NANOENCAPSULATED CORDYCEPS MEDIUM ENHANCES SKIN CELL REGENERATION

NAWAPOL UPATCHA

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นาโนแคปซูลของน้ำเลี้ยงถั่งเช่าช่วยส่งเสริมการสร้างเซลล์ผิวใหม่

นายนวพล อุปัชฌาย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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NANOENCAPSULATED CORDYCEPS MEDIUM ENHANCES SKIN CELL REGENERATION

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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นวพล อุปัชฌาย์: นาโนแคปซูลของน้ำเลี้ยงถั่งเช่าช่วยส่งเสริมการสร้างเซลล์ผิวใหม่ (NANOENCAPSULATED CORDYCEPS MEDIUM ENHANCES SKIN CELL REGENERATION) อาจารย์ที่ปรึกษา: รองศาสตราจารย์ ดร.ปริญญา น้อยสา, 60 หน้า.

คำสำคัญ: เทคโนโลยีการห่อหุ้มระดับนาโน/น้ำเลี้ยงถั่งเช่า/การต้านอนุมูลอิสระ/กลไกการกินตัวเองของเซลล์/การสมานบาดแผล/การสร้างคอลลาเจน/การสร้างเซลล์ผิวใหม่

สาเหตุหลักของโรคความเสื่อมรวมถึงความชราของผิวหนังและเป็นมะเร็งผิวหนังได้แก่ ความเครียดจากปฏิกิริยาออกซิเดชันที่เกิดจากอนุมูลอิสระ (Reactive Oxygen Species หรือ อนุมูลอิสระ) Cordycepin ซึ่งเป็นสารประกอบที่ออกฤทธิ์ทางชีวภาพของ *Cordyceps militaris* เป็นสมุนไพรที่มีศักยภาพหลากหลายเช่น มีฤทธิ์สำหรับต้านอนุมูลอิสระและต้านมะเร็ง จุดมุ่งหมายของการศึกษาครั้งนี้คือเพื่อประเมินการสังเคราะห์คอลลาเจนและอิลาสตินของเซลล์ไฟโบรบลาสต์ที่เหนี่ยวนำด้วยไฮโดรเจนเปอร์ออกไซด์ที่กระตุ้นให้เซลล์ไฟโบรบลาสต์เกิดความเครียดออกซิเดชัน เซลล์ไฟโบรบลาสต์ถูกรักษาด้วยสาร Cordycepin 1 μM, สาร Cordycepin 1 μM และ CMP 0.1 μM และกลุ่มควบคุมที่เป็นบวกคือกลุ่มที่ได้รับการสัมผัสกับไฮโดรเจนเปอร์ออกไซด์ 30 นาที และรักษาด้วยสาร Cordycepin 1 μM, สาร Cordycepin 1 μM และ CMP 0.1 μM ตัวบ่งชี้ความแก่ชราของเซลล์และได้รับการประเมิน ได้แก่ การเพิ่มจำนวนเซลล์ การกำจัดสารอนุมูลอิสระที่เกิดขึ้นภายในเซลล์ การแสดงออกของยีนคอลลาเจนและอีลาสติน การแสดงออกของยีนต้านอนุมูลอิสระ และการแสดงออกของยีนสร้างโปรตีนคอลลาเจนชนิดที่ 1 (*COL1A1*) นอกเซลล์ CMP สามารถเพิ่มจำนวนเซลล์ในสภาวะปกติ นอกจากนี้ CMP ยังลดสารอนุมูลอิสระที่เกิดจาก การเหนี่ยวนำด้วยไฮโดรเจนเปอร์ออกไซด์ โดยเพิ่มการแสดงออกของ *COL1A1* และยีนที่เกี่ยวข้องกับแมทริกซ์ที่อยู่นอกเซลล์ นอกจากนี้ CMP ยังควบคุมการแสดงออกของยีน *COL1A1* นอกจากนี้ CMP ยังส่งเสริมการแสดงออกของแมทริกซ์ที่อยู่นอกเซลล์ (COL1A1 และ Elastin) หลังจากเกิดความเครียดออกซิเดชันที่เกิดจากไฮโดรเจนเปอร์ออกไซด์ และ CMP สามารถยับยั้งไฮโดรเจนเปอร์ออกไซด์ที่เกิดจากความเครียดออกซิเดชันได้อย่างมีประสิทธิภาพ

สาขาวิชาเทคโนโลยีชีวภาพ ลายมือชื่อนักศึกษา_____

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The major cause of degenerative diseases, including skin aging and skin cancer, is oxidative stress induced by reactive oxygen species (ROS or free radicals). Cordycepin, a bioactive compound of *Cordyceps militaris* is a potential harbor for herbal drugs which has antioxidant and anti-cancer effects. The present study aimed to evaluate the synthesis of collagen and elastin treated with cordycepin on dermal fibroblast cells (HDFs). HDFs were cultured and compared in two conditions (normal and oxidative stress conditions). In normal conditions, HDFs were treated with 1 μM cordycepin, 1 μM cordycepin medium, and 0.1 μM develop cordyceps medium-loaded nanoparticles (CMP). In the oxidative stress condition, HDFs were exposed to hydrogen peroxide (H_2O_2) and post-treatment with 1 μM cordycepin, 1 μM cordycepin medium, and 0.1 μM CMP. The senescent phenotypes of the HDFs cell were evaluated, including cell proliferation, scavenging, intracellular ROS, expression of collagen and elastin synthesis genes, expression of the antioxidant gene, and expression of extracellular matrix protein collagen type 1 (COl1A1). Here, CMP could increase cell proliferation and reduce H_2O_2-induced ROS. The CMP was 60.91 ± 10.69 - 323.4 ± 66.97 nm, the consequence of this was the increase of the COL1A1 expression and extracellular matrix genes. It was an important finding that CMP could efficiently inhibit oxidative stress induced by H_2O_2 and activate autophagy of HDFs. Altogether, CMP presents novel nano compounds with a high potential to be applied in cosmeceutical products.

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LIST OF ABBREVIATIONS

%	=	Percent
℃	=	Degree Celsius
µg	=	Microgram
µm	=	Micrometer
µM	=	Micromolar
3D-skin	=	Three-dimensional skin
ROS	=	Reactive oxygen species
AMPK	=	5' adenosine monophosphate-activated protein kinase
ART	=	Mono-ADP-ribosyl transferase
UV	=	Ultraviolet radiation
ATP	=	Adenosine triphosphate
CMP	=	Cordyceps medium loaded nanoparticles
CM	=	Cordycepin medium
C	=	Cordycepin
HDFs	=	Human dermal fibroblast cells
PCR	=	Polymerase Chain Reaction
CO_2	=	Carbon dioxide
COL1A1	=	Collagen 1 type I
H_2O_2	=	Hydrogen peroxided
GPX	=	Glutathione Peroxidase
SOD	=	superoxide dismutase
Ctrl	=	Control
DAPI	=	4′,6-diamidino-2-phenylindole
ATG5	=	Autophagy Related 5
ATG12	=	Autophagy Related 12
DMEM	=	Dulbecco's Modified Eagle Medium
DMSO	=	Dimethyl sulfoxide
DNA	=	Deoxyribonucleic acid

LIST OF ABBREVIATIONS (Continued)

mm	=	millimeter
ECM	=	Extracellular metrix
KRT18	=	Keratin 18
g	=	Gram
min	=	Minute
GAPDH	=	Glyceraldehyde 3-phosphate dehydrogenase
hrs	=	Hours

CHAPTER 1
INTRODUCTION

1.1 Significance of the research

The major cause of degenerative disease including skin aging and cancer skin was oxidative stress occurring reactive oxygen species (ROS). The skin is the largest organ of the body. Skin is organized into primal layers, epidermis, and dermis. The epidermis, of ectodermal origin, was the outermost tissue of the skin that covers most of the body (Alhajj et al. 2017). Reactive oxygen species (ROS) are naturally produced by normal cell function and a play role in cellular signaling (Wang et al. 2016). That was a contract with environmental stress including UV radiation, pollution, and chemical reagents. So, it occurs skin is damaged by ROS formation. That was important to protect that can induce oxidative stress to occur from ROS such as superoxide, and hydrogen peroxide (Bischof et al. 2015). Long-time human skin was made up of three key parts: Reaction which was damaging to the skin human. Resulting in the danger of dermal fibroblast. Dermis ware consists of 2 types of proteins, namely collagen, and elastin. These proteins are important for the repair and recovery of human skin after injury or degradation of collagen (Borrelli et al. 2018).

Nanoparticle technology in 2023 is growing interest in pharmaceutical production. Research into drug delivery is moving from the micro to the nanoscale. Applications of nanoparticle technology as pharmaceutical or drug components or constituents of drugs are novel approaches for controlled release, targeted delivery, and enhancement of bioavailability (Khan et al. 2015). Polymer capsules created at the nanoscale provide advantages such as controlled medication release, degradation, and high absorption (Chu et al. 2013). But we expect the versatile Cordyceps. After undergoing the process of nanoparticle technology will be more efficient. It can also be used in various industries, including food supplements and cosmetic ingredients.

Cordyceps is a genus of entomopathogenic fungus that includes about 600 species. The genus Cordyceps is classified under the order Hypocreales; family Ophiocordycipitaceae and phylum; Ascomycota (Tuli, Sandhu et al. 2014). Cordyceps is a genus of entomopathogenic fungus that includes about 600 species. *Cordyceps militaris* is a species of medicinal mushroom capable of producing the bioactive compound Cordycepin (3'-deoxyadenosine) (Tuli, Sandhu et al. 2014), which has pharmaceutical effects for antioxidant (Olatunji, Feng et al. 2016), anti-inflammatory (Lei, Wei et al. 2018, Yang, Zhou et al. 2020), and tissue remodeling process effects (Kim, Shin et al. 2021). Cordycepin can be produced from cordyceps in both submerge culture and solid-state fermentation, resulting in a range of biologically active compounds such as polysaccharides, cordycepin, adenosine, amino acid, organic selenium, ergosterol, sterols, cordycepic acid, superoxide dismutase, and vitamins (Shih, Tsai et al. 2007).

In addition, decreased levels of growth factors, reduced amount of collagen, abnormal accumulation of elastin, and reduction in the epidermal and dermal thickness are often indicative of clinical signs such as xerosis, wrinkles, sagging, blemishes, and all signs of skin aging. In previous studies, we found that cordycepin increases the rate of cell migration of intracellular fibroblasts, helping the skin stimulate the production of Collagen and accelerating tissue regeneration through Adenosine Receptor-Mediated Wnt/β-Catenin Pathway Stimulation by regulating GSK3b activity (Kim, Shin et al. 2021). However, we may be able to enhance the effectiveness of cordyceps extract or cordycepin extract in rejuvenating skin, stimulating cell growth, and improving wound healing (Mathew-Steiner, Roy et al. 2021). Therefore, in this study, it was interesting to develop the protocol of cordyceps medium loaded nanoparticles (CMP) and nanotechnology for improve quality and investigate bioactivities of the develop cordyceps medium loaded nanoparticles (CMP) *in vitro*.

1.2 Research objectives

1.2.1 To improve the protocol of product nanoencapsulation of cordycepin medium (CMP).

1.2.2 To obtain knowledge of the nanocap of the cordycepin medium enhance the collagen synthesis *in vitro*.

1.2.3 To establish the Cordycepin (C), Cordyceps medium (CM) and Cordyceps medium loaded nanoparticles (CMP) involved in the antioxidant activity on HDF cells.

1.2.4 To establish the Cordycepin (C), Cordyceps medium (CM) and Cordyceps medium loaded nanoparticles (CMP) involved in the autophagy process on HDF cells.

1.2.5 To obtain knowledge of the nanocap of the cordycepin medium enhance the wound healing process.

1.3 Research hypothesis

Internal dermal cells consist of human dermal fibroblast cells. It is commonly used in wound healing tests to develop and evaluate potential for clinical use. In cosmeceuticals, human skin cells are used as a role model for demonstrating the efficiency of skin care products and toxicity testing. The key role of cell rejuvenation is to increase gene expression through the extracellular matrix activation of COL1A1, elastin, and cell proliferation. By 2023, pharmaceutical products will be more focused on nanoparticle technology. The nanoscale is being studied in drug delivery research instead of the microscale. For controlled release, targeted delivery, and improved bioavailability, nanoparticle technology applications such as pharmaceutical or drug constituents or components of drugs are novel approaches. Nanoscale-produced polymer capsules have advantages like controlled medication release, low degradation, and high absorption. We are therefore interested in reducing cordycepin medium to nano size and evaluating the substance's potency in skin rejuvenation.

1.4 Scope of thesis

The primary purpose of this study is skin cell regeneration through collagen synthesis, antioxidation and autophagy of human dermal fibroblasts (HDFs) via Nano encapsulated cordyceps medium. Frist, we develop cordyceps medium-loaded nanoparticles (CMP) and test the absorption, Secretion of protein. Thereafter, these CMP were demonstrated the characteristics of percent yield, EE, LE, and particle sizes. Second, we will investigate the bioactivities of the development (CMP) in fibroblast cells. Third, we will stimulate the test in two conditions compare between: Normal condition & oxidative stress conditions. Finally, we will be interested in Skin Cell Regeneration through Collagen Synthesis, Antioxidation, and Autophagy.

1.5 Expected results

The nanoparticles revealed the mechanism of intracellular collagen synthesis. CMP is an exciting role because it controls collagen synthesis and all cell proliferation. This expression was confirmed by protein expression and wound healing activity.

The wound healing process is a very complex natural wound healing process. Awareness education will improve wound care. especially chronic wounds. The principle of preparing a dressing bed is the basis for wound healing. Nowadays, there are more innovations in bioengineering, biochemistry, and biotechnology. To be used in wound care, we need to study more to bring things. And to suit the wound and research and invent new things to heal faster, better wounds, recover faster, less scars.

CHAPTER 2
LITERATURE REVIEWS

2.1 Human dermal fibroblasts

2.1.1 The structure of skin

Our typical health and well-being depend on healthy skin. Healthy skin keeps the balance of fluids, controls body temperature, and serves as the body's first line of defense against infections and viruses. The largest organ in the human body is the skin, with a surface area of 2 m^2 (Lawton 2019). That is the skin and its appendages (hair, nails, and certain glands). The thickness of the skin, which makes up 15% of the adult body weight, ranges from 0.1 mm at the eyelids, where it is thinnest, to 1.5 mm at the thickest point (palms of the hands and soles of the feet) (Kolarsick, Kolarsick et al. 2011). In hypodermis, there are multiple layers to the skin. The layer underneath the dermis is primarily made up of fat. Our bodies are protected from toxins, germs, and fluid loss by the epidermis, the outermost layer that is visible to the unaided eye and touchable. There are 5 keratinocyte cell sublayers in it. Keratinocytes make up the bulk of the epidermis. Spindle-shaped structures form as it matures, and keratin is produced (protein fibers) (Hughes 2001). The basement membrane is the flexible, multilayered tissue that connects the epidermis to the dermis underneath the epidermis. The numerous functions of the skin's appendages, such as the follicles, sebaceous, and sweat glands, are also important. The innermost layer that produces keratinocytes is called the basal layer. Keratinocytes (or stratum basale), prickle layers, known as stratum spinosum Keratinocytes, will develop into a spindle-shaped structure and form keratin (protein fiber) Stratum granulosum or granule layer when keratin begins the cells form solid granules that are converted to keratin and epidermal lipids as they push forward. Cells in the clear layer the stratum corneum (stratum lucidium) is compressed, flattened and almost indistinguishable from each other. The horny layer (also known as the stratum corneum), depending on the location of the body, is the outer layer. It is the outermost layer of the epidermis and consists of an

average of 20 flattened dead cell sub-layers, desquamation, a process that causes regular shedding of these dead cells. Sweat and sebaceous gland pores are also found in the horn layers (Lawton 2019).

2.1.1.1 Epidermis layer

The epidermis, it made up from multiple layers of keratinocytes. The outermost of the layers of the skin, making up about 90 percent of the cells there. Additionally, keratinocytes produce and secrete glycolipids, which are made up of a portion of fat and a portion of sugar. Glycolipids aid in limiting the ease with which water can enter and exit the body (Chambers and Vukmanovic-Stejic 2020, Lee, Hong et al. 2021).

