 mM oleic acid-treated cell samples on the 1st day

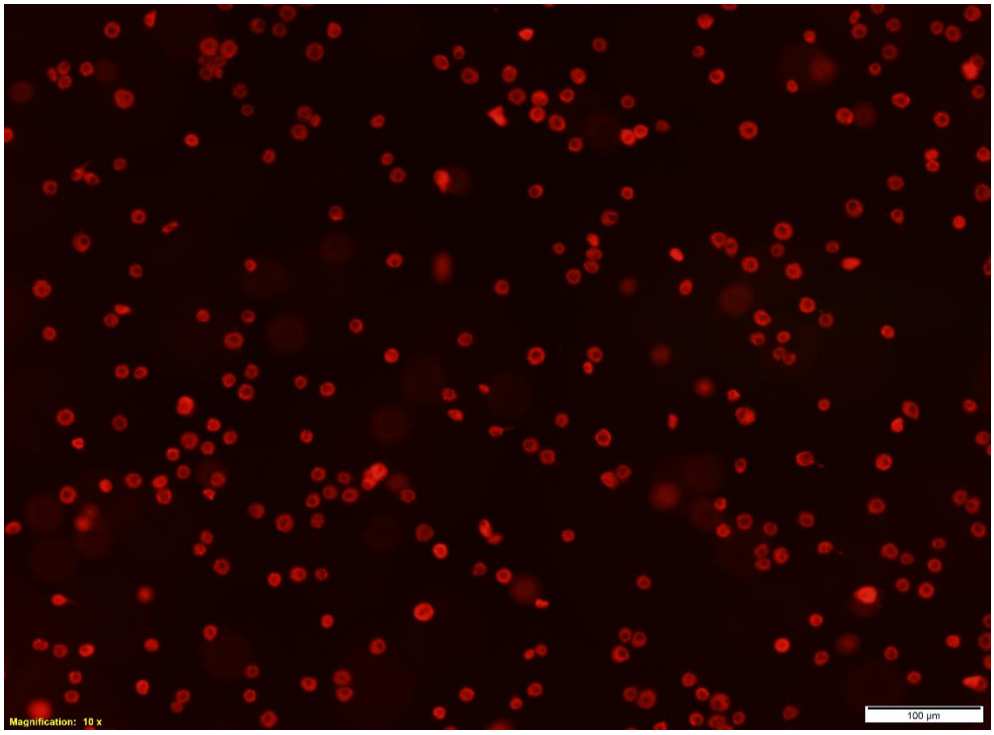


Figure A. 77. Fluorescence image of 0.1 mM oleic acid-treated cell samples on the 2nd day

