

# Effects of Hyperglycemia and Doxorubicin on Aging Induction Optimization: Focus on the Role of p53 and mTOR in Human Dermal Fibroblast Cells

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## Abstract

Aging, a kind of physiological process, is influenced by a number of different biological and genetic factors, the driver for all age-related diseases. Aging of the skin can use samples of human fibroblasts (preputium) which are induced with high glucose media and doxorubicin using cell culture methods. Aging is measured using markers at the genetic level, namely p53 and mTOR. The p53 protein is one of the most important markers of apoptosis and the mTOR protein plays an important role in the proliferation, growth, motility, survival, autophagy of cell, including the synthesis of protein. Therefore, the optimization strategy of cell aging is well suited to physiological aging conditions. In this review, we explore the optimization of aging in fibroblast cell cultures induced by high-glucose media and doxorubicin and review signs of aging, namely the biomarkers p53 and mTOR. The hope to be able to know aging is close to the original condition so that it becomes an opportunity as well as a challenge for the transition of basic research into developing interventions. Several major electronic databases, including Embase, PubMed, Cochrane, and CINAHL were used to select articles from the period between February 2013 and February 2023. From 5 available literatures, we demonstrated the activity of high-glucose media and doxorubicin as induction of fibroblast senescence. High-glucose media and doxorubicin showed an aging effect by increasing p53 and mTOR markers in fibroblast cell cultures. It is hoped that, with this article on aging optimization, insights into future research directions can be achieved.

**Keywords:** *Hyperglycemia, Doxorubicin, Aging Induction, Optimization, p53, mTOR, Human Dermal Fibroblast Cells.*



## A. INTRODUCTION

Aging refers to a coordinated process which allows a progressive decrease in physiological function, leading to impaired skin function. This decline is mediated by many factors, including those which are intrinsic and extrinsic, including cellular senescence, contributing to the decrease in tissue function in old age. As people age, cells in their skin accumulate, leading to changes in the skin's microenvironment. These changes can promote skin aging, as young cells switch to an aging-associated secretory phenotype (SASP) that significantly change the skin microenvironment (Weinmüllner et al., 2020)

The structure and physiology changes of the skin happens as a natural consequence of aging and contribute to a decrease in skin health (Lephart, 2018). The mechanism for accelerating skin aging, mainly due to increased reactive oxygen species (ROS), mtDNA mutations, telomere shortening, and hormonal changes is produced by excessive exposure to various factors (Tobin, 2017). Hyperglycemia is

thought to be a condition closely related to accelerated skin aging.

There is a hypothesis that assumes that hyperglycemia becomes a stimulating factor for aging. This claim has been backed up by some empirical evidence in low-to-human model organisms (Bartke, 2008). Aging of the skin is also associated with hyperglycemia. For instance, research suggests that excessive subjection to hyperglycemic conditions may lead to preterm cellular senescence in the dermal fibroblasts (Dekker et al., 2009). In addition, patients with diabetes shows a higher cross-linking degree in their collagen. Cross-linking in collagen prevents effective restoration, thereby leading to the skin premature aging (Gunn et al., 2009).

Aging is also correlated with cancer. Aging interacts with cancer in multiple ways with several overlapping aging-causing molecular pathways. Aging is an integral part of many causes of cancer and also has an impact on treatment response, but chemotherapy treatment can cause cell death, often through apoptosis, which clinically causes tumor regression (Damasceno, 2016). Many types of chemotherapy drugs cause DNA damage (breakdown of DNA strands or cross-linking) leading to cell death. The cancer drug doxorubicin works by preventing the re-sealing of the DNA double helix by inhibiting topoisomerase 2, which triggers DNA damage and thus can lead to aging (Ewald et al., 2010).

Skin aging can be measured using various markers at the genetic level, including p53 and mTOR. The p53 protein is one of the most important markers of apoptosis and is easily identifiable because of its abundance in keratinocytes. It has been reported that inhibiting the expression of p53 can lower the chance of apoptosis in skin cells. It subsequently slows down the aging process, but the condition also promotes tumor development. This shows that it is imperative to maintain p53 expression as a physiological balance in skin cells, which is important to consider when designing basic cosmetic drugs (Gritsenko et al., 2017).