Stratum lucidum, this layer can be found on the palms and soles. The stratum lucidum is a smooth, seemingly translucent epidermal layer. Keratinocytes on the stratum lucidum die and flatten. The association between keratinocyte and eleidin proteins results in these cells being transparent and impervious to water.

The stratum corneum is the outermost layer of the epidermis and is the strongest of the 30 layers of corneocytes. These cells have a flat morphology and fewer nuclei. But there is an accumulation of keratin fibers. An increase in keratin, known as cornification It consists of 3 fundamental factors (1) Substitution of cytoskeletal frameworks with compact proteins for intracellular organelles and their contents. Cross-linking of proteins at the cell edge to form a corneal-encapsulated cell. and the fusion of corneocytes to form physiologically dead multicellular structures (Eckhart et al., 2013). Derived from the stratum granulosum, Granulosum stratum, and Keratinocytes. Formed and shifted from stratum granulosum and stratum spinosum due to their proximity to each other. These cells look interesting. Keratin protein, which consists of many fibers and keratohyalin. (Keratohyalin) thickens the cell membrane and forms lamellar granules inside the cell in the stratum granulosum. Keratin and keratohyalin are mixed to produce keratohyalin. a large number of keratinocytes. When cells die Only keratin remains due to the decay of the nucleus and other cell organelles. The cell membrane and keratohyalin form the stratum lucidum Hair and nails are strengthened by the stratum corneum and the stratum basal structure. This layer is also known as the basal cell layer or stratum germinativum. The stratum

germinativum is the lowest layer of the epidermis and is located near the dermis. the most from this condition keratinocytes will be created the basal stratum contains melanocytes and other cells. including Merkel cells, the brain experiences sensation as a result of the stimulation of sensory nerves by Merkel cells, which act as receptors. The outside of the hands and feet are filled with these cells. The pigment melanin, which gives skin and hair their color, is produced by melanocyte cells. Both genetics and external factors affect the outermost layer of the skin, causing aging. These include drinking too much alcohol, smoking and overexposure to UV light. These all-cause wrinkles and uneven or thinning skin thickness. The function of the epidermis is important to the human body. The epidermis has the ability to protect against injury and illness from germs and fungi that can enter healthy skin. Acidic skin covers can help protect against pathogens due to their dryness and acidity (pH 4-6). In addition, the skin plays an important role in vitamin D production which is important for bone growth and preservation. The largest number of receptors for biological stimuli are heat, cold, touch, surface, pressure, and vibration. Found in the skin, the face, palms, fingers, soles, nipples and genitals all have sensory receptors. importance of skin This includes regulating body temperature or response to grilling. Skin, however, is linked to nonverbal communication or human social acceptance.

2.1.1.2 Dermis layer

The dermis layer, which consists of dense, wavy connective tissue. Composed of sweat glands, arteries, and other components, the papillary dermis and reticular dermis are the two layers that make up the dermis. The most superficial of the two is the papillary dermis, located just below the dermis junction. It consists of loose connective tissue with collagen, reticular fibers, elastic fibers and capillaries and quite thin Strong fibroblastic structures are the result of loose connective tissue. The extracellular component of the skin consists of structural molecules that form fibers, nanofibers, and a protein cell matrix. Filament-forming molecules provide extracellular matrix (ECM) structure by assembling rigid proteins into a complex three-dimensional framework. Filament-forming molecules provide extracellular matrix (ECM) structure by assembling rigid proteins into a complex three-dimensional framework. Proteoglycans and glycosaminoglycans (GAGs), which are the majority of non-fiber-forming molecules.

It works to create an active, dynamic and osmotic environment (Tracy et al., 2014). Collagen, the structural component responsible for fibrous formation, accounts for 77% of the dry and fat free weight of the skin. The protein with the highest levels of tensile strength and overall fertility is collagen. The skin also contains the proteins fibrin, fibronectin, vitronectin, elastin and fibrillin. Hyaluronan (HA), decorin, versican and dermatopontin. which has a negative charge and likes water Proteoglycans are some of the proteoglycans found in the skin. The ECM component is present in healthy skin and slowly regenerates damaged skin. The reticular dermis is the deeper and thicker layer of the dermis, which lies above the subcutaneous layer of the skin. It contains dense connective tissue which includes elastic fiber, collagen fiber, fibroblasts, blood vessels, mast cells, lymphatic and nerve ending. Fibroblasts are mesenchymal cells with many vital functions during development and in adult organisms. are responsible for producing the extracellular matrix forming the dense connective tissue of the skin and play major roles in wound healing.

2.1.2 Fibroblast cultures

The epidermis, which is the top layer of the skin, it is the stratified squamous epithelium, which is mainly composed of keratinocytes different (Dehdashtian, Stringer et al. 2018). The main constituents (cells) of the epidermis are known as keratinocytes, which also produce the protein keratin. The main cell found in the dermis is the fibroblast, and its role is to produce cytokines, growth factors, and hormones, which promote proliferation and the ability to shape the fibroblast or other cells. Including production and release of extracellular matrix such as collagen, elastin, and another chemical reagent (Cichorek and Wachulska et al. 2013).

Using fibroblast cells isolated from these fibrotic disorders, there is therefore important interim research on abnormalities of ECM components such as type I and III collagen. These cells are created from keloid and hypertrophic scar tissues from skin biopsies. These cells can be extracted from debrided burn tissue or live skin. A qualified specialist taking a skin biopsy is the first step in the generation of dermal fibroblasts for therapy. The biopsy is subsequently delivered to a good manufacturing practice (GMP) cell culture processing unit for the enzymatic isolation, culture, expansion, and purification of dermal fibroblasts. For a therapeutic application to be

successful, cell culture techniques must advance in order to increase the cells without sacrificing their distinct cellular identity and regenerative capacity. (Thangapazham and Darling et al. 2014). Some of these developments include technology to begin producing decent numbers of cells with the desired potential. The optimal location to harvest according to the desired therapeutic indication. and storage and delivery of fibroblasts to clinical sites for use. The fact that fibroblasts preserve intact cell cycle checkpoints and lack mutations in oncogenes and tumor suppressors is a significant benefit of fibroblasts. After fibroblasts are injected into mice, toxicology studies show no evidence of tumor production or oncogenic change. The development of dermal fibroblast products includes many animal models in pig and rodent for preclinical evaluation of dermal fibroblasts in addition to this safety profile (Thangapazham and Darling et al. 2014). As a result, normal fibroblasts are a desirable model for research on the control of the cell cycle, DNA repair, and apoptosis. In significant part, cancer cells accomplish these characteristics by reactivating and altering numerous cellular programs that are typically used throughout development. During embryogenesis and tissue homeostasis, these programs regulate coordinated events such cell proliferation, migration, polarity, apoptosis, and differentiation.

The application of cell culture technologies in biology, medical research, and applications has grown significantly. Through generating fibroblast primary cultures, researchers can obtain representative cells that have preserved the majority of their original features and functions, providing a crucial starting point for further cell biology and cell engineering (Siengdee, Klinhom et al. 2018). Whatever stromal cell that lacks characteristics for a more identifiable mesenchymal lineage is called to as a "fibroblast." Because of this ambiguous definition and the difficulty, we have in identifying distinct cell markers, we tend to think of fibroblasts as uniform, static cells (Tracy *et al.*, 2014).

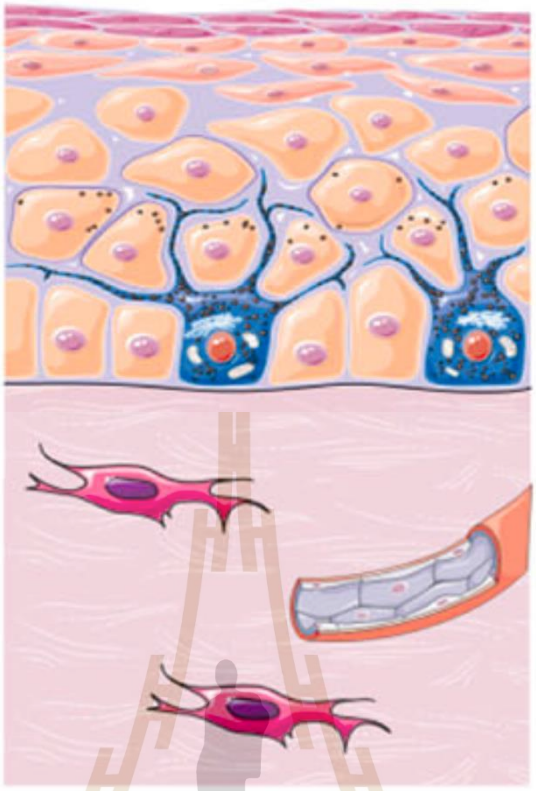


Figure 2.1 Human dermal fibroblast cell icon (Henrot and Laurent et al. 2020).

2.1.3 The application of fibroblasts

The potential of fibroblasts to reprogram skin for the aim of skin rejuvenation in aging skin is being understood to develop new therapies for skin rejuvenation. Virtually every aspect of skin development, including skin morphogenesis, embryonic growth, and physiological circumstances depend on fibroblasts. Cell treatments for a range of skin conditions have been developed as a result of the discovery that dermal fibroblasts have the ability to regenerate in skin repair and rejuvenation. The spindle-shaped appearance of these cells on tissue culture plastics defines them. For instance, the dermis of the skin has many subpopulations of fibroblasts with distinct morphologies and physiological roles in each of the dermal layers. Fibroblasts express varying amounts of collagen and different ratios of collagen type I and III mRNA depending on the depth of the dermis where they are located. Fibroblasts partnering with keratinocytes, immunological cells, and endothelial cells to enhance the signaling molecule at injury sites,

fibroblasts play a role in wound healing. Inflammatory leukocytes are triggered by signaling molecules released by platelets. White blood cells, fibroblasts and endothelial cells can aid the healing process by enhancing the chemical gradient that occurs when platelets are activated. spontaneous inflammatory phase cell aging Bacterial infection, reduced oxygen, and starvation. All affect the occurrence of chronic wounds. Chronic wounds are wounds where normal healing has been thrown away. This causes the healing process to prolong despite proper treatment. Most fibroblasts found in chronic wounds show signs of premature aging. It also showed abnormal morphology as well as reduced migration and propagation activities. The p38-MAPK pathway was upregulated, which reduced proliferation. Inflammation, oxidative stress, and UV radiation all activated the p38-MAPK cytokine pathway. send signals to the nucleus that cause differentiation and maturation (Raffetto *et al.*, 2008).

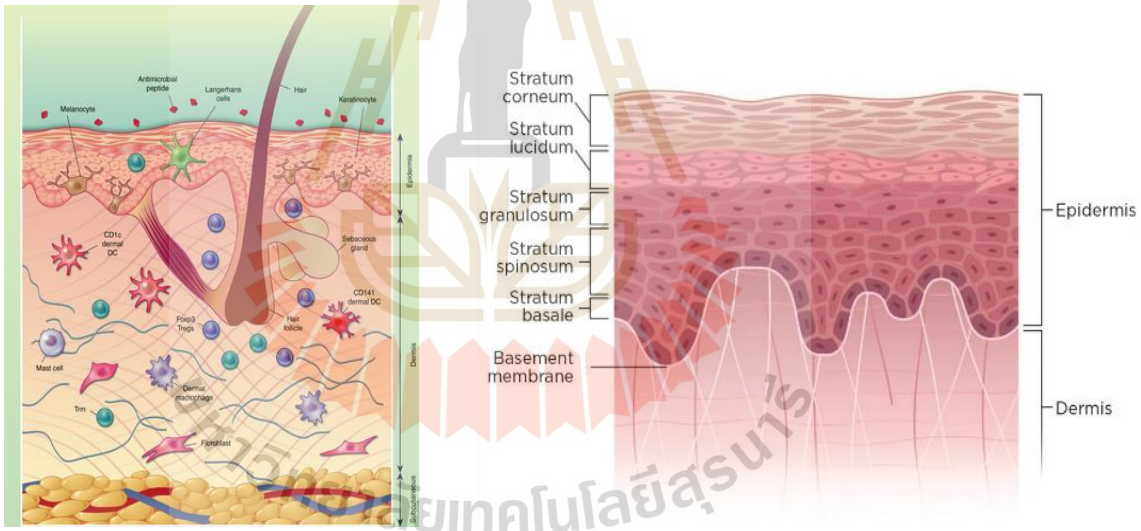


Figure 2.2 Skin layers and structure. (A) Skin layers (Chambers and Vukmanovic-Stejic 2020). (B) Skin 1: the structure and functions of the skin (Lawton 2019).

2.1.4 The skin disease and factors of the disease

Skin aging and skin cancer are become increasingly common in humans because air pollution in the world, the result from NASA's Jet Propulsion Laboratory, the amount of carbon dioxide (CO_2) in the atmosphere has increased from 365 parts per million (ppm) in 2002 to more than 400 ppm today, contributing to global warming and climate change. As a result of the greenhouse effect and subsequent global warming, more UVA and UVB rays are reflected back down to the Earth's surface. affecting life on earth, especially the humans. How effect in human, Ultraviolet radiation (UVA, UVB, and UVC radiations) will cause mutations and damage of skin in the animal. It is the shortest wavelength radiation. Originally, ozone would have filtered out all this radiation. But now, because of human pollution. UV radiations have affected another thing including senescent, wound healing of skin. The report describes the wound healing after the patient passes the x-ray machine. The wound is not pass x-ray machine healing faster than pass x-ray 4 times.

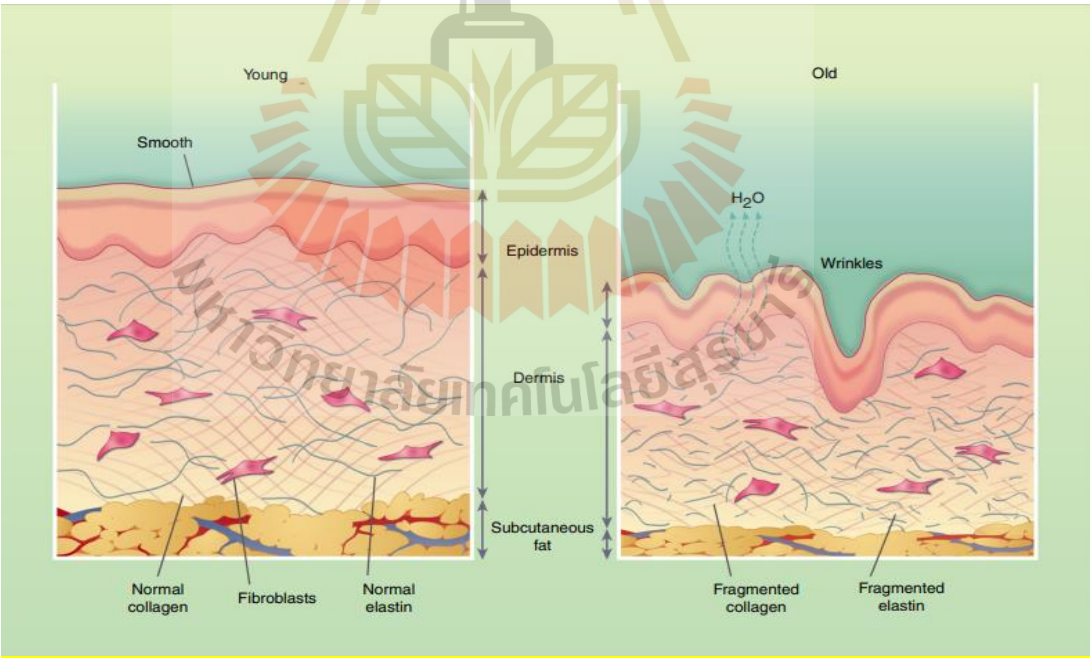


Figure 2.3 Structural changes in human skin with age. Young skin structure (left) and compared with older skin structure (right). Older skin has fragmented elastin and collagen (Chambers and Vukmanovic-Stejic 2020).

2.2 Wound healing

Skin needs to be immediately recovered following the damage in order to sustain its functions. The extracellular matrix, resident skin cells, cytokines, chemokines, growth factors, and regulatory molecules all play a role in this process, as do secondary blood mononuclear cells, chemokines, growth factors, and resident skin cells. Three consecutives, overlapping phases have been used to arrange the complex skin restoration process. the phases of remodeling, proliferative, and inflammatory activity. Hemostasis and inflammation of the cutaneous neuropathy make up the inflammatory process. They begin as soon as the injury occurs and last about an hour. After a 24 h period of rapid recruitment of neutrophils to the injured tissue. There was a subsequent decline throughout the following week. Inflammatory monocytes and macrophages gradually Infiltrate wounds and grow in the second stage after being damaged.