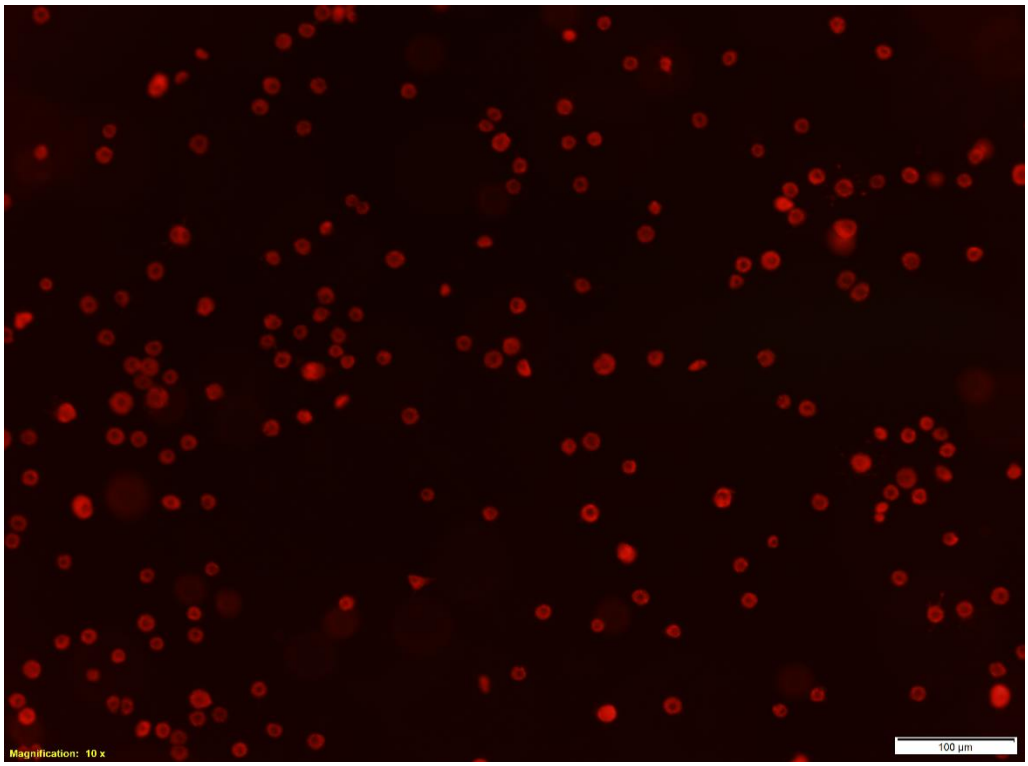


Figure A. 78. Fluorescence image of 0.1 mM oleic acid-treated cell samples on the 3rd day

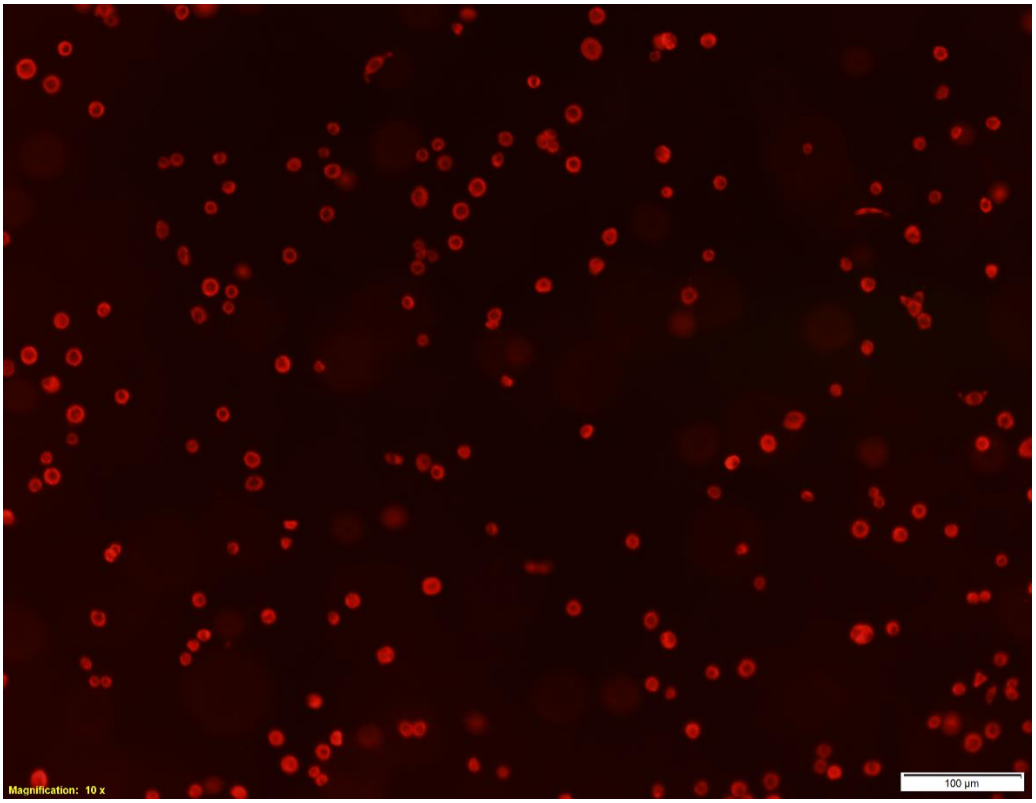


Figure A. 79. Fluorescence image of 0.2 mM oleic acid-treated cell samples on the 1st day