The mTOR protein is known as serine/threonine kinase, belonging to the related phosphoinositide kinases family. This protein plays an important role in the growth, survival, motility, proliferation, autophagy, and protein synthesis of cells (Laplante & Sabatini, 2012). All of these mTOR-influenced pathways are mechanisms that may also explain the aging process. In addition, mTOR also has a crucial part in controlling metabolism and spatial control by regulating the cytoskeleton actin (Laplante & Sabatini, 2012). Interestingly, observations made in model animals indicate that long-term mTOR hindrance is correlated with substantial life continuation (Anisimov et al., 2010).

## **B. METHOD**

### **1. Search strategy**

This research was conducted following the guidelines of Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols. A systematic search was performed by searching all related articles from these databases: Embase, PubMed, Cochrane, and CINAHL from the time of publication from February 2013 to February 2023. We use several keywords such as “human dermal fibroblast”,

“hyperglycemia”, “doxorubicin”, “aging”, “p53”, and “mTOR”. Keywords are combined in such a way using Boolean operators and MeSH descriptors, so that various appropriate combinations are formed. After the initial search, the authors assessed the appropriateness of each publication. Data we generate from the database search were then coded, analyzed, and qualitatively compared.

## 2. Study Selection

The initial stage of article selection and review is carried out by adjusting the criteria to the title and abstract. Inclusion criteria were defined, including studies regarding optimization of senescence in fibroblasts by administration of high-glucose media and doxorubicin with signs of p53 and mTOR. We included experimental studies, including *in vitro* and *in vivo* studies, and systematic reviews. Other research designs, including thesis results and reviews were not included in the inclusion criteria. We confirm selected articles indexed in the Scimago Journal and Country Rank (SJR).

In the next step, exclusion criteria are also determined, such as population-focused studies or other interventions. Data were drawn from selected studies, including study population, details of interventions, outcomes, and main findings. The data is synthesized using a qualitative approach. Themes were identified across studies, including the effectiveness of the intervention and the reviews were checked for errors and bias.

## 3. Data Collection

Studies that meet the requirements will be summarized and retrieved namely the name of the author, country, purpose of study, and main findings. Then arranged in a table that was created in Microsoft Excel 2010.

# C. RESULT AND DISCUSSIONS

## 1. Human Dermal Fibroblasts

Fibroblasts play an important role as cellular component of connective tissue and provide the structural framework. These cells do this through their ability to produce components of the extra cellular matrix, like collagen, because this is an abundant protein in the human body. Fibroblasts also play a role in regulating process in biology, such as wound healing, fibrosis and cancer (Mijit et al., 2020). In particular, human dermal fibroblasts are the most significant parts of the skin layer which, depending on their location, will have different characteristics and functions. (S. Xu et al., 2014)

Dermal fibroblasts in human play an important role in drug testing or toxicological screening (McAuley et al., 2017). *In vitro* culture of human fibroblasts can also provide a model for understanding important processes such as wound healing, extracellular protein synthesis, drug delivery, cell migration and senescence, both under pathological and physiological conditions. This interest arose as a consequence of research showing that human dermal fibroblasts could be reprogrammed into pluripotent stem cells (Otero et al., 2012). Their use has paved a new pathways to better

understand many human diseases, as well as creating new therapeutic approaches. Fibroblasts isolated from skin tissue show various uses, especially in the context of regenerative medicine (Otero et al., 2012).

## 2. Aging and Hyperglycemia

There are several mechanisms which help describe the ways in which glucose can contribute to the skin aging process. This process is mostly related to how advanced glycation end products (AGEs) is formed. Advanced glycation end products (AGEs) are a kind of substances that are manifested in the final stages when reaction during glycation happens when glucose, free amino groups proteins, nucleic acids, or lipids and other reducing sugars meet (Noordam et al., 2013).

On the surface of the epidermis, there is a structure, known as the skin barrier. This barrier can suppress the loss of water and help preserve sebaceous membranes and stratum corneum's protective attributes. Yokota et al. study revealed that AGEs can decrease ceramide (CER) and cholesterol (CHOL) content in the epidermis. They do this by lowering the expression of ceramide synthase (CERS3) (Yokota et al., 2017). Lee et al's study also suggested that the process of glycation can affect the function of epidermal structural proteins such as filaggrin and transglutaminase-1. This can cause damage to the skin barrier (Lee et al., 2017).