They peak during the vegetative stage. then began to decline over the next two weeks. before eventually taking over as the main mononuclear cells in the tissue repair process. Immediately after injury Circulating white blood cells travel to the skin and reach a plateau after 4 days before starting to deteriorate. They had to stay there for another two weeks. Regeneration of tissues that are reproduced in the early stages of the proliferation phase Including scar formation to restore the integrity of the skin are both tasks performed during the third phase. This begins two weeks after the injury. Several months may pass by the end of this update. Cellular properties of acute wound healing and damage regeneration (Figure 2.3). These procedures are described in detail in the following sections.

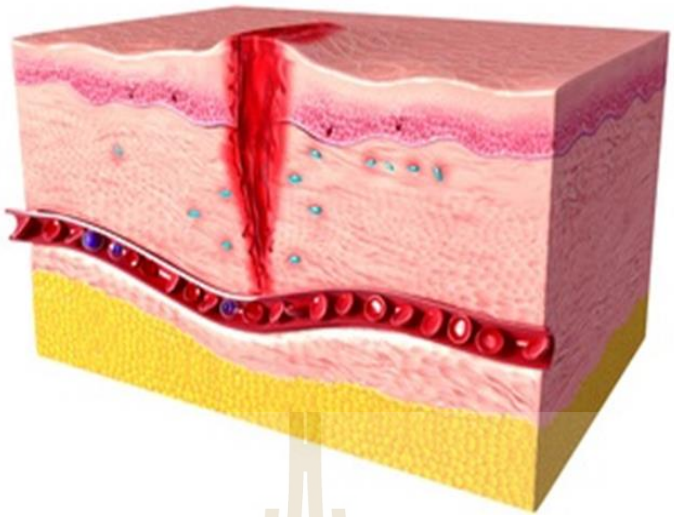


Figure 2.4 Tissue repair of human skin (https://www.woundsource.com/blog/four-stages-wound-healing).

2.2.1 Hemostasis phase

When a wound occurs, a blood vessel is torn, causing bleeding. The body will react to the blood vessels causing Vasoconstriction and platelets aggregation, which can occur by the chemical substance from the injury cell as a stimulus. Leading to the formation of blood clot (Coagulation cascade), which contains various coagulation factors. In the blood clot, it contains Fibrin, which is in the form of a meshwork, causing the bleeding to stop and cover the wound in the early stages. It will also provide a framework for cells in the Wound healing process to enter if the Fibrin meshwork does not complete, it can affect Wound healing. Platelets, besides being the initial cell of the Wound healing process, by Aggregation itself also releases various Cytokine to stimulate the Wound healing process to continue, such as Platelet Drived Growth factor (PDGF), Transforming growth factors, Fibroblast Growth Factor -2 (FGF-2), etc.

2.2.2 Inflammatory phase

This phase occurs within 10-30 minutes after injury which will be born vasodilation, increased capillary permeability, complement activation, white blood cell (pmn, monocyte) migration causes pain, swelling, redness and heat at the wound area.

When the white blood cell enters the wound, it destroys cell debris, bacteria, and the injured extracellular matrix by releasing enzyme protease to digest. In addition, macrophage, which is a cell that is important to wound healing, also secretes many growth factors to stimulate growth. Proliferation such as angiogenesis epithelization fibroblast proliferation and migration and collagen synthesis etc.

2.2.3 Proliferation phase

The wound has granulation tissue, angiogenesis, and wound (Contracture and epithelization) which will have different cells to replace the inflammatory. Cell granulation tissue to create a new extracellular matrix, mainly collagen produced by fibroblast with cytokine as a stimulator to cause fibroblast increase, fibroblasts migration, and collagen synthesis. Collagen also contains essential substances for this process, such as vitamin c, iron, and oxygen, which are essential cofactors for collagen formation. Hydroxylation of proline and lysine etc.

Angiogenesis which is stimulated by Macrophage and Tissue Hypoxia by Macrophage, which is a cell that plays an important role in every process of Wound Healing, secretes substances such as Transforming Growth Factor to stimulate Endothelial cells to create new blood vessels.

Wound Contraction Fibroblast formation, in addition to collagen and other extracellular matrix (Fibronectin, Proteoglycan) plays an important role in wound contracture formation by itself moving to the edge of the wound. and change to Myofibroblasts which has the power to pull the edges of the wound together. There is more wound contracture in 2nd healing wounds than in primary closure wounds.

2.2.4 Remodeling phase

The Remodeling Phase or the Maturation Phase may have a traumatic process. Treatment, which will begin about 20 days, what will happen later and can continue, the next step is not remembered what else needs to be, e.g., a tape recorder, the duration of the wound healing, is not possible. Things can't remember. At this stage, the wound will improve (Increase Tensile Strength), which will cause Collagen Cross Link and collect information on looking at various cells here (Decreasing Cellularity,

which during this time the wound is (Scar). The amount of blood that will occur causes collagen to be created and doesn't break down more collagen until all causes of creation and destruction. Collagen softened, flattened, flattened scars which required lightening in which the experimental process was controlled by the scar macrophage. Mature and healthy skin is no more than 80% of normal skin and does not need fat, sweat glands, or pores, so it does not dry out more easily than normal skin.

The key function of resident and peripheral immune cells, as well as the microenvironment and their interactions during the wound healing process (Wilkinson and Hardman 2020). Wounds are classified into two types: acute wounds and chronic wounds. Acute wound is healing wounds occurring in natural process. Chronic or non-healing wounds occur when a wound is not healed by the acute healing process but is interrupted for a time.

The qualities of acute wound fluid and chronic wound fluid are opposite each other. TNF-2 and IL-1 levels are highest in acute wound fluid 2-3 days after wound onset and thereafter significantly decline. later Fluid from chronic wounds consistently contains significant levels of pro-inflammatory cytokines. In contrast to chronic wound fluid, which contains small levels of these growth factors as well as matrix metalloprotease (MMPS), which breaks down the ECM, acute wound fluid contains substantial amounts of growth factors such PDGF, IL-6, TGF-2, and TGF- to boost the proliferate phase.

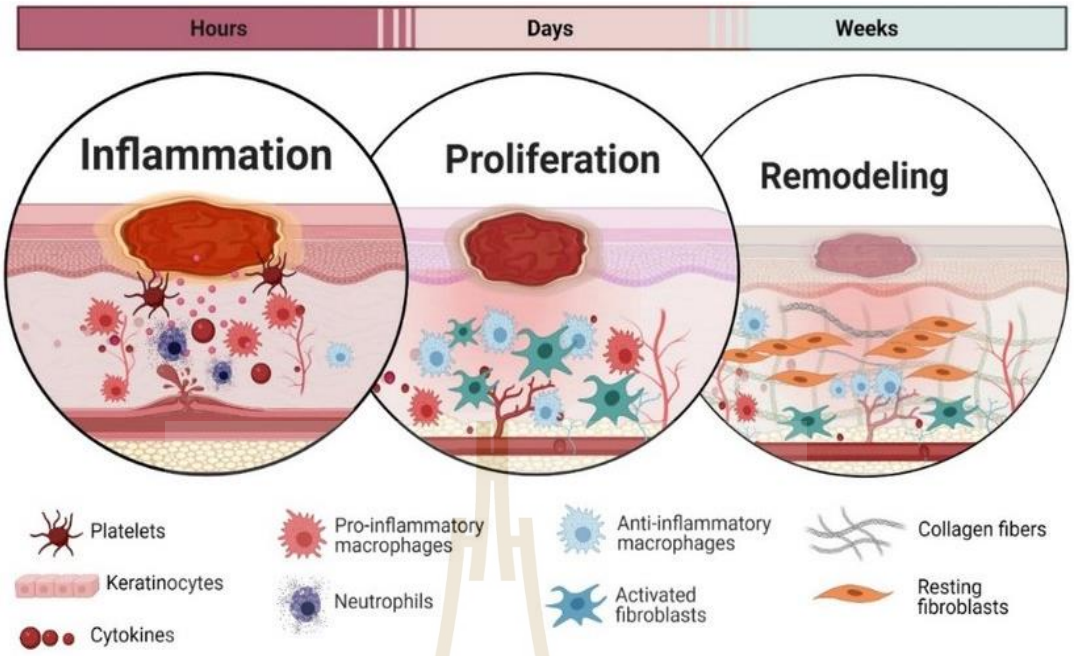


Figure 2.5 Timeline of occurring wound healing process in natural mechanism (Chambers and Vukmanovic-Stejic, doi:10.1111/imm.13152)

2.2.5 The relevance of collagen to wound healing

There are 2 distinct portions of the dermis: the lower reticular dermis and the upper papillary dermis. The papillary dermis, which is found right below the dermal-epidermal junction, creates conical upward projections called dermal papillae. The interdigitation of these projections with epidermal rete ridges increase the area of the dermal-epidermal interface and improves dermal and epidermal adhesion. The papillary dermis is composed of a loosely coiled bundle of collagen, elastic fibers, fibrocytes, blood vessels, and nerve terminals. The reticular dermis is composed of dense collagen fibers, thicker elastic fibers, deep epidermal appendages, vascular networks, and nerves. Collagen fibers, which make up the main substance of the dermis, are created by fibroblasts. More than 90% of dermal fibers are interstitial collagen. Mainly type I and III, which give the skin tensile strength and mechanical resistance. Elastic fibers are responsible for repairing the physical structure of the skin after deformation. and these fibers extend from the laminadenza of the epidermis to the papillary dermis. Elastic fibers are thinner in the dermis and thicker and lie horizontally in the dermis.

The matrix produced by industrial floor materials is composed of epidermal fibers and cells. It is widely distributed throughout the papillary dermis and is composed of glycoproteins and proteoglycans. Some of the cellular components in the dermis are dermal dendrocytes, mast cells, and fibroblasts. Fibroblasts are the main cells in the dermis that form the dermal fibers and base material. Compared to net-like skins, they are more numerous. The metabolically inert fibroblasts called fibrocytes are dormant. Collagens phagocytose fibroblasts and call “fibroblasts”.

2.3 Autophagy

2.3.1 Autophagy and skin wound healing

Your body can recycle and disassemble old cell components through autophagy, which helps your cells function more effectively. When your cells are under stress or lack nourishment, a natural cleaning process starts. The potential role of autophagy in preventing and treating disease is being researched. A fundamental biochemical mechanism for maintaining cellular and organismal homeostasis is autophagy. In numerous experimental settings, pharmacological and genetic therapies that disrupt autophagy responses either induce or exacerbate illness. Mutations in autophagy-related mechanisms frequently result in serious human diseases. Here, we review and analyze preclinical evidence connecting autophagy failure to the etiology of significant human diseases, such as cancer and cardiovascular, neurologic, metabolic, pulmonary, renal, infectious, musculoskeletal, and ocular disorders.

As one of the main quality-control guardians in the cell, autophagy is crucial in preventing stressors as well (Conway, Akpinar et al. 2020). Recent research has shown that autophagy is crucial to several stages of wound healing. In particular, autophagy has an anti-infection effect during the inflammatory phase and negatively regulates the inflammatory response, which prevents excessive inflammation from causing tissue damage.

Generally, when an injury occurs in the skin, the dermis has a natural wound-healing mechanism. But sometimes this process to be slow because a loss of fibroblast function and decrease response to tissue injury. In dermis layer has important cells line namely “fibroblast”, that are the most common cell in connective tissue and

connective tissues permanent residents. They secrete the fibers (notably collagen and elastin) and ground substance of the extracellular matrix, which is important for wound healing.

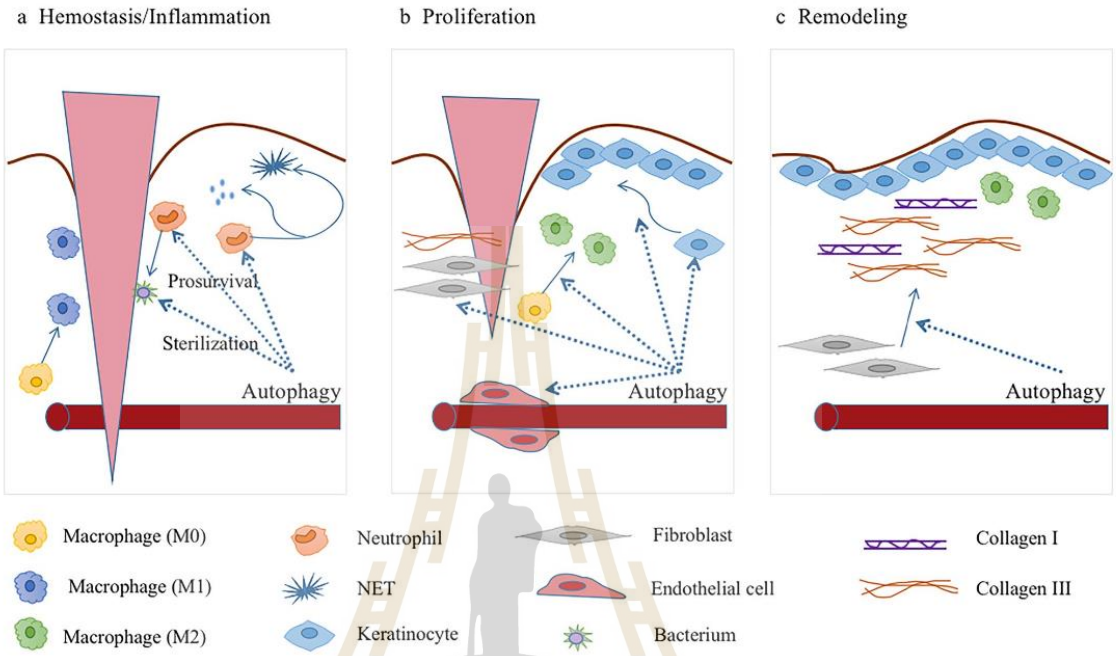


Figure 2.6 The function of autophagy in skin wound healing (Ren and Zhao et al. 2022). At the hemostasis/inflammatory (a), proliferative (b), and remodeling (c) phases of wound healing, autophagy plays a role in controlling the process. The survival, proliferation, and migration of neutrophils, macrophages, endothelial cells, keratinocytes, and fibroblasts are all encouraged by autophagy, which makes it easier for them to carry out their biological activities and hastens the healing of wounds.

2.4 Cordyceps, and cordycepin

2.4.1 CORDYCEPS botany

Cordyceps is a genus of entomopathogenic fungus that includes about 600 species. The genus Cordyceps classified under order Hypocreales; family Ophiocordycipitaceae and phylum: Ascomycota (Tuli, Sandhu et al. 2014). The natural habitat of this medicinal fungus is high altitude areas (10,000 ft. to 14,000 ft.) altitude of the Himalayas including Nepal, China, Tibet, and India (Meena, Pandey et al. 2010).

Cordyceps in the naturally formed by when caterpillars feed on mushroom spores that fall to the ground during the summer. The butterfly's body contains spores. When the winter arrives to avoid the cold, the caterpillars will dig about 15 cm into the soil. Lower temperatures weaken the caterpillars in the winter, allowing the acquired mushroom spores to germinate. By sucking the worm's nutrients and minerals. and started spreading the fibers throughout the worm and devouring the worm's brain, spending the entire winter growing and sucking up all the worm's nutrients. Mushrooms can be seen in the summer (Tuli and Sharma et al. 2013).

(A) (B)

Figure 2.7 The timeline growing of cordyceps and cordyceps found in natural (https://www.flickr.com/photos/PaulKbibliodyssey.blogspot.com/2010/06/fungisdanicis.html).