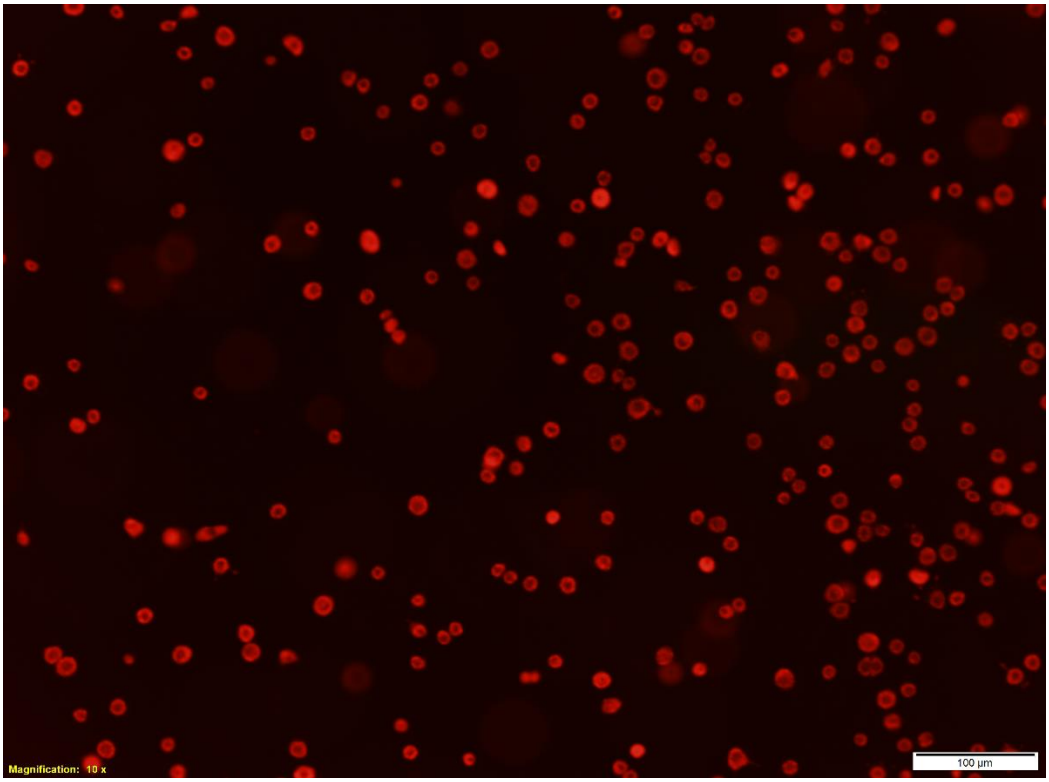


Figure A. 80. Fluorescence image of 0.2 mM oleic acid-treated cell samples on the 2nd day