The epidermis layer primarily constitutes keratinocytes that has a significant role in maintaining skin health. Plethora of research have demonstrated that stratum corneum whole structure may go down when the presence of lipid synthesis in the epidermis is lowered. A study using 3D skin model clearly revealed that  $\alpha 1$  and  $\beta 6$  integrins in the basal layer of epidermal cells were overly expressed when AGEs's influence is present. Consequently, keratinocyte cells structure can become irregular, the cytoplasm undergoes vacuolization, making the stratum corneum thinner (Pagoon et al., 2014).

In the dermis layer, the presence of AGEs speed up the deposition and accumulation of fibroblasts in the skin by lowering the circulation of the CatD enzyme. thereby accelerating photoaging (X. Xu et al., 2018). A study showed that AGE activates ROS, induces p38/JNK expression, activates the transcription factor FOXO1, and finally causes fibroblast apoptosis (Alikhani et al., 2007). AGEs can also easily gather in the extracellular matrix of the dermis, a process that can change the balance between synthesis and degradation of the extracellular matrix and eventually causing disruption of skin homeostasis. The skin model Lee et al. confirmed that under glycation conditions, the synthesis of MMP-1 and extracellular matrix (ECM) was reduced, even collagen and elastin degradation occurred. Accumulation in the long run can cause the destruction of the protein structure, subsequently changing the shape of collagen and elastin fibers. It makes them unable to keep their biomechanical properties and functions (Lee et al., 2017).

### 3. Aging dan Doxorubicin

Aging is the body's natural protection against the cells malignant transformation. Some major events in carcinogenesis process include DNA damage and hyperactivation of oncogenes. This process can induce cell senescence and inhibit the emergence of cancerous cells. In addition, studies also show that chemotherapy drugs can instigate cell senescence. (Ewald et al., 2010).

Chemotherapy is regarded as the most important way of treating cancer. Drugs chemotherapy not only instigate the occurrence of apoptosis, but it can also help promote cancer cell senescence. Doxorubicin is one of the most widely used chemotherapy drugs. It works by instigating DNA cross-linking that causes autophagy, cell apoptosis and senescence. Different from apoptosis, senescence is mostly in a stable condition along with active metabolism. Doxorubicin is able to prompt cell apoptosis within 24 hours depending on a dose given by inhibiting proliferation and inducing cell death. (Cristofalo et al., 2003)

Doxorubicin is a chemotherapy used to treat many cancers, including leukemia, lymphoma, and solid tumors of the breast, ovary, or endometrium. The antitumor effect of doxorubicin has been reported on several signaling pathways. First, it inhibits DNA proliferation and synthesis by intercalating into DNA, eventually resulting in cell cycle arrest. Second, it facilitates apoptosis at the cell surface. This mechanism causes damage and disruption of mitochondrial DNA, which causes damage to the mitochondrial membrane, release of cytochrome C, and activation of caspase-9, and further activation of caspase-3. (Kurniawati et al., 2015)

In addition to inducing DNA damage and oxidative damage, doxorubicin produces toxins and inhibits growth in human fibroblasts. Cells treated with doxorubicin accumulated in the G2/M phase through topoisomerase II inhibition, and this treatment induced cell death/apoptosis through altering the kinetics of the p53 pathway. (Kurniawati et al., 2015) Furthermore, doxorubicin changed the expression patterns of many genes involved in cell cycle regulation such as p53, apoptosis and cell damage. The p53 tumor suppressor gene, which is an important transcription factor involved in the cell cycle, regulates cell cycle modulators such as the kinase inhibitor p21 which is rapidly induced by genotoxic reagents. (Kurniawati et al., 2015)

The above finding which shows that low concentrations of the active drug induce senescence points to the potential for therapeutic aging (TIS). Doxorubicin can produce premature aging. Administration of doxorubicin at higher doses damage DNA and promote stress which is associated with more pronounced apoptosis, while induction of aging can be obtained with the use of lower doses that are given continually (Kurniawati et al., 2015).