2.4.2 Pharmacological of cordyceps

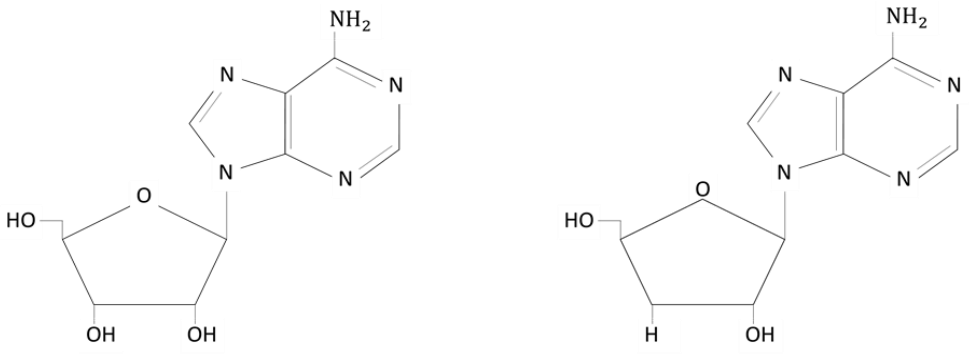
Mushroom *Cordyceps militaris* is produced as an antibiotic production. (Cunningham, Manson et al. 1950). The best quality cordyceps was the Cordyceps Sinensis species. Pharmacologically active compounds like cordycepin, adenosine, cordymin, and exopolysaccharides (Adnan, Ashraf et al. 2017). The main constituent of this fungal extract is a unique bio-metabolite known as cordycepin or (3'deoxyadenosine), which has anti-inflammatory properties. antioxidant and an effective anticancer agent (Borchers, Keen et al. 2004, Zaidman, Yassin et al. 2005, Yoon, Park et al. 2018). Nutritional value in Cordyceps, it contains a wide range of

nutritious components such as amino acids, essential vitamins and minerals (B1, B2, B12 and C), proteins, sterols, nucleosides and other trace elements. as well as many types of carbohydrates including monosaccharides Oligosaccharides and other polysaccharides Cordycepin was found to be of medicinal importance (Hur 2008, Yang, Feng et al. 2009, Yang, Li et al. 2010). Several bioactive substances including polysaccharides, cordycepin, adenosine, amino acids, organic selenium, ergosterol, sterols, cordycepic acid, superoxide dismutase (SOD) and vitamins (A, E, B2, B3 and C) are produced by themself (Hughes 2001).

Cordycepin acts as a nucleoside analog and shares its structure with the nucleotide adenosine in cells. 3'hydroxyl (Fig. 2B) (Fig. 2A). Adenosine is a nitrogenous base that acts as a cellular nucleoside necessary for DNA and RNA formation and other chemical reactions. in living cells Some transcription-related enzymes are unable to differentiate between cordycepin and adenosine. Thus, cordycepin can prevent the end of the RNA chain, which infers the functional pattern of cordycepin in the process. a number of biomolecules (Tuli, Sandhu et al. 2014). In cells, cordycepin is converted to 5'mono-, di- and triphosphates. that disrupt the activity of enzymes involved in de novo purine production, such as ribose-phosphate pyrophosphokinase and 5-phosphoribosyl-1-pyrophosphate aminotransferase (Soltani, Malek et al. 2018).

2.4.3 Cordycepin interferes in signaling partway

It has been observed that cordycepin shortens the poly-A tail of m-RNA, which has an additional effect on m-RNA stability within the cytoplasm. It has been shown that some miRNAs are more sensitive to polyadenylation of cordycepin than other mRNAs, inhibiting cell adhesion and decreasing focal adhesions. In addition, cordycepin may inhibit cell translation and growth by activating open AMP. Activates kinase (AMPK) in an unspecified way. hence the closure of the mTOR signaling pathway (Wong et al. 2010).



(A) Adenosine (B) Cordycepin (3'deoxyadenosine)

Figure 2.8 The chemical structures of bioactive compounds (Cordycepin and adenosine).

2.4.4 Medical applications of Cordyceps

There are many pharmacological and medicinal uses for Cordyceps strains. The main constituent of Cordyceps extract is a biological metabolite called Cordycepin (3'deoxydenosine), which has anti-inflammatory properties. antioxidant and effective anti-cancer Protective effects of inflammatory harm in disorders such as acute lung injury (ALI), osteoporosis. and stroke.

Osteoporosis is chronic disease is a problem in the elderly. It is detected only when the bone is broken. by directly affecting life whether the movement, pick up things. At present, the new diagnostic and treatment has been studied extensively. Osteoporosis is bone mineral density (BMD) decreases that the cause of easily broken bone. From the report in 2015, Zhang success in study cordycepin protect against estrogen deficiency-induced osteoporosis in rat models. Cordycepin can counteract the bone loss in this experimental. Result show that 20 μg/mL oral administration of cordycepin decreased ALP activity and tartrate resistant acid phosphatase activity (Zhang, Deng et al. 2015) previous work revealed that cordycepin protects against osteoporosis as a result of increased bone generation and inhibited bone loss under different mechanisms including mitigate osteonecrosis via a decrease in ROS (Dou, Cao et al. 2016), protect the femoral head in alcohol-induced osteonecrosis via increases beta-catenin (Chen and Zhu et al. 2017).

Stroke is one common disease involving cerebrovascular. The result of which the brain to lack blood supply to cultivate and divided into two types: ischemic stroke and hemorrhagic stroke. Ischemic stroke is most cases calculation about 80% of all strokes. It may be caused by blood clots that form in another area and then circulate to the brain (Iadecola 2017). This causes narrowing of the blood vessels in the brain. Treatment guidelines for this disease can be divided into two types: the control blood flow by use drugs to break down blood clots within the blood vessels It has been reported to be associated with inflammation stroke and brain injury (Iadecola 2017).

Cordyceps have increases energy in the cell in vitro via beta-adenosine triphosphate (ATP) in 2001 GUOWEI DAI et al.: study about cordycepin improves state bioenergy status in mouse liver. The result showed that ATP increases in the liver of mouse at concentration 200 or 400 mg/kg per day for 7 days by 12.3% and 18.4% respectively. That enhances cellular energy in the cell form of ATP (adenosine triphosphate). Upon hydrolysis of phosphates from ATP, a lot of energy is released which is further used by the cell (Dai, Bao et al. 2001). Numerous studies conducted in the past on cordyceps have shown that cordyceps have many beneficial properties. Including the ability to kill the embryo. Anti-inflammatory, anti-diabetic, antioxidant and anti-cancer (Cao, Shang et al. 2019). Other studies have found that cordycepin can protect rat heart tissue from ischemic/reperfusion damage. by promoting the production of the antioxidant enzyme HO-1 and the Akt/GSK-3b/p70S6K pathway (Park, Kang et al. 2014). Our results showed that by reducing ROS generation induced by H2O2 exposure in BM-MSCs (Wang, Yin et al. 2015).

2.5 Nanotechnology

The use of biological processes, organisms, or systems to create a product that aims to raise people's living standards is called biotechnology. On the other hand, Nanotechnology is a field of engineering that deals with the creation of mechanical and electronic devices. Nanomaterials are simply materials made of very small molecules. The material contains particles or constituents with external dimensions on the nanoscale ranging from 1 to 100 nm (Mahmoudi, Hofmann et al. 2012). Many biological systems found on Earth are at the nanoscale. By combining all biological

principles Scientists can obtain laboratory results using principles of chemistry, physics, and nanotechnology and engineering for manufacturing and Characterization of nanoscale biological devices and molecules using biosystems based on these facts resulting in a new technology called “nanobiotechnology” (Roco 2003).

2.5.1 Nanobiotechnology

Biotechnology, therefore, nanotechnology is a combination of nanotechnology and biotechnology. Both conventional microtechnology and molecular biology methods are used. Using biological concepts and methods biotechnology manages cellular, molecular, and genetic processes to produce goods and services. and is used in a wide range of fields from agriculture to medicine and other industries (Sahoo, Parveen et al. 2007).

The most important clinical application of nanotechnology will be in drug development (Khan, Khan et al. 2015) found that the advent of nanomaterials as a targeted drug delivery commodity to combat communicable and non-communicable diseases has overcome the limitations. limitations of conventional drug delivery systems such as specificity, bioavailability, biocompatibility, and biodegradability. Recently, interest in carbohydrate-based polymers in drug delivery systems (DDS) has increased due to their biocompatibility. Better encapsulation performance Environmental friendliness, low cost and drug release control. Researchers have begun to focus on starch as a possible option for drug delivery. food packaging and biomedical applications as fillers. dispersant and binders Due to abundant supply, cost-effective, environmentally friendly, and naturally biocompatible. This is because the dough has better loading performance. Sustained slow release of therapeutic compounds, Siddha and Ayurvedic formulations, and bioactive compounds to the targeted area. Despite these benefits native starch is limited by its poor functional properties. Therefore, physical, chemical, and enzymatic pretreatment and surface treatment are critical to optimizing the pharmaceutical applications of starch. such as DDS, implants, stents, skin, and ophthalmic systems. The current review focuses on production. Surface pretreatment, modification (using physical, chemical and biotechnology methods) and characterization of powder nanoparticles into small and nano DDSs. and used in the treatment of infectious diseases cancer therapy and treatment of neurodegenerative

diseases (Sivamaruthi, kumar Nallasamy et al. 2022).

In addition to cellulose and chitin, starch is one of the most common biopolymers on earth (Tharanathan 2005). Corn is the primary source of starch produced worldwide. Large amounts of other types of starch, including cassava, potato, and wheat starch, are also produced. The only part of these crops that has any commercial value is the starches (Zhu 2020). Cassava is grownup in 48 out of 76 provinces in Thailand have ripe cassava fields. The five provinces with the largest cassava plantations are Chachoengsao, Kamphaeng Phet, Chaiyaphum and Nakhon Ratchasima, which account for 25% of Thailand's total output. With approximately 50% of the market, Thailand is the largest exporter in the world. 11 million tonnes of cassava products were exported in 2017 and the target is 10.6 million tonnes in 2018, so it would be good if this research and study would help. Let this agricultural product.

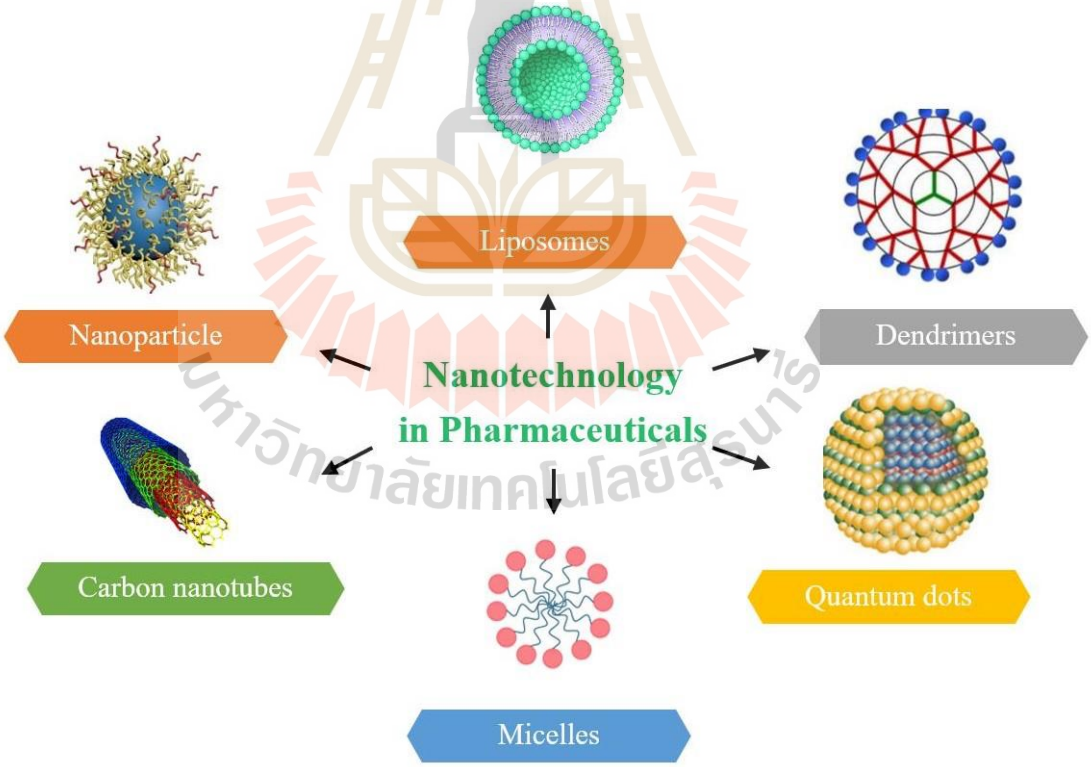


Figure 2.9 The various pharmaceutical science applications of nanotechnology (Jadhav, Chawra et al. 2020).

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Material

3.1.1 Reagents and chemicals

Cordycepin standard and cordycepin medium derived from Laboratory of Cell-Based Assays and Innovations (CBAI), School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology. The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO), were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from HyClone® (HyClone, Logan, UT).

3.2 Method

3.2.1 Fabrication of cordycepin medium-loaded nanoparticles

The preparation method of cassava starch nanoparticles was modified from physical treatment (Kaokaen, Jaiboonma et al. 2021). These methods are comprised of three steps in which (1) starch was extracted, (2) CM was encapsulated, and (3) aqueous nanosuspensions of CM-loaded cassava starch nanoparticles (CMP).

First, 4% cassava starch powder was dissolved in 1,000 ml of DI water under magnetic stirrer for 30 minutes and added to ethanol (dropwise) ratio 1:1.5 into slurry of hot starch and stirred for 30 min. Then, the hydrolyzed starch was collected by centrifugation at 10,000 rpm for 5 min. The hydrolyzed starch was transfer to a hot air oven (60 °C) for 1-2 days.

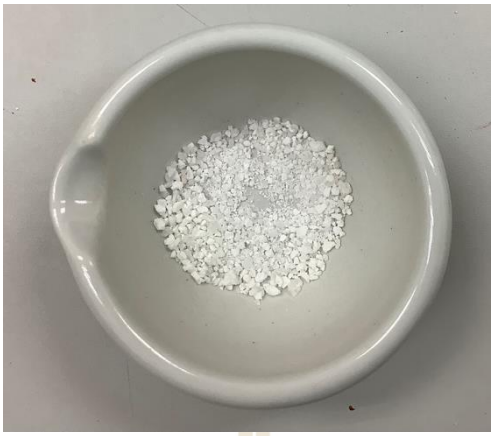


Figure 3.1 Preparation of modified cassava starch.

Second, 6% of hydrolyzed starch powder from physical treatment was mixed with 2.5 mM of cordyceps medium while continuously stirring for 30 min. The solution was added dropwise with ethanol at a ratio of 1:1.5 into a starch solution and stirred at room temperature for 24 h. After incubation, the encapsulated nanoparticles were collected by centrifugation at 10,000 rpm for 5 minutes.

Figure 3.2 Preparation of encapsulation process.

Finally, 0.1% of CMP were dissolved by 10 ml of DI water and sonicated by Ultrasonicator at 40% amplitude for 10 min (pulse on 5 minutes and pulse off 10 minutes) to disperse and reduce the size of nanoparticles. The aqueous suspension of CMP was collected at room temperature for future use.

(A) (B)

Figure 3.3 (A) Before encapsulation process particles is crushed with a mortar and (B) After encapsulation process particles are crushed with a mortar.

3.2.2 Characterization of CMP

CMP were prepared followed by the previously described. After ultrasonication, the concentration of cordycepin was determined by high performance liquid chromatography (HPLC). The calibration curves were created with standard cordycepin. Encapsulation efficiency (EE) and loading efficiency (LE) were calculated using the following equations, respectively:

$$EE\% = \frac{\text{mass of Cordycepin– loaded nanoparticles (post– concentration)}}{\text{Total mass Cordycepin added (post–concentration)}} \times 100 \quad (1)$$

$$LE\% = \frac{\text{mass of Cordycepin– loaded nanoparticles}}{\text{Total mass of particles (mass of cordycepin and cassava starch)}} \times 100 \quad (2)$$

3.2.3 Cytotoxicity assays

MTT cell survival assay was used to determine the effect of cordycepin (C), cordyceps medium extract (CM), CMP, and hydrogen hydroxide on cell viability. The human dermal fibroblast cell line maintained in DMEM, supplemented with 10% FBS and 1% penicillin-streptomycin and 1% L-glutamine. The fibroblast cells were seeded in 96-well plates at a density of 1.5×10^4 cells/well and incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24 h. These cells were treated with a varying concentration of C, CM and CMP or hydrogen peroxide (H_2O_2) for 24 h. The MTT solution were added to each well at the final concentration of 0.25 mg/ml before further incubation for 4 h at 37°C in the darkroom. The formazan crystal was solubilized in DMSO. The absorbance was measured at 570 nm by using a microplate reader (BMG Labtech, Ortenberg, Germany). The 50% inhibitory concentration (IC50) was obtained from the dose-response curve of percent viability (Y) versus concentration assessed (X) and calculated with a linear regression performed using Microsoft Excel.