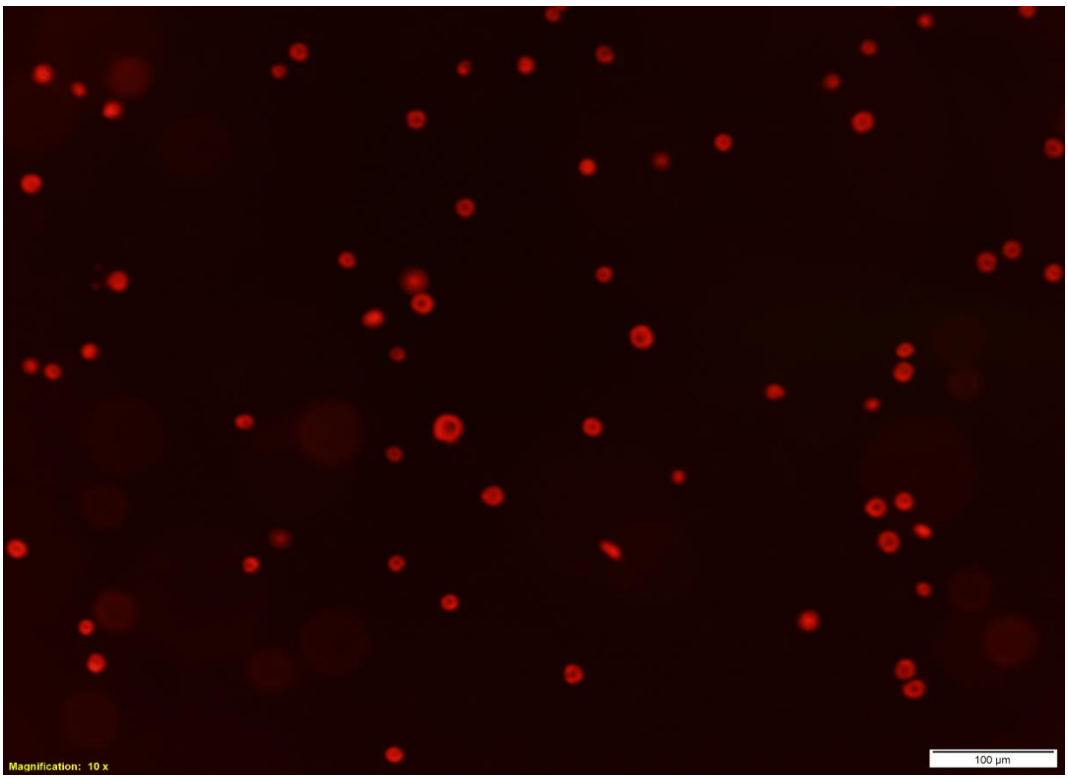


Figure A. 81. Fluorescence image of 0.2 mM oleic acid-treated cell samples on the 3rd day