### 4. Role of p53 in Aging

p53 is a tumor suppressor protein which is known as the genome guard. This protein integrates many different physiological signals that exist in mammalian cells. p53 is particularly responsive to stress signal and it becomes functionally active. When it is active, it triggers temporary cell cycle arrest, permanent cell cycle arrest or cell death

(apoptosis) (cellular senescence). Two potent tumor suppressor mechanisms include apoptosis and cellular senescence. They work irreversibly to prevent cells damage from undergoing neoplastic change. However, these processes can also significantly decrease progenitor tissue or stem cells that are competent in proliferation. Current empirical evidence shows that p53 can actively repair damaged DNA which plays a crucial role in aging (De Boer et al., 2002). These studies have implication to the role of p53 in senescence and implicate it as an important regulator in the aging process among many organism (Maier et al., 2004).

A convincing evidence on the direct role of p53 in promoting aging phenotypes was provided by Tyner et al. They examined exons 1-6 of the p53 coding sequence. This study used rats that were induced by a mutant p53 allele (allele m) containing exons 7-11 (hereinafter referred to as p53<sup>+/m</sup> mice). This study found that these mice were resistant to cancer, but their life span is 20-30% shorter. Besides, the mutant mice seem to show signs of faster aging in the skin, lymphoid organs, liver and skeletal muscles. This acceleration is mainly driven by stem cells decreased ability to produce adequate numbers of differentiated progenitors and cells that are mature (Tyner et al., 2002).

Another study conducted by Maier et al. used mice that are transgenic and that overly emit p44 (hereinafter referred to as P<sup>+/+</sup> mice). The p53 gene is a natural p53 isoform that does not have a major transactivation domain. The study found that mutant mice had a much lower possibility of cancerous cells, but most of the P<sup>+/+</sup> mice died within 1 year which was strongly suspected due to the mechanism of premature aging. The early aging phenotype of these mutant mice is associated the unusually high activity of the p53 which rise the activity of the IGF signaling pathway (Maier et al., 2004). This pathway has promoted senescence in such a diverse specieses as fruit flies, nematodes and mice (Blüher et al., 2003; Guarente & Kenyon, 2000).

## 5. Role of mTOR in Aging

The rapid development in genomics-based technologies have shown promising markers at the transcriptomic, cellular levels metabolism, and the genomic for skin problem like aging. Research has shown that dysregulation of mammalian target of rapamycin (mTOR) is involved in a wide range of diseases, from diabetes to various types of tumors, inflammatory skin disorders, and skin cancer (Mice et al., 2010). Recent studies have also demonstrated that mTOR plays an important role in skin cell growth regulation, proliferation, and differentiation. The maintenance of skin homeostasis and morphogenesis depends on functioning mTOR signals to regulate keratinocyte differentiation and activate the epidermal stratification program, which is concurrent with hair follicle formation (Ding et al., 2016).

Changes in the mTOR pathway can regulate protein synthesis, and this can negatively affect the proliferation and growth of the cells. This subsequently results in phenotypically diverse skin diseases. Keratinocytes can inactivate Akt/mTORC1 by giving an early signal in healthy skin differentiation which uses mTOR as proliferation control (Calautti et al., 2005). Several cellular processes can be used as signs of aging. These include epigenetic changes, telomere attrition, genome instability, loss of

proteostasis, cell senescence, stem cell exhaustion, dysfunction in mitochondria and changes in communication between cells. In some of these processes, mTOR has been predicted to have a significant role. (Huang, 2020)

The mTOR enzyme can be located in two distinct locations, namely mTORC1 and mTORC2. The mTORC1 enzyme modulates some important stages in protein synthesis which works to control the genes expression that support cell proliferation and survival. The two most important target of mTORC1 signaling are known as 4EBP1 and S6K. They act as a modulator in the translation initiation process. By inhibiting mTORC-dependent translation, researchers found that this can extend cell lifespan by providing protection against a number of different pathological conditions related to aging. (Huang, 2020)

In mitochondrial metabolism, mTOR is seen as an important modulator of oxidative stress. It works by increasing oxidative stress metabolism as well as promoting mitochondrial biogenesis (Fang et al., 2016). Mitochondria is significant source of reactive oxygen species (ROS) which can be very important in the aging process. In this case, ROS are primarily resulted from a different NADPH oxidase (Nox) isoform. The main defense system against the damaging effects of ROS is nuclear factor erythroid-related factor 2 (Nrf-2). This is a transcription factor which help to manage the translation of common anti-oxidant enzymes like catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD). Through the use of PGC-1 $\alpha$  pathway, mTOR can result in various defense functions of ROS, which affect the acceleration of cellular aging (Zhao et al., 2017).