3.2.4 Scavenging the Hydrogen peroxide – oxidative stress on human dermal fibroblast cells

The dermal fibroblast cells were seeded in 96-well plates at a density of 1.5×10^4 cells/well and incubated for 24 h. These cells were separated into normal condition and oxidative stress condition. For normal condition, the cells were treated with the optimize concentration of cordycepin standard, CM and CMP (1 μM, 1 μM and 0.1 μM, respectively) for 24 h while oxidative stress condition, the cells were pre-treated with 1 mM of H_2O_2 and post-treated with the optimize concentration of cordycepin standard, CM and CMP (1 μM, 1 μM and 0.1 μM, respectively) for 24 h. The cell viability was determined by MTT assay and compared with the control cells as 100% viability.

3.2.5 RNA Isolation and Reverse Transcription (RT-PCR)

Gene expression was examined by RT-PCR. Human dermal fibroblast cells were cultured and transferred into the 6-well plates at 80% confluence. In normal condition, these cells were treated with C, CM and CMP (1 μM, 1 μM and 0.1 μM, respectively) for 24 h. While pre-treated with H_2O_2 and replace with C, CM and CMP

(1 µM, 1 µM and 0.1 µM, respectively) in oxidative stress condition. After incubation, total RNA was extracted with Macherey-Nagel Kit (Macherey- Nagel, Düeren Germany). Total RNA was then converted into cDNA by ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO CO., LTD., Japan). The PCR was conducted in a BioRad/T100 Thermal cycler (BioRad, CA, USA) with specific primers, and amplified cDNA products were identified by electrophoresis using a 1.5% agarose gel. The gel was visualized using Red safe staining and gel documentation. Normalization of the relative expression level of a target gene with the *GAPDH* gene as an internal control was used to determine the relative expression level of a target gene. The expression of the type I collagen genes will be performed by PCR (BioRad, USA) will be used the primer shown in Table 3.1.

3.2.6 Immunofluorescence assay

Human dermal fibroblast cells were cultured and in 24-well plates upper sterilized cover slip at 80% confluence. After cell treatment, the treated cells were fixed with 4% paraformaldehyde for 30 minutes at room temperature. Then, the cells were washed with PBS twice and permeabilized with 3% bovine serum albumin (BSA) in 0.1% triton-X 100 of PBS for 20 minutes at 4 °C. After 20 minutes, the cells were incubated with primary antibody (1:500 of Anti-collagen type 1 Rabbit pAb, EMD Millipore Corp., USA). All incubations were performed overnight, and then the samples were incubated with the secondary antibody (Alexa Flouor™ 488 goat, Life technologies corporation, USA) (1:1,000) for 30 minutes. Nuclei were stained with DAPI and observed under the fluorescence microscope (ZOE™ fluorescence cell imager, BioRad, USA).

Table 3.1 Primer used in the experiment.

Gene	Primer	Sequence (5'-3')
Collagen and Elastin synthesis	*Collagen1A1* (Forward)	CTGGATGGATTGAAGGGACA
	Collagen1A1 (Reverse)	CAACACGTCCTCTCTCACC
	KTR18 (Forward)	CAGCAGCAGCTTCAGTACCAGTGTCTA
	KTR18 (Reverse)	GGTGTAGGTATCATAACTCCGCCCATT
	Elastin (Forward)	CCGCTAAGGCAGCCAAGTATGGA
	Elastin (Reverse)	AGCTCCAACCCGTAAGTAGGAAT
	Vimentin (Forward)	CCAGATGCGTGAAATGGAAG
	Vimentin (Reverse)	TGAGTGGGTATCAACCAGAG
Antioxidant	*SOD* (Forward)	CTAGCGAGTTATGGCGAC
	SOD (Reverse)	CATTGCCCAAGTCTCCAAC
	GPX (Forward)	CGCCAAGAACGAAGAGATTC
	GPX (Reverse)	CAACATCGTTGCGACACAC
	Catalase (Forward)	TCCGGGATCTTTTTAACGCCATTG
	Catalase (Reverse)	TCGAGCACGGTAGGGACAGTTCAC
Autophagy	*ATG5* (Forward)	TGGCTGAGTGAACATCTGA
	ATG5 (Reverse)	AAGTAAGACCAGCCCAGTT
	ATG12 (Forward)	GAGACACTCCCATAATGAA
	ATG12 (Reverse)	GTAGGACCAGTTTACCATC
	P62 (Forward)	GGTGCAAGAAGCCATTTAGG
	P62 (Reverse)	GCCATTAGGCAAGCTATGTG
House Kipping Gene	*GAPDH* (Forward)	ACCTGACCTGCCGTCTAGAA
	GAPDH (Reverse)	GCTCAGGGGCCTTTGGACATCTCTT

3.2.7 Estimation of ROS generation

ROS were measured with the non-fluorescent probe 2′,7′-dichlorofluorescein diacetate (DCFH-DA). DCFH-DA passively spreads into cells and is deacetylated by esterases to form nonfluorescent 2′,7′-dichlorofluorescein (DCFH). DCFH reacts with ROS to form the fluorescent product DCF, which is trapped inside the cells. To appraise intracellular ROS levels of C, CM and CMP in normal and oxidative stress condition. After cell treatment, the treated cells were incubated with DCFH-DA, an indicator of general oxidative stress, for 30 min at 37 °C under 5% CO_2. Consequently, the cells were washed two times with PBS. The fluorescence intensity was excited and determined by using fluorescence microplate reader.

3.2.8 Monodansylcadaverine (MDC) assay

The 24 well plate cells at 80% of confluence in 400 µl of culture media (DMEM containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 g/ml streptomycin) on a 24-well tissue-culture dish containing a cover glass for each well. To appraise MDC levels in normal and oxidative stress condition. After cell treatment, the treated cells were incubated with Monodansylcadaverine (MDC), a fluorescent compound for the identification of autophagic vesicles, for 24 h at 37 °C in an atmosphere of 95% air and 5% CO_2. The cells were wash 3 times with PBS to remove the media and add 400 µl of paraformaldehyde (PFA; Sigma) to fix the cells, Incubate the cells for 2 h at 37 °C. Subsequently, the cells were washed 3 times with PBS before incubated with 0.05 mM of MDC at 37 °C for 30 min. The cells were determined under fluorescence microscopy (MDC has an autofluorescence at 365 and 525 nm wavelength, for excitation and emission, respectively).

3.2.9 Wound healing assay

The 6 well plate cells at 80% of confluence in 2,000 µl of culture media [DMEM containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 g/ml streptomycin] on a 6-well plate. To appraise wound healing in normal and oxidative stress condition. Use pipet tip 10 µl scrape surface area 6 well plate into a straight line and then removed medium. The treated cells were incubated for 24 h at 37 °C in an atmosphere of 95% air and 5% CO_2. The cells were determined under light microscopy at the incubation time (0, 12, 24, 48 h).

3.2.10 Cellular uptake

The 6 well plate cells at 80% of confluence in 2,000 µl of culture media (DMEM containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 g/ml streptomycin) on a 6-well plate. To appraise wound healing in normal and oxidative stress condition. Kept the supernatant at the incubation time (0, 15, 30, 60, 120, 240, and 480 min). The treated cells were incubated for 24 h at 37 ℃ in an atmosphere of 95% air and 5% CO_2. The cells were determined under HPLC.

3.2.11 BCA protein assay

The 6 well plate cells at 80% of confluence in 2,000 µl of culture media (DMEM containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 g/ml streptomycin) on a 6-well plate. To appraise wound healing in normal and oxidative stress condition. Kept the supernatant at the incubation time (0, 15, 30, 60, 120, 240, and 480 min). The treated cells were incubated for 24 h at 37 ℃ in an atmosphere of 95% air and 5% CO_2. The cells were determined by BCA protein assay (Pierce™, Thermo scientific, USA).

3.2.12 Statistical analysis of data

Each experiment was observed in Triplicate. The data were presented as mean ± SD and Statistical analysis was performed using SPSS (version 26.0, SPSS Inc., USA). Significant differences between the treatment and control were determined by one-way ANOVA analysis, followed by student T-tests, and *$*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. control cells. Significance vs. hydrogen peroxide control cells was indicated as follows: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.$*001.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Results

4.1.1 Characterization of C, CM, and CMP

To characterize these nanoparticles, we evaluated the solution of C, CM and CMP was determined by using Malvern/Zetasizer-zs machine, result shows, The average peak particle size of C was 0.4 and 125.2 nm. The CM was 1.4 and 275.1 nm. The CMP was 89.3, and 279.7 nm (Figure.1A). In this study, the nanoparticle size ranges between 0.1-300 nm of C, CM, and CMP. The percentage of yield, percentage encapsulation efficiency (EE), percentage loading efficiency (LE). The optimized conditions for creating encapsulated nanosuspensions of C, CM, and CMP. Hydrolysis of cassava starch (CS) with percentage yields of 64.70. Encapsulation by using hydrolyzed cassava (HC) with percentage yields of 79.67, percentage EE were 50.29 and percentage LE were 8.09.

Table 4.1 Characterization of CMP.

Method	% Yield	%Encapsulation efficiency (EE)	%Loading efficiency (LE)	Average particles size (nm)
C	-	-	-	0.4-125.2
CM	-	-	-	1.4-275.1
CMP (Kaokaen P, 2020)	50.90	17.63	3.46	116.67 ± 86.22
Improve CMP (This study)	79.67	50.29	8.09	184.5 ± 95.2

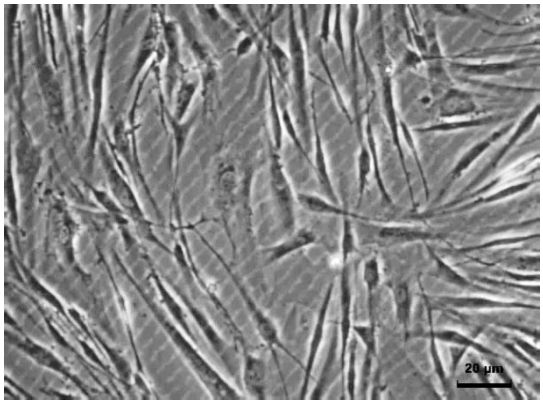


Figure 4.1 Human dermal fibroblasts show the spindle shaped.

4.1.2 Cytotoxicity and cell viability of C, CM, and CMP in HDF cells

To evaluate the toxicity effects of C, CM, and CMP. HDF cells were treated for 24h, and cell viability was determined by using MTT assay. Results showed that C, CM can stimulate the proliferation of HDF cells at concentrations of 1 µM, while CMP can stimulate the proliferation of HDF cells at a concentration 0.1 µM. The half-maximal inhibitory concentration (IC50) of C, CM, and CMP on HDF cells was 1,439, 519, and 106 µM, respectively. (Figure.4.2). Thus, these results demonstrate low concentrations of CMP enhanced efficiencies of the proliferation of HDF cells when compared with C and CM treatment.

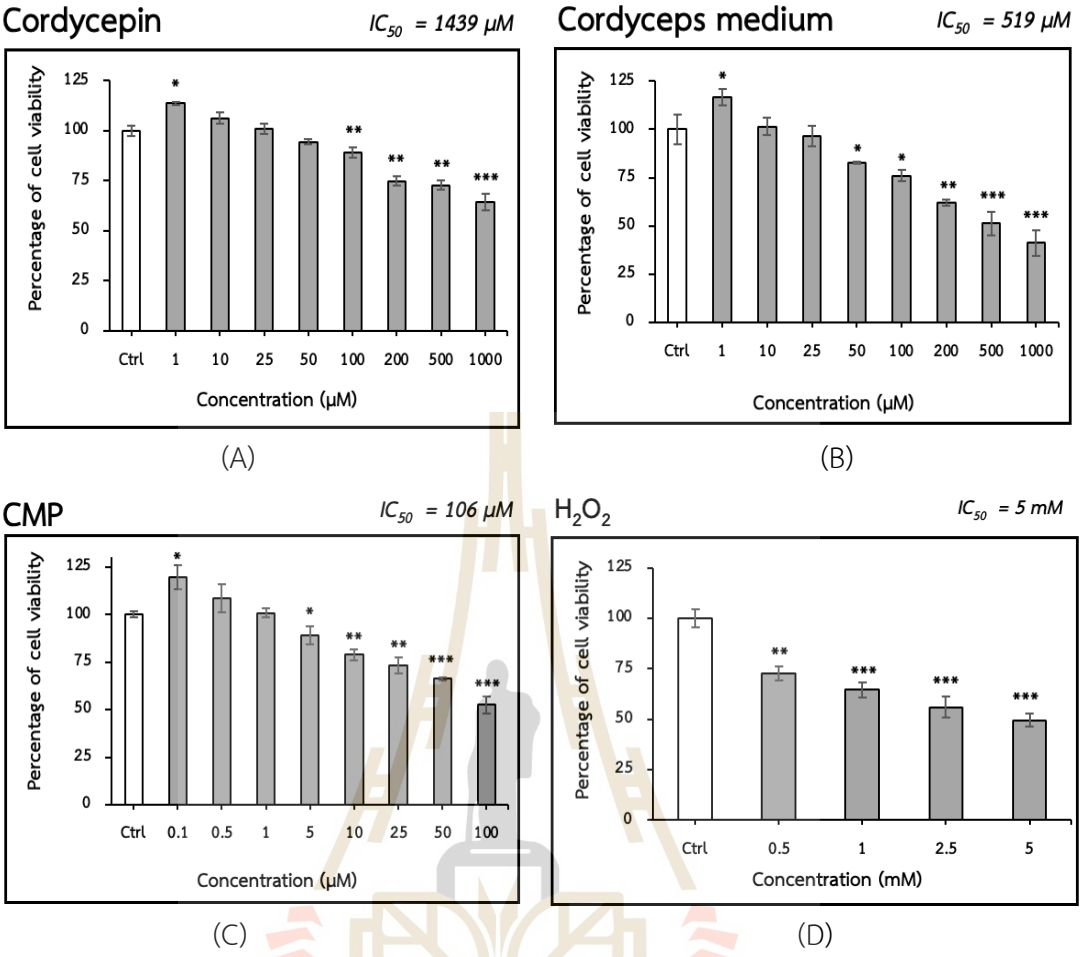
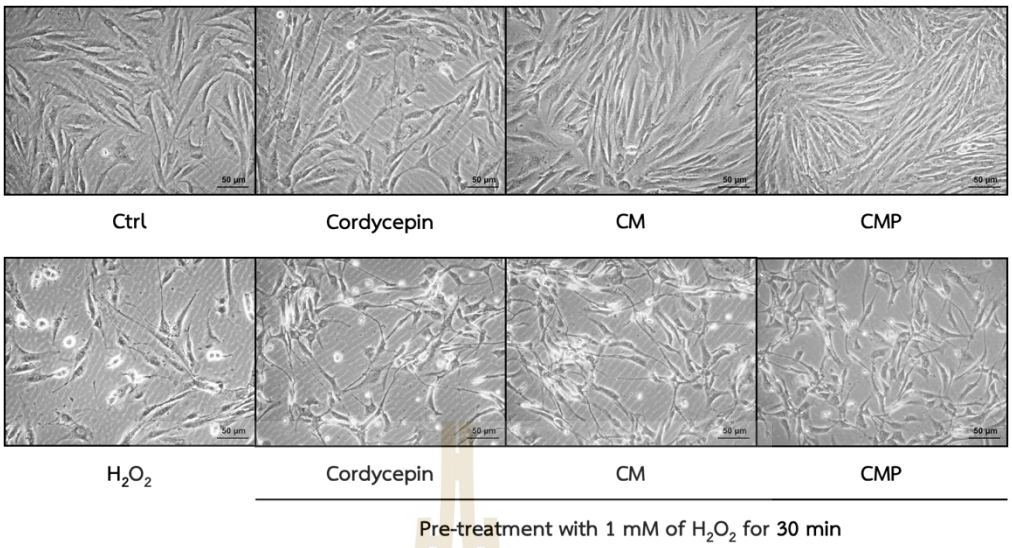


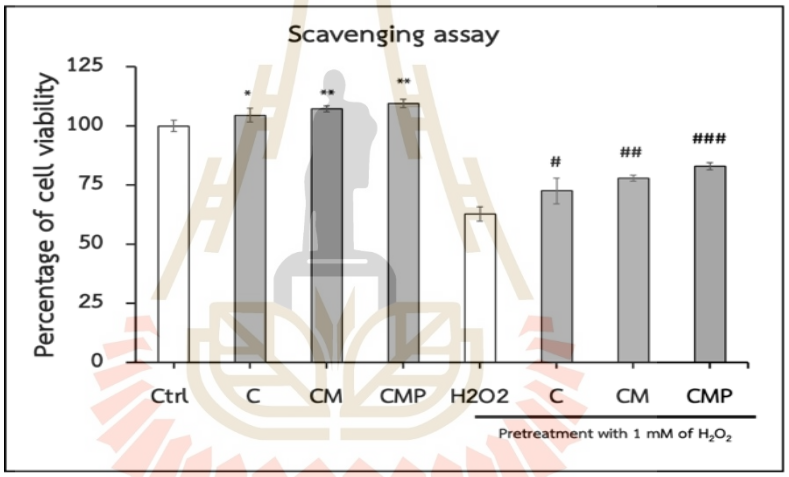
Figure 4.2 Cell viability and cytotoxic testing. The HDF cells were treated with C (A), CM (B), and CMP (D) for 24 hours, and then, treated with H_2O_2 for 30 min (D). The treated cells were determined by MTT assay. Data were presented as mean ± SD (n = 3) **p < 0.05, **p < 0.01, and ***p < 0.001 vs. control cells.*

4.1.3 The scavenging of Hydrogen peroxide – oxidative stress on HDF cells

To determine the effects of hydrogen peroxide concentration to assess the damage caused by oxidative stress and evaluate the scavenging effect of C, CM and CMP (Figure 4.3). From the result, we found that CM and CMP can increase the cell proliferation of HDF at 1 μM and 0.1 μM, respectively. In stress condition, we found that 1 mM of hydrogen peroxide treatment can cause damage and oxidative stress in HDF cell death 37 % while the percentage of cell viability post-treatment before hydrogen peroxide with C, CM and CMP were 73, 78, and 83, respectively.