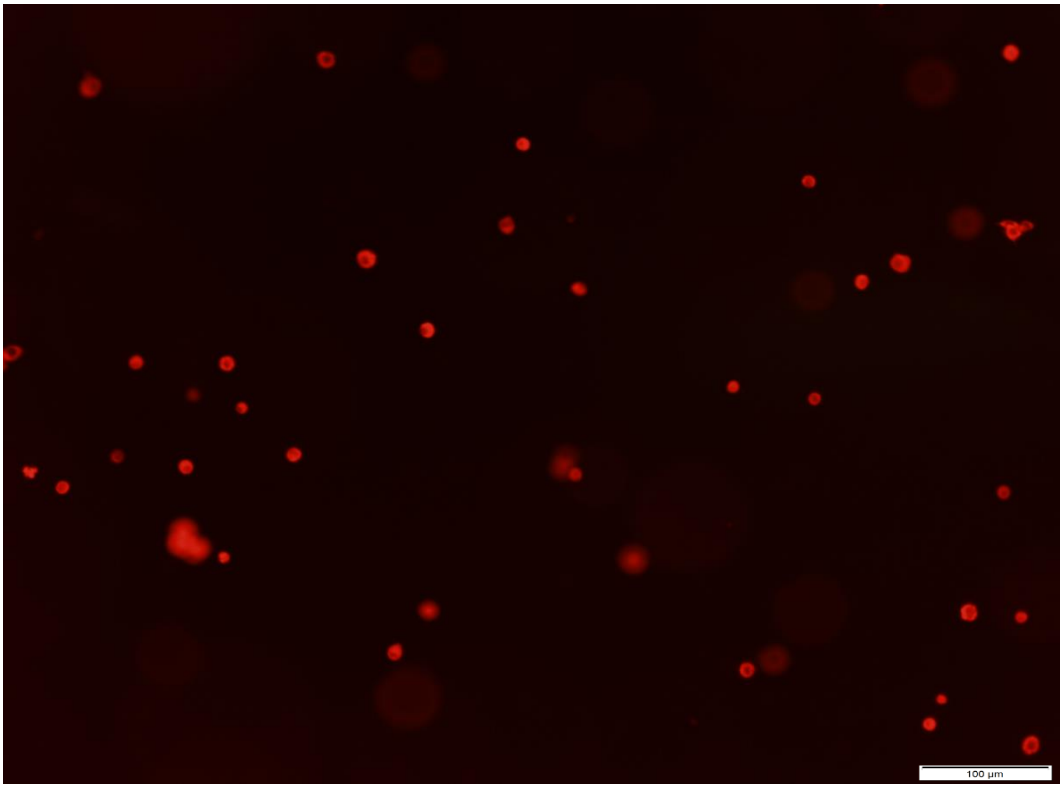


Figure A. 82. Fluorescence image of 0.3 mM oleic acid-treated cell samples on the 1st day

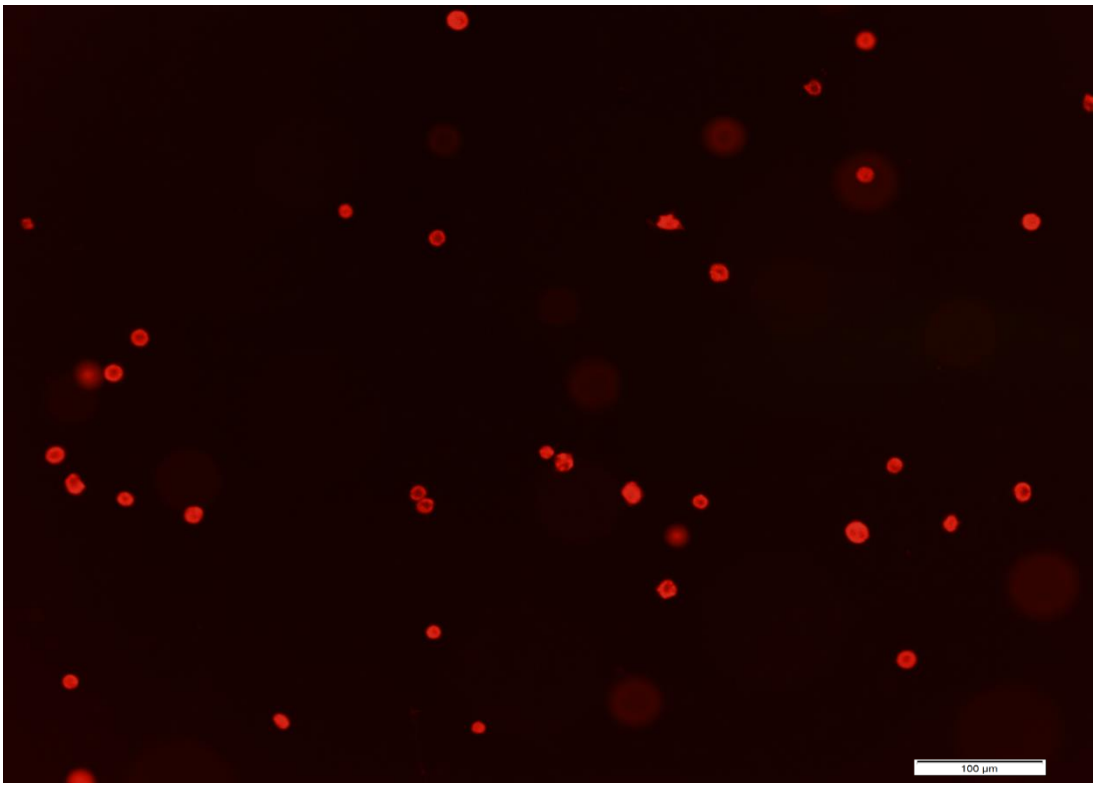


Figure A. 83. Fluorescence image of 0.3 mM oleic acid-treated cell samples on the 2nd day