Aging is characterized by an increase in systemic pro-inflammatory mediators. Aged cells are reported to release a series of pro-inflammatory substances (SASP), including TNF- $\alpha$ , IL6, and IL1. The inflammatory process causes increased expression of MMP9, ICAM-1, VCAM-1, and VSMC which lead to matrix remodeling and is associated with aging (Dysfunction, 2017). Transcription factors regulate pro-inflammatory cytokines that are responsive to redox potential through the signaling of mTOR (Conn & Qian, 2011).

The mTOR pathway is undeniably the most important factor in the process of tissue stem cell activation by promoting their proliferation and release from a dormant state. The activation of stem cell can accelerate tissue turnover and repair. In the long run, however, this leads to massive decrease in the stem cell pool. This suggests that mTOR inhibition may be an effective approach to conserving the stem cell pool, thereby retaining the ability to repair tissue damage and inhibit aging (Conn & Qian, 2011)

Researchers found that mTOR signaling can promote cellular senescence. This is a phase of decline in cellular function that goes with its development and maturation. Loss of replication ability is a sign of cell life without activation of the apoptotic process. mTOR active state that happen when the cell cycle is clocked will put cells into an senescent state. These cells can eventually become hypertrophic, as well as showing hyperdifferentiation and a pro-inflammatory state. Furthermore, they no longer have the capacity to proliferate. This loss is one of the hallmarks of an aging cell phenotype. This phase can result in cell injury and depleted physiological cell capacity, which lead

to cell senescence (Conn & Qian, 2011).