(A)



(B)

Figure 4.3 Scavenging assay. The morphology of HDF cells after treated with the conditions (A). The data of scavenging assay (B). The HDF cells were treated with C, CM, and CMP for 24 hours and pre-treated with H_2O_2 for 30 min. The treated cells were determined by MTT assay. Data were presented as mean ± SD (n = 3) *$p < 0.05$, **$p < 0.01$ vs. control cells. Significance vs. hydrogen peroxide control cells was indicated as follows: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$.

4.1.4 Gene expression of Collagen-, Elastin- synthesis, and Protein expression of Collagen Type 1 on HDF cells

4.1.4.1 Gene expression of Collagen-, Elastin- synthesis of on HDF cells

To determine the gene expression of collagen related genes on HDF treatment with C, CM, and CMP in normal and oxidative stress condition. The results show that the treatment with C, CM, and CMP in normal condition the expression of collagen- and elastin- synthesis including *Collagen type 1 (Col1A1), Elastin, Keratin 18 (KTR18),* and *Vimentin.* These genes were assessed by RT-PCR. As shown in Figure 4.4, the expression of *Collagen type 1 (Col1A1)* of C, CM, and CMP were significantly increased when compare the control group (untreated) at 1.85, 2.10, and 2.44-fold. *Elastin* was 2.10, 2.20, and 6.67-fold. *KTR18* was 1.95, 4.37, and 5.53-fold. *Vimentin* was 0.88, 0.96, and 0.94-fold, respectively. In the oxidative stress condition, the expression of *Collagen type 1 (Col1A1)* of C, CM, and CMP was significantly increased when compare the control H_2O_2 were 1.16, 1.57, and 1.91-fold. *Elastin* was 2.43, 2.58, and 3.79-fold. *Keratin 18 (KTR18)* were 3.26, 3.82, and 3.91-fold. *Vimentin* were 0.88, 0.96, and 0.94-fold, respectively.

4.1.4.2 Protein expression of Collagen Type 1 on HDF cells

To determine the production of Collagen Type 1 on HDF cells. HDF cells was evaluated by Immunofluorescence staining (Figure 4.5). In normal condition, the results showed that the expression of collagen in C, CM, and CMP groups was 2.45, 9.06, and 10.98-fold, respectively. which were significantly increased compared with the control group (Figure. 2C). In oxidative stress condition, the results showed that the expression of collagen in C, CM, and CMP groups was 1.45, 5.78, and 8.41-fold, respectively when compare the control H_2O_2.

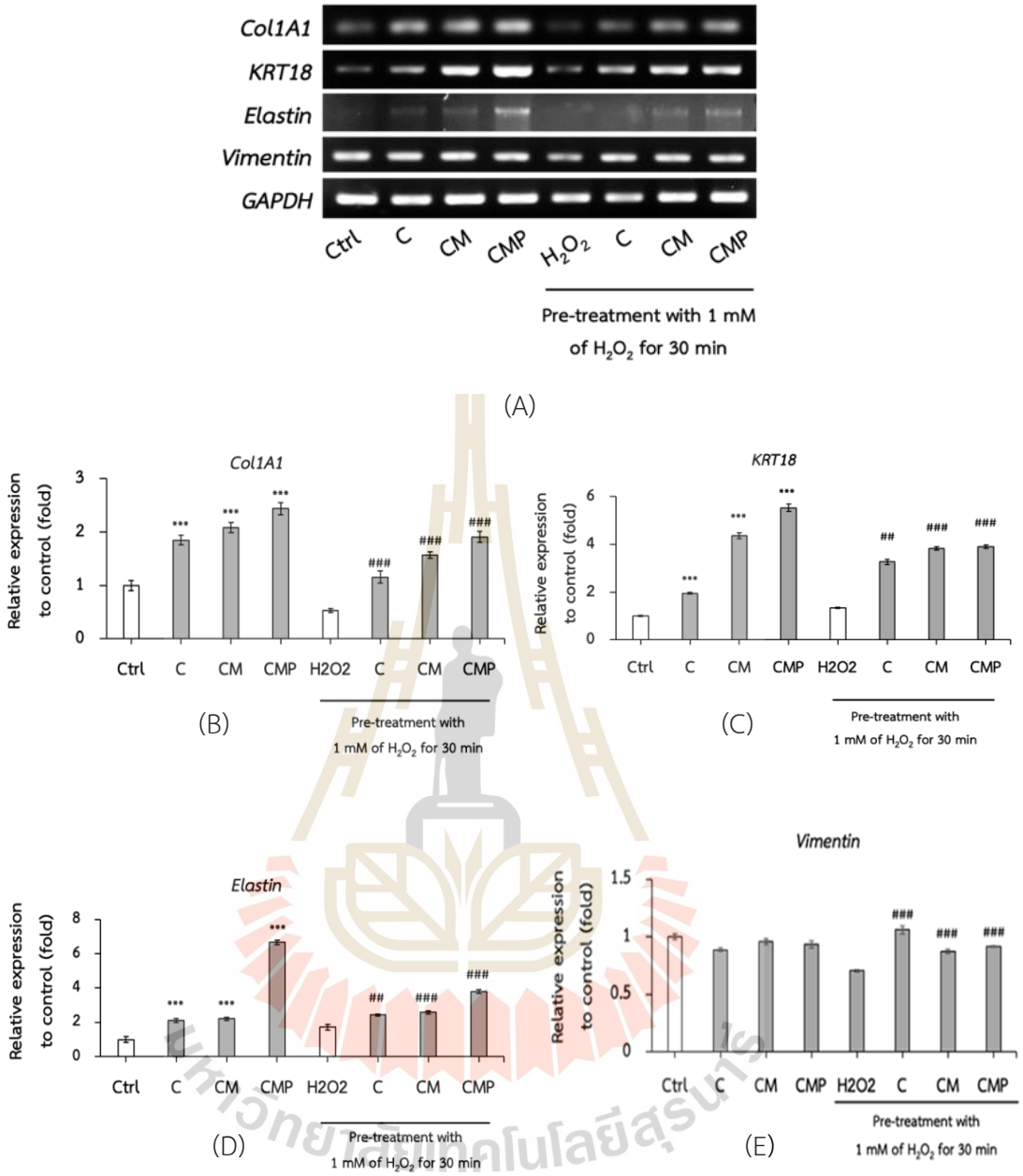


Figure 4.4 Gene expression of Collagen-, Elastin synthesis on HDF cells. The gel-based PCR (A). The expression of *Col1A1* (B)*, KRT18* (C)*, Elastin* (D) *and Vimentin* (E) of HDF cells. The mRNA expression levels of genes relative to controls (fold) after HDF cells were treated with a concentration (1 μM, 1 μM and 0.1 μM) of C, CM and CMP, respectively. Significance vs. control cells was indicated as follows: *$*p < 0.05$, $**p < 0.01$, and $***p < 0.001$* vs. control cells. Significance vs. hydrogen peroxide control cells was indicated as follows: *$^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$.*

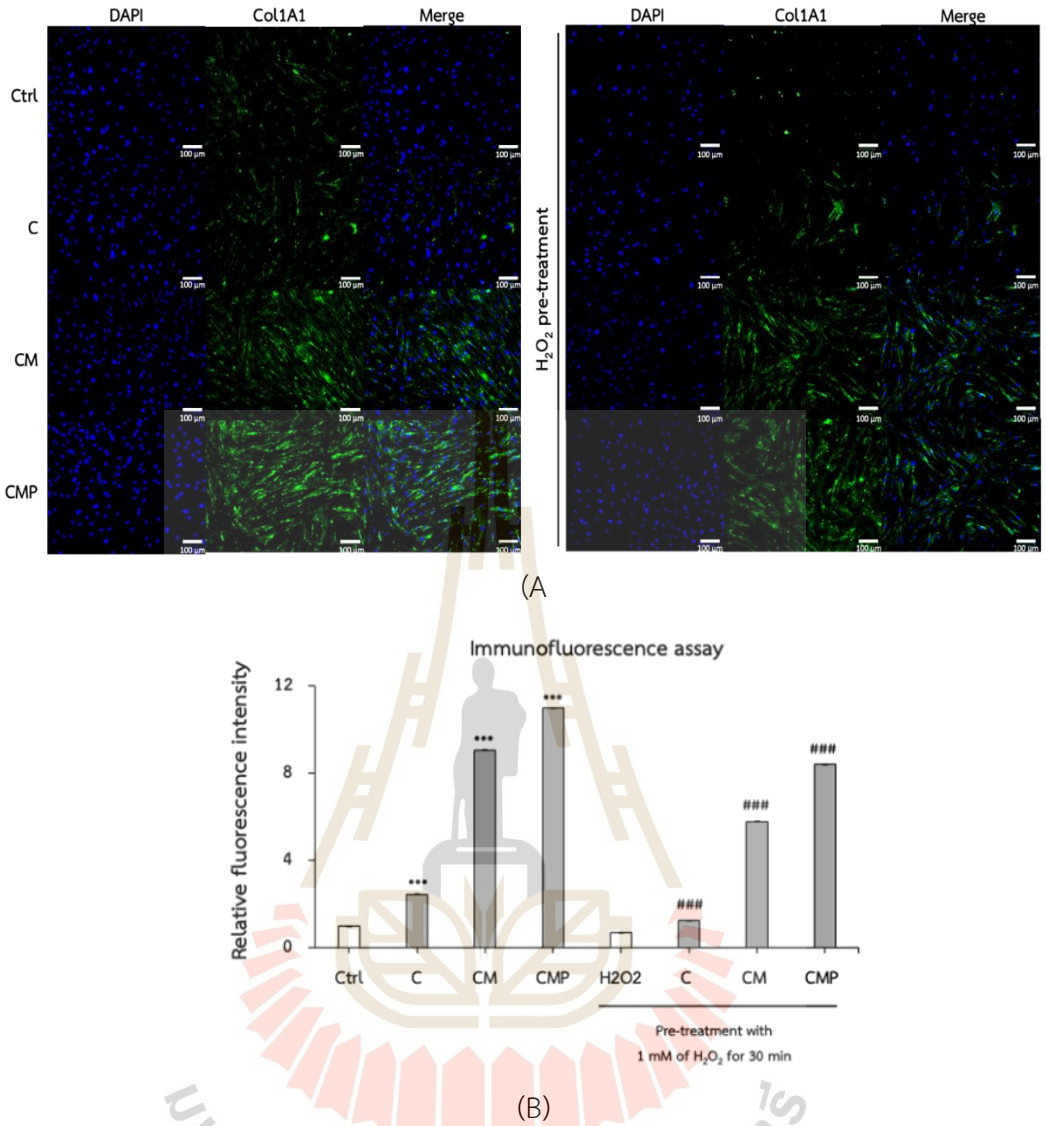


Figure 4.5 Protein expression of Collagen Type 1 on HDF cells. Expression of COL1A1 protein under fluorescent microscope (A). The mRNA expression levels of genes relative to controls (fold) after HDF cells were treated with a concentration (1 μM, 1 μM and 0.1 μM) of C, CM and CMP, respectively (B). Significance vs. control cells was indicated as follows: *$*p < 0.05$, $**p < 0.01$,* and *$***p < 0.001$* vs. control cells. Significance vs. hydrogen peroxide control cells was indicated as follows: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, *and* $^{\#\#\#}p < 0.001$.

4.1.5 Gene expression of antioxidant, and Protein expression of antioxidant on HDF cells

4.1.5.1 Gene expression of antioxidant on HDF cells

To determine the gene expression of antioxidant related genes (*Catalase, SOD,* and *GPX*) in normal and oxidative stress condition. These genes were assessed by RT-PCR. As shown in Figure. 4.6, the results show that the expression of *Catalase* in cell treatment with C, CM, and CMP were significantly increased 3.22, 3.84, and 3.50-fold when compared the control group. The expression of *SOD* was 2.51, 4.08, and 5.16-fold. *GPX* was 1.10, 1.93, and 2.09-fold, respectively. In oxidative stress condition, the expression of *Catalase* of C, CM, and CMP treatment was significantly increased 3.16, 3.94, and 4.58-fold. *SOD* was 3.19, 4.80, and 5.35-fold. *GPX* was 1.39, 1.59, and 2.25-fold, respectively.

4.1.5.2 Protein expression of antioxidant on HDF cells

To determine the ROS formation on HDF cells. The treated cells were evaluated by DCFH-DA assay in normal condition and pretreated with hydrogen peroxide, which induced intracellular oxidative stress in HDF cells (Figures. 4.7). The result showed that in normal condition the ROS formation levels of CM, and CMP treatment were significantly decreased when compare the control group were 0.99, 0.96, and 0.94-fold, respectively. In oxidative stress condition, the results showed that the ROS formation levels of C, CM, and CMP treatment were significantly decreased were 0.4, 0.5, and 0.6-fold, respectively when compare the control H_2O_2.