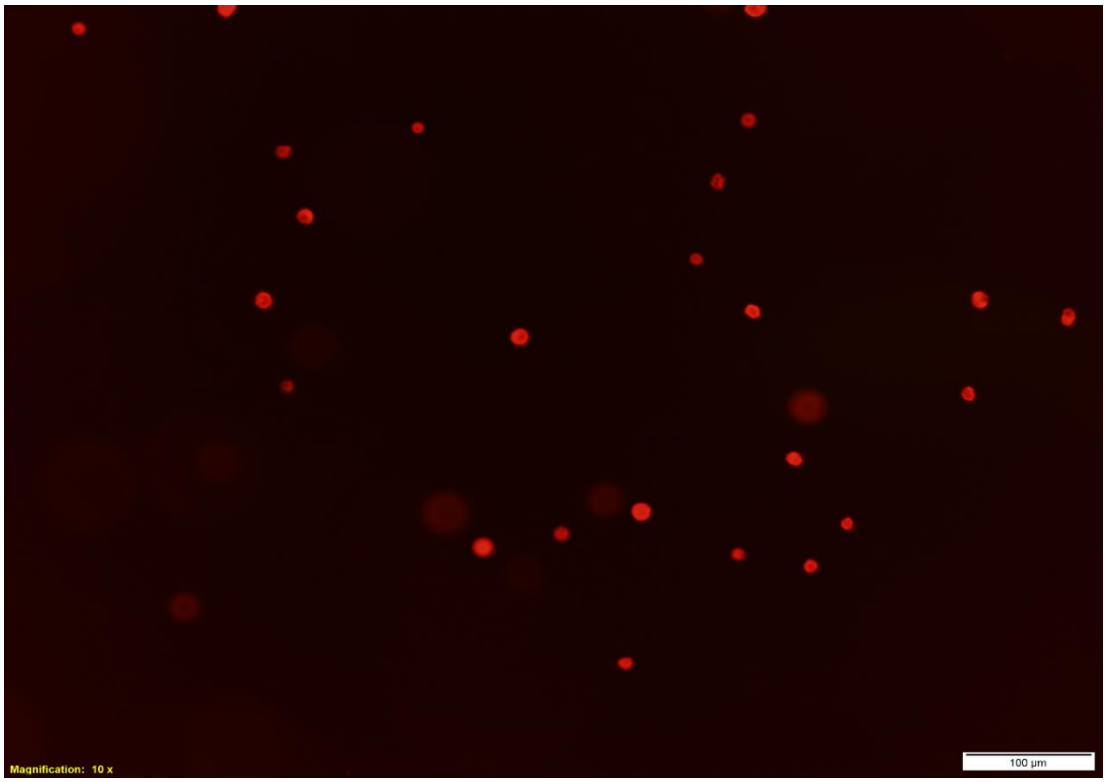


Figure A. 84. Fluorescence image of 0.3 mM oleic acid treated cell samples on the 3rd day