**Table 1. Studies on senescence induction in fibroblasts**

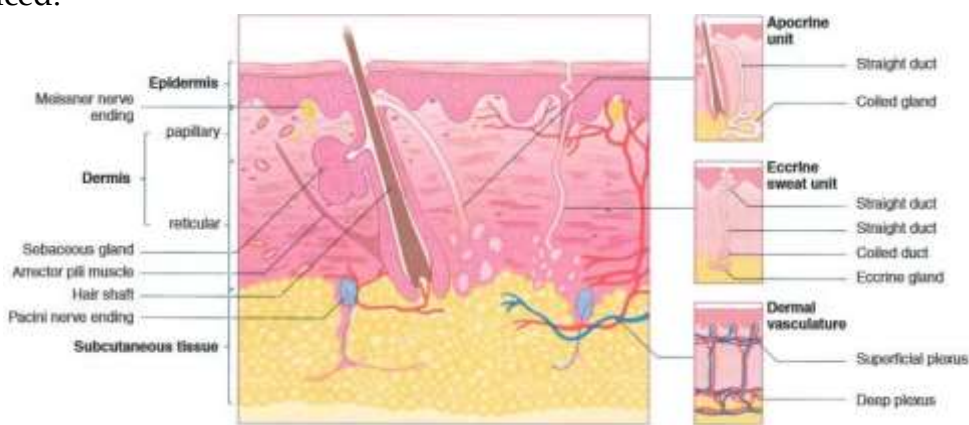
Authors	Country	Study Objective	Study Design	Study Group	Principal Findings
Prima Buranasi n, Koji Mizutan i et, al (Buranas in et al., 2018)	Japan	This study was intended to investigate changes in the properties of human gingival fibroblasts (HGFs) which is put under high concentration of glucose.	RCT	Gingival specimens were obtained from the non-inflamed periodontal tissues of one male (62 years old) and two females (27 and 68 years old) who represented. Primary HGFs were isolated from healthy gingiva and cultured with 5.5, 25, 50, and 75 mM glucose for 72 h. In vitro wound healing.	High concentration of glucose can inhibit the proliferation of gingival fibroblast cell in human High concentration of glucose can inhibit the proliferation of cells and promote death of cells in human gingival fibroblasts (HGFs)
Laeli Muntafia h, et al (Muntafiah et al., 2016)	Indonesia	The aim of this study is to explore the potential of pumpkin seed extract (PSE) as an anti-aging agent	RCT	Cells were treated using doxorubicin to induced cells senescence. Doxorubicin 250 ng/ml and give pumpkin seed, the antiaging agent as antioxidant at the dose of 100, 200, 400, and 800 µg/mL	The overall result of this study showed that PSE has anti-aging effect by decreasing SA-βgal activity. This anti-aging activity possibly due to the interaction between tocopherol to CYP 3A4 based on molecular docking.
Soydas, et al (Soydas et al., 2021)	Turkiye	This study examined high glucose in vitro effects and metformin at two different doses on the proliferation of cell, apoptosis, and gene expression RELA/p65, COL1A1, and COL3A1 in primary dermal fibroblasts. humans (HDFs)	RCT	HDF of skin tissue from five healthy, non-diabetic, elderly (over 55 years) female donors collected during aesthetic surgery at the Center for Plastic and Reconstructive Surgery. Effect of normal glucose (5.5 mM) and HG (50 mM HG) concentrations on HDF, with two doses of metformin (50 M and 500 M), investigated by immunostaining	Metformin can counteract many of the damaging effects of high glucose, particularly reduction cell proliferation and reduced COL1A1 expression, and slightly inhibited the effect of high glucose on NF-kB metabolism
Su-Jeong Kim, et al (Kim et al., 2019)	USA	Here, we examined the role of hypermethylation of the G-rich sequence factor 1 (GRSF1) promoter region, a mitochondrial RNA binding protein, in replication- and doxorubicin-induced cellular senescence. GRSF1 expression was lower in senescent fibroblasts, and GRSF1 knockdown induced senescence in human primary fibroblasts.	RCT	250 nM doxorubicin were utilized for as long as 24 h, to treat the main cells and the medium was then put back with completed medium every 3 days for two weeks	Methylation of the GRSF1 promoter rose in the process of cellular senescence. GRSF1 promoter methylation also rose twice as much in doxorubicin-instigated senescent cells. GRSF1 expression in senescent cell declined.
Nosrati, et al (Nosrati et al., 2023)	Austria	Human dermal fibroblasts (HDFs) were found in this study. It is treated with doxorubicin (doxo) to induce senescence. The senescent phenotype of these stress-induced premature senescent (SIPS) cells was confirmed with several markers. The expression of pro-fibrotic genes was quantified and finally, the impact of their secretome on the fibrotic response of non-senescent fibroblasts was assessed.	RCT	HDFs at passages 13–17 were seeded at 3500 cells/cm <sup>2</sup> . SIPS cells received the first dose of 150 nM doxorubicin one day and the second dose 4 days after seeding, one week after seeding the doxorubicin-containing culture media was changed to normal culture media and one week after that the experiments were conducted using these cells	The assessment of SIPS cell was performed: SIPS HDFs showed a significant increase in SA-β-gal activity with 75% of cells stained positive for this enzyme activity whereas only 7% of proliferating cells were positive. This study found that p21 expression was significantly modulated in SIPS cells compared to proliferating cells and quiescent cells, which further supports the validity of p21 as a senescent cell marker. As another marker of senescence, expression of lamin B1 was substantially lower in SIPS cells than proliferating cells.
					The data obtained are relevant with the observation that metformin has a protective effect in an in vitro model of aging 3T3 fibroblasts under high glucose



					conditions inducing cell proliferation, collagen I and III production, protection from apoptosis, and reduced NF-kB(p65) activity.
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### 6. Fibroblas as a Model of Aging

The skin is made of three different layers, consisting the epidermis, dermis, and hypodermis. They differ significantly in terms of structure and function (Figure 1). Structure of the skin also has a significant role in controlling body’s temperature by releasing water into the environment. The skin is the body’s largest organ which cover the entirety of the body’s surface. It constitutes 15% of the total body weight of an adult (Bergfelt, 2009). As biologically aged skin ages, the dermal-epidermal junction flattens, the extracellular matrix (ECM) atrophies, and collagen and elastin become disorganized and reduced.



**Figure 1. Anatomy of Skin** (Bergfelt, 2009)

Fibroblast is a crucial cellular element of the skin. It is accountable for numerous functions in the skin organ, like maintaining dermal homeostasis, keeping the physiological state of other skin layers, and renewing the extracellular matrix (ECM) by degrading used skin components and synthesizing new ones, particularly collagen and elastin. As aging continuous, skin fibroblasts experience significant morphological alteration. They also undergo changes in activity and proliferative potential. Dermal fibroblasts decreases when human is getting older, resulting in decreased ability in proteostasis maintenance of ECM components. This can make the skin thin, as it losses its flexibility, and elasticity. This process is indicated with the formation of wrinkles - all of which are signs of natural skin aging (Sadowska-Bartosz & Bartosz, 2020).