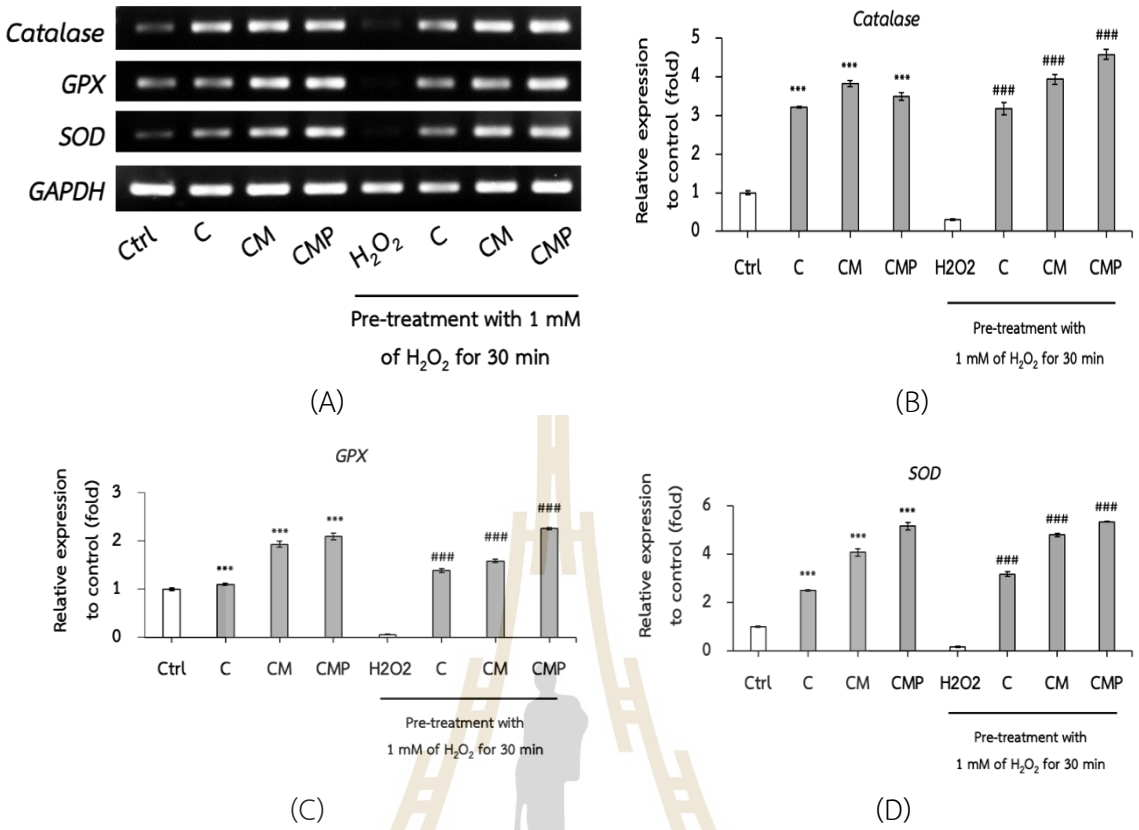


Figure 4.6 Gene expression of Antioxidant on HDF cells. The gel-based PCR (A). Expression of *Catalase* (B), *GPX* (C), *and SOD* (D) of HDF cells. The mRNA expression levels of genes relative to controls (fold) after HDF cells were treated with a concentration (1, 1 μM, and 0.1 μM) of C, CM and CMP, respectively. Significance vs. control cells was indicated as follows: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. Significance vs. hydrogen peroxide control cells was indicated as follows: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$.

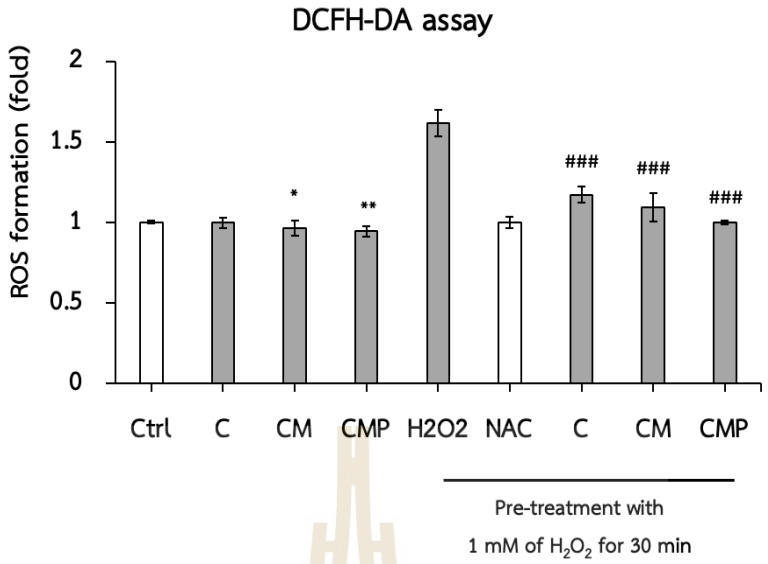


Figure 4.7 Protein expression of Antioxidant on HDF cells. The mRNA expression levels of genes relative to controls (fold) after HDF cells were treated with a concentration (1 μM, 1 μM and 0.1 μM) of C, CM and CMP, respectively. Significance vs. control cells was indicated as follows: *$*p < 0.05$, **$p < 0.01$,* and *$***p < 0.001$*. Significance vs. hydrogen peroxide control cells was indicated as follows: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, *and* $^{\#\#\#}p < 0.001$.

4.1.6 Expression of autophagy-related genes and Autophagic vacuoles formation on HDF cells

4.1.6.1 Expression of autophagy-related genes on HDF cells

To determine the gene expression of autophagy-related genes (*P62*, *ATG5* and *ATG12*) in normal and oxidative stress condition. These genes were assessed by RT-PCR as shown in Figures. 4.8. The results show that the *ATG12* expression of C, CM, and CMP treatment were 1.19, 1.16, and 1.44-fold, respectively. *ATG5* expression were 1.18, 1.63, and 1.90-fold, respectively. *P62* expression in C and CM treatment were decrease significant while CMP treatment were increase significant 1.40-fold when compared the control group. In the oxidative stress condition when compare the control H_2O_2, *ATG12* expression of C, CM, and CMP treatment were increase significant as 0.36, 0.76, and 0.76-fold, respectively. *ATG5* expression of C and CM were increase significant 1.67, and 1.66-fold. *P62* expression were increase significant 0.74, 1.01, and 1.00-fold.

4.1.6.2 Autophagic vacuoles formation on HDF cells

To determine the effect of autophagic vacuoles formation in HDF cells. The treated cells were evaluated by MDC assay (Figures. 4.9). In normal condition, the results showed that the relative fluorescence intensity of C, CM, and CMP groups was increase significant 1.48, 1.88, and 1.91-fold, respectively. when compare the control group (Figure. 4C). In oxidative stress, the results showed that the relative fluorescence intensity of C, CM, and CMP groups was increase significant 1.16, 1.87, and 1.93-fold, respectively.

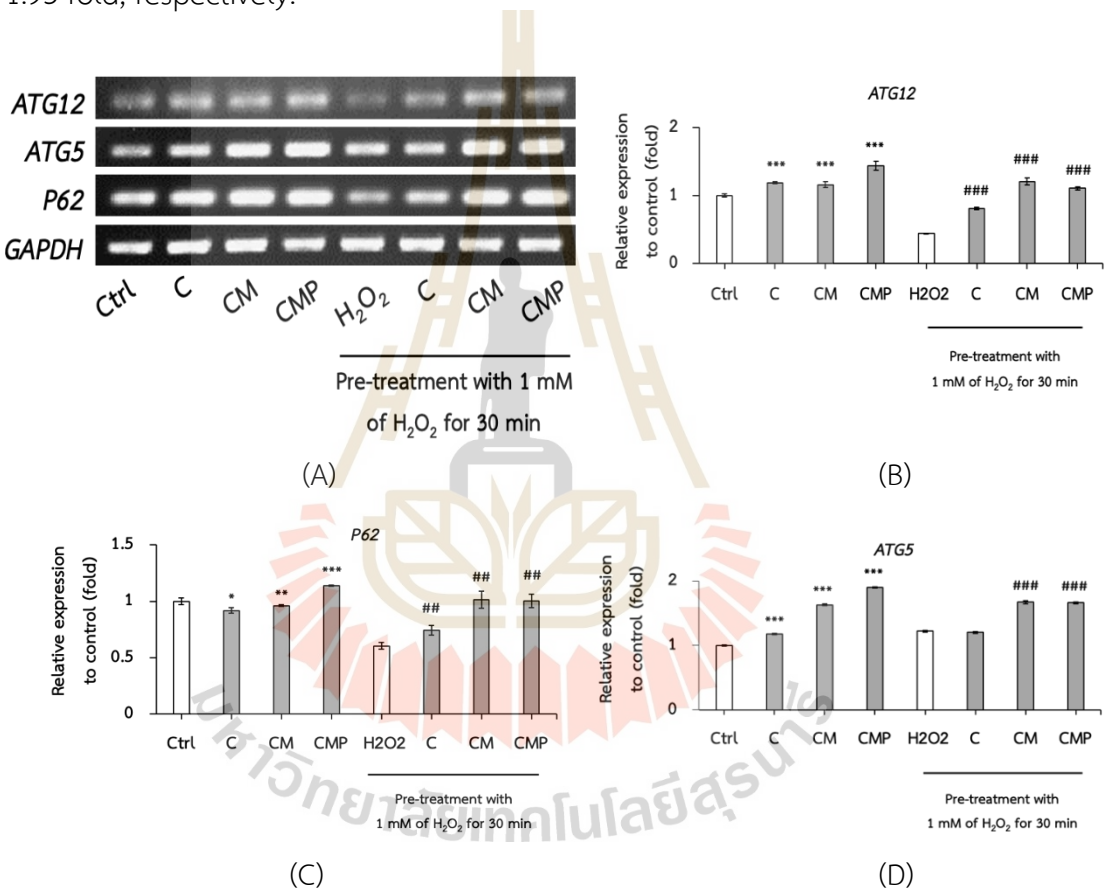


Figure 4.8 Gene expression of Autophagy on HDF cells. The gel-based PCR (A). Expression of *ATG12* (B)*, P62* (C)*, and ATG5* (D) of HDF cells. The mRNA expression levels of genes relative to controls (fold) after HDF cells were treated with a concentration (1 μM, 1 μM and 0.1 μM) of C, CM and CMP, respectively. Significance vs. control cells was indicated as follows: *$*p < 0.05$, $**p < 0.01$*, and *$***p < 0.001$*. Significance vs. hydrogen peroxide control cells was indicated as follows: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, *and* $^{\#\#\#}p < 0.001$.

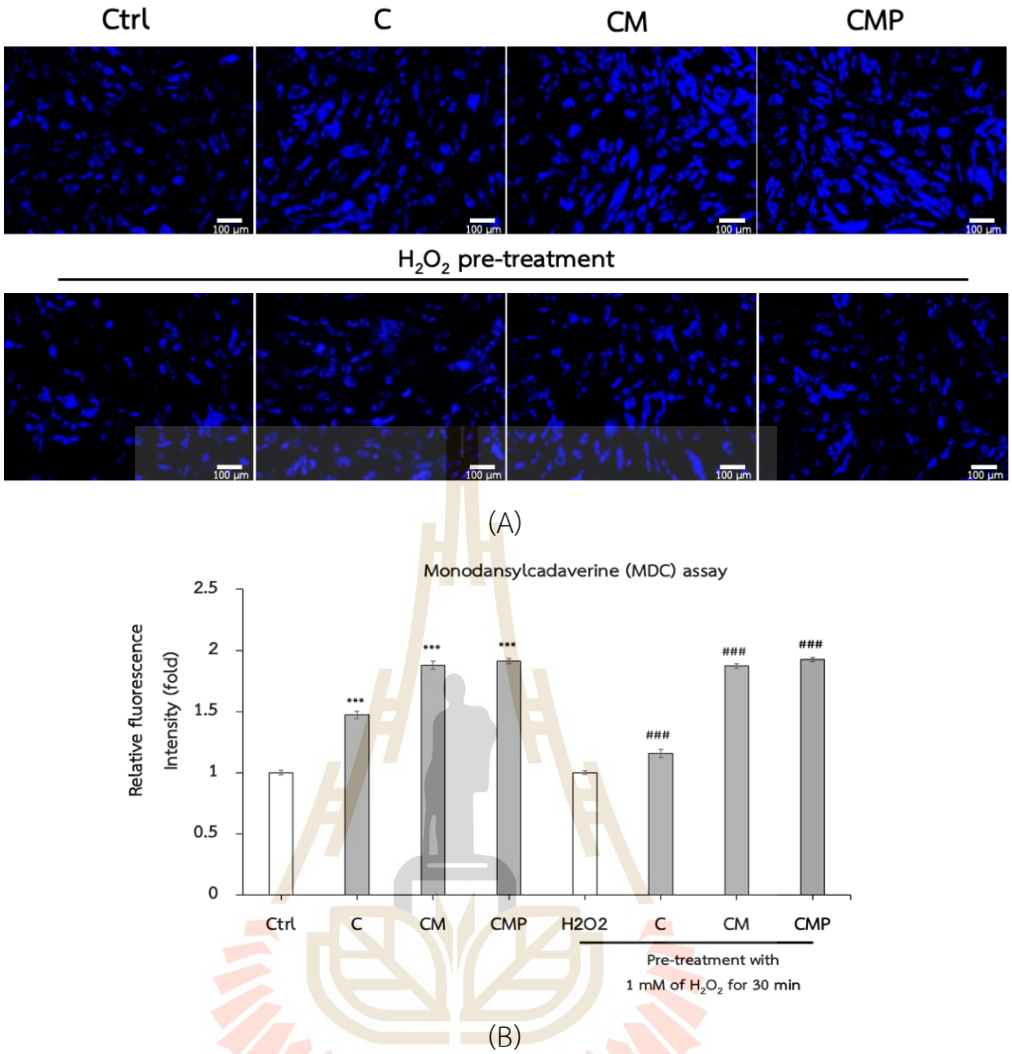


Figure 4.9 Protein expression of Autophagy on HDF cells. Expression of autophagic vacuole formation under fluorescent microscope (A). The mRNA expression levels of genes relative to controls (fold) after HDF cells were treated with a concentration (1 µM, 1 µM and 0.1 µM) of C, CM and CMP, respectively (B). Significance vs. control cells was indicated as follows: *$*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. Significance vs. hydrogen peroxide control cells was indicated as follows: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$.*

4.1.7 Efficiency of the Absorption level, secretory protein level, and wound healing on HDF cells

4.1.7.1 Efficiency of the Absorption level on HDF cells

To determine the effect of the absorption level, secretory protein level, and wound healing on HDF cells. The cordycepin absorption level in the suspension medium was evaluated by HPLC (Figure. 4.10). The normal condition results showed, the concentration of cordycepin at incubation times (0, 15, 30, 60, 120, 240, and 480 min.) of C was 5.52, 5.35, 5.13, 4.29, 3.79, 2.98, and 2.64 μM, respectively. The CM was 5.50, 4.29, 3.44, 1.34, 0.82, 0.35, and 0.24 μM, respectively. The CMP was 5.47, 4.11, 2.29, 1.17, 0.81, 0.29, and 0.19 μM, respectively.

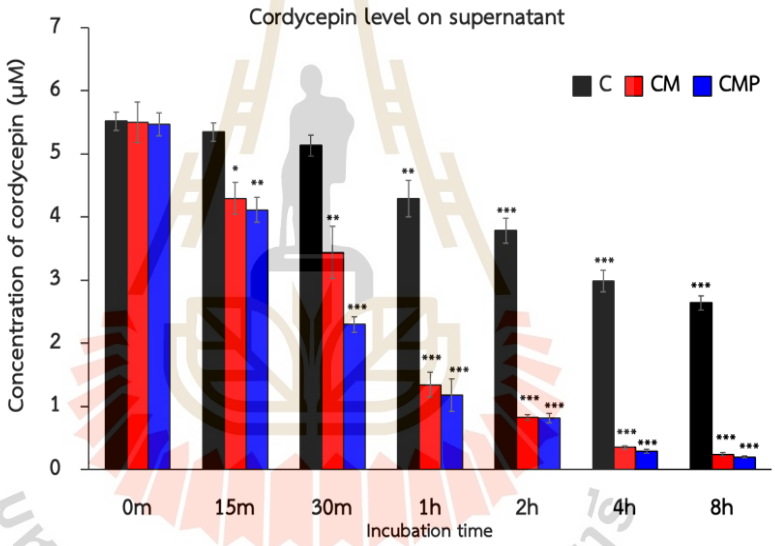


Figure 4.10 Efficiency of the Absorption level. The absorption level of relative to controls (μM) after HDF cells were treated with a concentration (1 μM, 1 μM and 0.1 μM) of C, CM and CMP, respectively. Significance vs. control cells was indicated as follows: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

4.1.7.2 Efficiency of secretory protein level on HDF cells

To determine the effect of secretory protein level of HDF treatment by BCA assay. After incubation time of C, CM and CMP, the supernatant was collected and evaluated the protein concentration. The results showed, the concentration of secretory protein level at times (15, 30, 60, 120, 240, and 480 min.) of C was 15.5, 41.9, 71.9, 81.8, 89.6 and 108.5 µg/ml, respectively. The CM was 44.4, 49.6, 77.0, 93.3, 98.5 and 117 µg/ml, respectively. The CMP was 97, 101, 100.1, 111.2, 115.5 and 129.3 µg/ml, respectively.