GC-MS Analysis

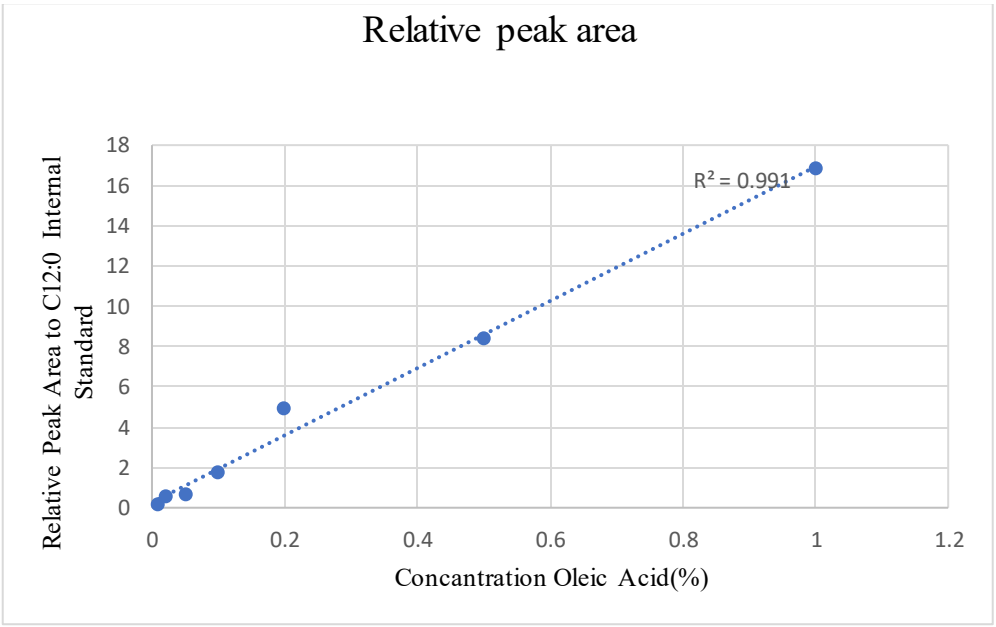


Figure A. 85. Calibration curve of oleic acid using C:12 internal standard

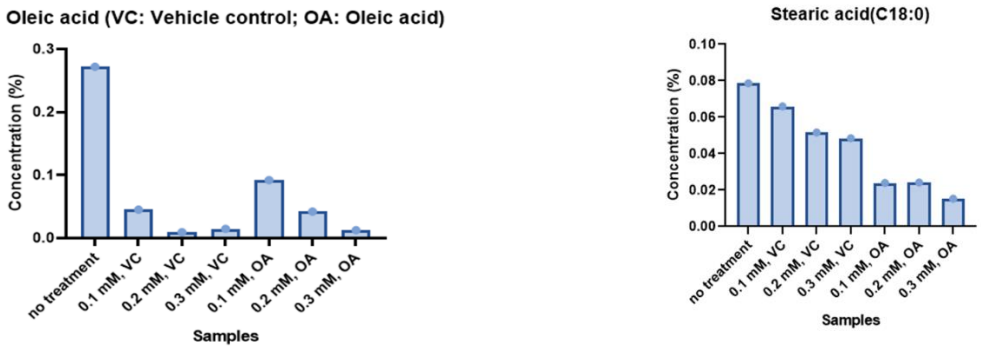


Figure A. 86. GC-MS analysis results of oleic acid and stearic acid change in insect cells

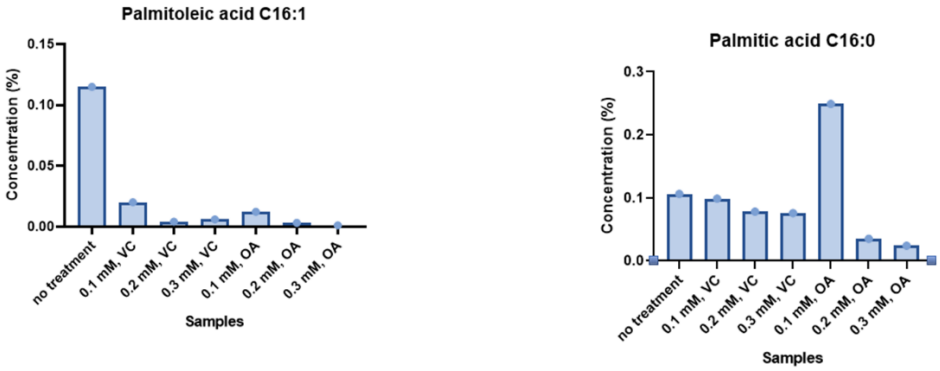


Figure A. 87. GC-MS analysis results of palmitoleic acid and palmitic acid change in incubated insect cells