Hayflick and Moorhead showed that human fibroblasts which are grown in vitro eventually attained senescence. Shortening in tholmere is seen as an intrinsic mechanism that inhibits replication of human cells. (Sadowska-Bartosz & Bartosz, 2020) Telomeres are repeated nucleotide sequences that protect the ends of chromosomes linearly. Successive telomere friction during cell division causes telomere shortening which in turn triggers DNA damage resulting in cell cycle arrest (increased p53) and cellular senescence. Whereas mTOR has the role of mediating and integrating growth signals, thus mTOR will regulate cell growth. In fibroblasts whose growth rate is reduced due to aging, mTOR biomarkers are found to be relatively two-fold higher.

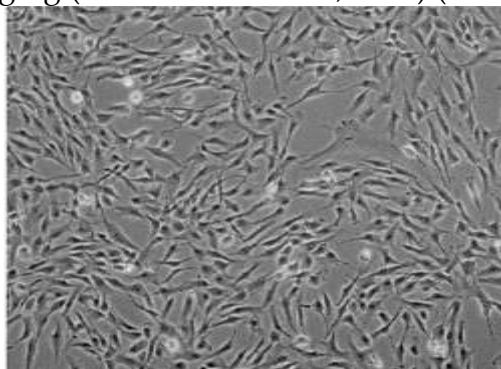
(Jäger et al., 2022).

Fibroblasts were explained in the late nineteenth century. These are ordinary cell type of connective tissue, with an enlarged morphology, fusiform shape. These play a crucial role in several physiological processes including extracellular matrix synthesis (ECM), the process of inflammation regulation, epithelial differentiation, and wound recovery. Besides, fibroblasts also play a role for the growth factors in secretion. Fibroblasts help to scaffold other cell types, as they act as the most significant cell mediator for fibrosis and tissue formation in a scar (Fernandes et al., 2016).

Primary fibroblast culture is increasingly important in aging research. In addition, fibroblasts which are emerged out of skin biopsies, for example, can become a powerful medium to investigate normal skin physiology or the states of a specific disease. (46) Fibroblasts have various functions in forming the basic framework of tissues and organs. Under homeostatic conditions, fibroblasts are responsible for synthesizing elastin and collagen. Elastin and collagen are examples of major proteins existing in the extracellular matrix (ECM). The extracellular matrix (ECM) prevent the skin from degrading matrix metalloproteinase structures (Dick et al., 2021).

During stressful stimuli, fibroblasts adapt to the environment and possess homeostatic capabilities to respond to and transmit local signals. In the event of injury, fibroblasts can change phenotype and synthesize the extracellular matrix needed to replace injured tissue. Under pathological conditions, extracellular matrix is produced in excess, and collagen is stored in a disordered manner which often results in irreversible organ dysfunction or disfiguring appearance. (Dick et al., 2021)(Berlanga-Acosta et al., 2020).

Human fibroblasts found in cell culture have the ability to express regulatory behavior, human genetic, and metabolism. Using cell culture can allows mechanism change investigation that happen while cells are subjected to predictable and reproducible damage in a controlled environment. Fibroblast culture is a useful tool for examining questions and hypotheses relevant to aging in biology. In fact, the human fibroblast model has been valuable for elucidating the cellular basis of some of the mechanisms underlying aging (Abdul Malik et al., 2020) (Cristofalo et al., 2003).



**Figure 2. Fibroblast Structure with 100x Magnification Fibroblast Cell Culture**

Source: Jain et al. (2017)

Cell culture is defined as laboratory methods which allow eukaryotic and prokaryotic cells growth under physiological conditions. It was found as early as the

20th century being introduced to examining tissue maturation, and growth as well as vaccine development and virus biology, and how genes play a role in disease and health. Experiment using cell culture are as equally varied as types of cell that can be grown in vitro. In a clinical context, nevertheless, cell culture is commonly related to model systems creation that investigate basic cell biology, disease mechanisms replication, or examine the level of toxicity of new drug compounds (Pérard-viret et al., 2020).

Cell culture offers numerous advantages and one of them is its potential to control genes and molecular pathways. Moreover, the uniformity of a certain cell type and a well-formed culture system can navigate the discomfiting environment, thereby allowing data generation with high reproducibility and consistency. This condition cannot be guaranteed when examining entire organ systems (Pérard-viret et al., 2020).

Human cell culture has become a widely acknowledged technique that make studies of metabolism and physiology of humans possible, something that are not always possible in vivo. Using tissue slices and biopsies is another alternative, even though they maintain the structure in vivo, and must be used right away because the cell is only viable in a short time. If isolated from tissue, it is possible for them to establish cell cultures for periods ranging from days to weeks. Skin biopsy is one way of obtaining cell from normal tissue, only if clinical procedures and ethical considerations have been sought. Another possible way include obtaining diseased tissue (eg liver tumor biopsy) that is removed by surgery as part of treating patient's disease (Pérard-viret et al., 2020).

With the presence of cell culture, individual cell types behavior cannot be influenced by systemic variations that may emerge during normal homeostasis in vivo. The cell culture is commonly a suspension of cells that are dispersed and extracted from the original tissue through mechanical, chemical or enzymatic dissociation. Primary cultures are done under laboratory conditions that require strictly high sterility, involving controlled environment with a certain temperature, gas and pressure. It must closely resemble the in vivo environment so that cells are can survive and proliferate under a controlled condition. Despite many years of human cell culture and rapid advances in molecular biology techniques, very sensitive analytical techniques can be used, giving rise to the increasing need for cell culture of human body's organ or tissue (Pérard-viret et al., 2020).

Cell culture allows the physiochemical environment control (pH, O<sub>2</sub> and CO<sub>2</sub> osmotic pressure, and temperature). This can give numerous advantages in cytology. Therefore, immunostaining can be conducted much easier. It is possible for cultures to be exposed to reagents at low temperatures and in defined concentrations with direct access to cells. Tissue samples are commonly varied, but the cultured cell lines can become a uniform constitution after one or two passages. This is due to the fact that cells are randomly mixed at each transfer and are selective. The culture conditions lean towards producing homogeneous cultures of the most robust cell types. Thus, it is possible to re-experiment with high similarity and this can reduce statistical differences (Hudu et al., 2016).

It is imperative to perform culture techniques under strict aseptic conditions, because usual contaminants such as fungi, yeast and bacteria can increase in size much more quickly than cells in mammals. It is imperative to have strict environmental control similar to cells from multicellular animals which usually do not exist in isolation. Therefore, cells cannot keep their independent existence without the required support. There is another limitation including the relatively high price for consumables and media, cross-contamination, and de-differentiation. Dedifferentiation is defined as the excessive growth of undifferentiated cells that reduce phenotypic characteristics of the tissue from the isolated cells. This can be solved under the appropriate conditions with selected media. (Pérard-viret et al., 2020) (Hudu et al., 2016).

Conversely, 2D culture has limitations because it cannot completely copy natural physiological and microenvironmental conditions like the physiology, structure, living tissue biological signals, and interactions of cell-matrix. In 3D cell culture, there is cell communication with the ECM, which does not exist in 2D, which will control cell growth, proliferation, and function. Cells cultured in 2 dimensions have been compelled to change various complex biological attributes including apoptosis, regulation of transcription and receptor expression, anti-apoptosis, cell proliferation and cell invasion (Hudu et al., 2016).

#### D. CONCLUSION

Aging can be caused by various kinds, for example, hyperglycemia and also the cancer drug, doxorubicin with the human fibroblast cell culture method. Hyperglycemia with high glucose media causes an increase in ROS. ROS are constructed through free oxygen radicals. This process produces oxidative stress. ROS at the high level can inhibit cellular functions to migrate, proliferate, and synthesize the extracellular matrix of fibroblasts and keratinocytes. Doxorubicin, which is a cancer medication, has shown significant effect, especially inducing aging. Doxorubicin regulates apoptosis by changing DNA and its mechanisms like intercalation of DNA, disruption of DNA cure by inhibiting type IIA topoisomerases, and the production of ROS. It is hoped that, with this article on optimizing aging fibroblasts with the aim of mimicking natural aging conditions, insights into future directions of aging research and aging therapy can be achieved.

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