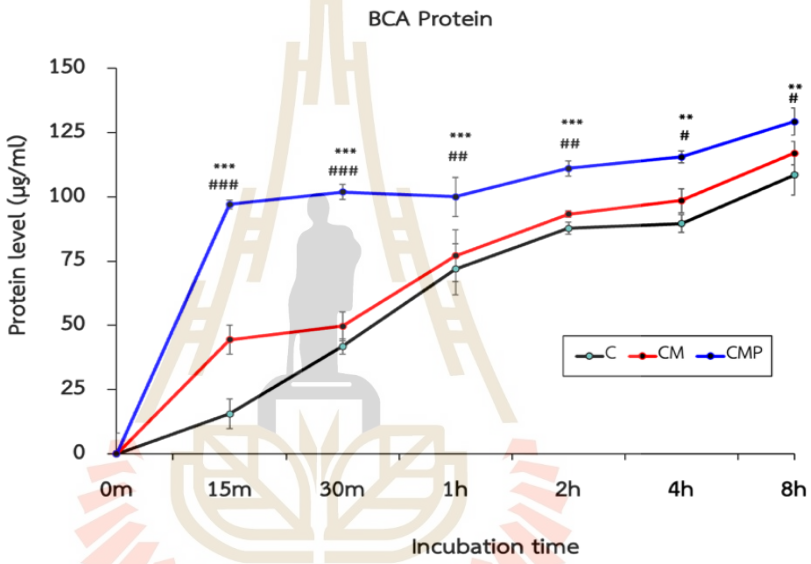


Figure 4.11 The efficiency of secretory protein level on HDF cells. HDF cells were pre-scattered before treated with C, CM and CMP for 12, 24 and 48 hours. The gap area was determined by ImageJ program. Values were expressed as mean ± SD (n = 3). Significance vs. the C condition was indicated as follows: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. Significance vs. the CM condition was indicated as follows: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$.

4.1.7.3 Efficiency of wound healing on HDF cells

To determine the wound healing on HDF cells by scattering assay, in normal condition, HDF cell were scraped and treated with C, CM and CMP or challenge with H_2O_2 in oxidative stress condition. The gap area was analyzed by ImageJ program. The results showed, the gap area (μm^2) at times (0, 12, 24, and 48 hrs.) of untreated control was 197984, 173081, 163990, and 40348 μm^2, respectively. The gap area of C treatment was 194094, 161957, 161623, 126385 and 26965 μm^2, respectively. The gap area CM treatment was 194220,152030, 133688, 96799 and 19722 μm^2, respectively. The gap area CMP treatment was 197061, 149402, 137209, 66910 and 14563 μm^2, respectively. In oxidative stress condition at times (0, 24, and 48 hrs.) of untreated control was 199878, 197652 and 85450 μm^2. The gap area of C treatment was 199720, 184798 and 13985 μm^2, respectively. The gap area CM treatment was 199618, 151532 and 9074 μm^2, respectively. The gap area CMP treatment was 199842, 114597 and 5847 μm^2, respectively.

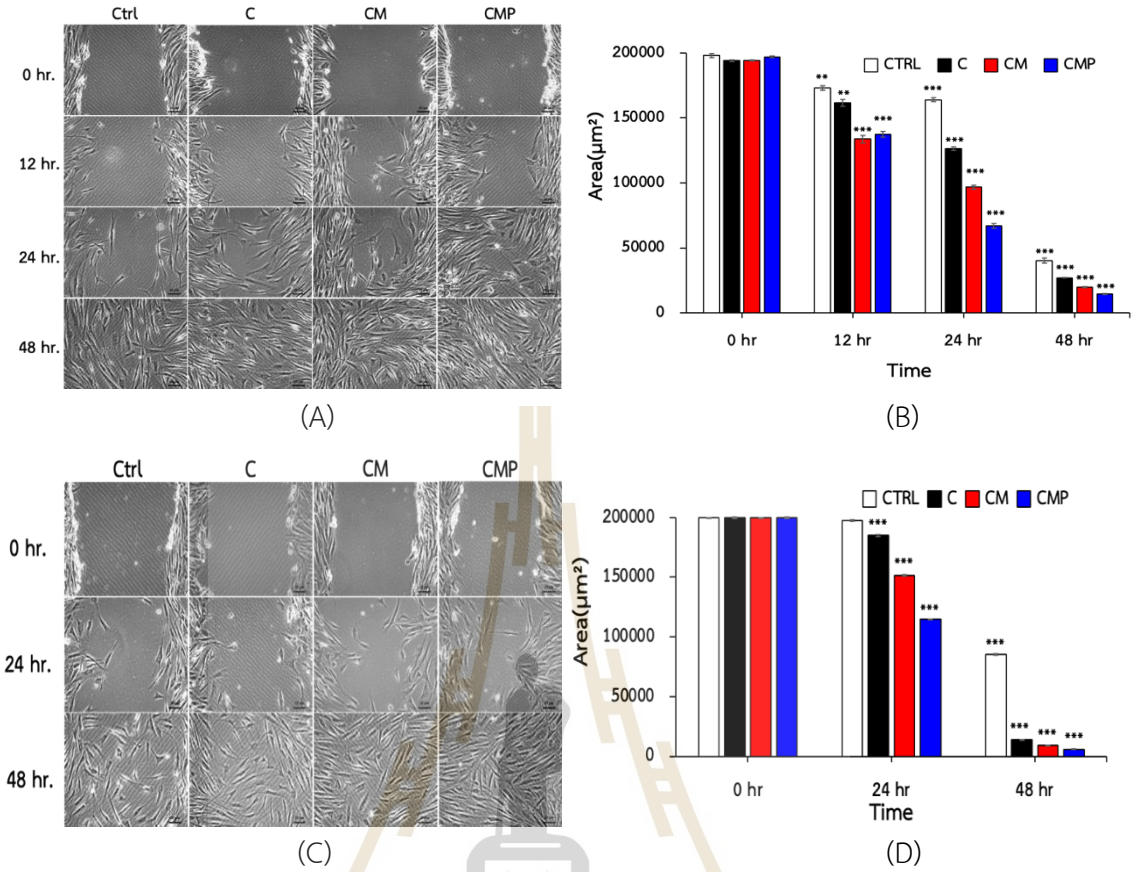


Figure 4.12 The efficiency of wound healing on HDF cells. Morphology of HDF cells in normal condition (A). The data of wound healing assay, The HDF cells were pre-scattered before treated with C, CM, and CMP for 0, 12, 24, and 48 hours (B). Morphology of HDF cells in normal condition (C). The data of wound healing assay, The HDF cells were pre-scattered before treated with C, CM, and CMP for 0, 24, and 48 hours (D). The gap area was determined by ImageJ program. Values were expressed as mean ± SD (n = 3). Significance vs. control cells was indicated as follows: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

4.2 Discussion

The develop CMP in this study, that improve from older protocol (Kaokaen, Jaiboonma et al. 2021). The protocol in this study could founded the optimum condition for produced the nanoparticles of cordyceps medium. The size of the encapsulated nanoparticles used in this study (size distribution ranging from 0.1-300 nm) was slightly larger than those of previous studies (Rajeswari, Moorthy et al. 2011, da Silva, Correia et al. 2017). Moreover, similar to Athira's 2018 study about the preparation and evaluation of a water soluble octenyl succinylated cassava starch-curcumin nano formulation found that the spherical morphology and a size range of 10–50 nm (Athira, Jyothi et al. 2018). However, Nano-encapsulated suspensions made from nanoparticles may be stored at room temperature for long periods of time without the risk of aggregation. Therefore, using the new and improved procedure the resulting nanoparticles are thus significantly more stable. It was also discovered that the material cloud effectively promoted cell proliferation when nanoparticles were introduced (also known as nano-suspension) to HDF cells. It is possible that the encapsulated extracts are more suitable for use in dry powder form, such as dietary supplements, due to their larger size than the nanoparticles. Before it is absorbed in the small intestine, cordycepin is less likely to be lost due to tapioca starch. Encapsulated nanoparticles are also suitable for use in solutions. It is the main ingredient in mouthwash. or as a drip to treat oral health problems including dry mouth (Silvestre, Minguez et al. 2009). Furthermore, the treatment of HDF cells with nanoparticles showed that the nanoparticles promoted the secretion of type I collagen protein within 24 h in two conditions. This is consistent with previous studies suggesting that the protein is important for cell proliferation and tissue remodeling effects (Price, Suk et al. 1975, Kim, Shin et al. 2021).

Evaluation of the cellular uptake of nanoparticles by HDF cells revealed that only a small number of encapsulated nanoparticles could enter to the cells (Herd, Daum et al. 2013). The oxidative stress was effective enough to cause cellular stress that induced cell death. As with the previous report, Multiple physiological functions including metabolism, protein translation, cell proliferation and survival Relying on AKT stimulation (Wei, Zhou et al. 2019). Cordycepin efficiently encourages cell proliferation, which is linked to the AKT phosphorylation pathway's mechanism of action. Additionally, a 2018 Zhen Yang Cui study discovered that cordycepin's anti-tumor

mechanisms, which control signaling pathways by blocking the CCL5-mediated Akt/NF-B signaling pathway, suggest that cordycepin may be an attractive therapeutic candidate for the treatment of cancer (Jang, Yang et al. 2019). NF-KB is a transcription factor with key roles in activating genes during inflammation and wound healing (Mitchell and Carmody 2018). Counterbalancing, higher levels of cordycepin will be needed to block the growth of other cancer cells since the toxicity of cordycepin is reduced by transforming the molecule into less harmful molecules. The encapsulation technique may change the structure of the chemical, since the encased nanoparticles perform better than the unencapsulated cordycepin. However, before the molecule is taken up by cells to perform, encapsulated nanoparticles are active or cassava starch nanoparticles can shield cordycepin in the environment (or supernatants that include adenosine deaminase) (Ijaz, Gilani et al. 2020).

The ability of cordycepin and encapsulation to protect HDF cells from ROS was evaluated using experimental methods to evaluate the effects of cordycepin reported previously (Cheng and Zhu 2019). At this time, we found that cordycepin prevents oxidative-stress-induced inhibition. Effects of cordycepin and encapsulated particles on ROS scavenging in HDF cells were evaluated in accordance with previously reported results (He, Lee et al. 2019). Extracts from *C. militaris* significantly inhibited the generation of reactive oxygen species (ROS) induced by hydrogen peroxide. Based on this experimental data regarding ROS generation in HDF cells, the results are consistent with the experiments in normal cells (Park, Lee et al. 2014). Cordycepin increases cell viability, and cell proliferation, and reduces ROS accumulation in human cells line. Measuring levels of antioxidant gene expression in HDF cells revealed that low concentrations of cordycepin can stimulate antioxidant genes. This can also explain experimental results regarding the levels of secreted proteins from HDF. Cells treated with nanoparticles had a higher level of expression of antioxidant genes than controls. ROS may accumulate to higher levels within these cells, which may cause the observed enhanced level of gene expression.

In this research, we have succeeded in developing of cordycepin extract-loaded cassava starch nanoparticles (CXCSNPs), which were derived from the encapsulation of cordyceps medium. These nanoparticles are more effective in stimulating cell proliferation, absorption, and protein secretion, expression of collagen synthesis, antioxidant, and autophagy genes, ROS protection, and skin cell regeneration.

CHAPTER 5
CONCLUSION

Many collagen peptides have been identified from many different collagen types and have been shown to regulate processes such as cell proliferation, migration, apoptosis, and reduce angiogenesis. Fibroblast cells via the secretion of collagen precursors. In normal and oxidative stress condition, the nanoparticle of CM (CMP) can promote collagen synthesis in fibroblast cells line. The cell is located in the dermis. It is critical for skin cell regeneration if occur ROS damage to the skin layer slows this process. The CMP stimulates cell regeneration activity via stimulating collagen synthesis, antioxidation and autophagy. The result shows that the CMP is still better than CM in all terms. The CMP upregulated collagen synthesis genes and wound healing activity using concentrations less than C, and CM at 10 times. Thus, the encapsulated nanoparticle of CM can increase the efficiency of skin cell regeneration through antioxidation and autophagy *in vitro*.

REFERENCES

REFERENCES

Adnan, M., S. A. Ashraf, S. Khan, E. Alshammari and A. M. Awadelkareem (2017). Effect of pH, temperature and incubation time on cordycepin production from Cordyceps militaris using solid-state fermentation on various substrates. Cyta-Journal of Food 15(4): 617-621.

Athira, G. K., A. N. Jyothi and V. R. Vishnu (2018). Water soluble octenyl succinylated Cassava starch-curcumin nanoformulation with enhanced bioavailability and anticancer potential. Starch-Stärke 70(7-8): 1700178.

Borchers, A. T., C. L. Keen and M. E. Gershwin (2004). Mushrooms, tumors, and immunity: an update. Exp Biol Med (Maywood) 229(5): 393-406.

Cao, Z.-P., Y.-J. Shang, Q.-Y. Liu, B.-Y. Wu, W.-X. Liu and C.-H. Li (2019). Neuroprotection of cordycepin in NMDA-induced excitotoxicity by modulating adenosine A1 receptors. European journal of pharmacology 853: 325-335.

Chambers, E. S. and M. Vukmanovic-Stejic (2020). Skin barrier immunity and ageing. Immunology 160(2): 116-125.

Chen, Y. X., D. Y. Zhu, Z. L. Xu, J. H. Yin, X. W. Yu, J. Mei, Y. S. Gao and C. Q. Zhang (2017). The Protective Effect of Cordycepin On Alcohol-Induced Osteonecrosis of the Femoral Head. Cell Physiol Biochem 42(6): 2391-2403.

Cheng, C. and X. Zhu (2019). Cordycepin mitigates MPTP-induced Parkinson's disease through inhibiting TLR/NF-KB signaling pathway. Life sciences 223: 120-127.

Cichorek, M., M. Wachulska, A. Stasiewicz and A. Tyminska (2013). Skin melanocytes: biology and development. Postepy Dermatol Alergol 30(1): 30-41.

Conway, O., H. A. Akpinar, V. V. Rogov and V. Kirkin (2020). Selective Autophagy Receptors in Neuronal Health and Disease. J Mol Biol 432(8): 2483-2509.

Cunningham, K. G., W. Manson, F. S. Spring and S. A. Hutchinson (1950). Cordycepin, a metabolic product isolated from cultures of Cordyceps militaris (Linn.) Link. Nature 166(4231): 949.

da Silva, N. M. C., P. R. C. Correia, J. I. Druzian, F. M. Fakhouri, R. L. L. Fialho and E. C. M. C. de Albuquerque (2017). PBAT/TPS composite films reinforced with starch nanoparticles produced by ultrasound. International Journal of Polymer Science 2017.

Dai, G. W., T. T. Bao, C. F. Xu, R. Cooper and J. S. Zhu (2001). CordyMax(TM) Cs-4 improves steady-state bioenergy status in mouse liver. Journal of Alternative and Complementary Medicine 7(3): 231-240.

Dehdashtian, A., T. P. Stringer, A. J. Warren, E. W. Mu, B. Amirlak and L. Shahabi (2018). Anatomy and Physiology of the Skin. Melanoma, Springer: 15-26.

Dou, C., Z. Cao, N. Ding, T. Hou, F. Luo, F. Kang, X. Yang, H. Jiang, Z. Xie and M. Hu (2016). Cordycepin prevents bone loss through inhibiting osteoclastogenesis by scavenging ROS generation. Nutrients 8(4): 231.

He, M. T., A. Y. Lee, C. H. Park and E. J. Cho (2019). Protective effect of Cordyceps militaris against hydrogen peroxide-induced oxidative stress in vitro. Nutr Res Pract 13(4): 279-285.

Henrot, P., P. Laurent, E. Levionnois, D. Leleu, C. Pain, M. E. Truchetet and M. Cario (2020). A Method for Isolating and Culturing Skin Cells: Application to Endothelial Cells, Fibroblasts, Keratinocytes, and Melanocytes From Punch Biopsies in Systemic Sclerosis Skin. Front Immunol 11: 566607.

Herd, H., N. Daum, A. T. Jones, H. Huwer, H. Ghandehari and C. M. Lehr (2013). Nanoparticle geometry and surface orientation influence mode of cellular uptake. ACS Nano 7(3): 1961-1973.

Hu, M. S., M. R. Borrelli, W. X. Hong, S. Malhotra, A. T. M. Cheung, R. C. Ransom, R. C. Rennert, S. D. Morrison, H. P. Lorenz and M. T. Longaker (2018). Embryonic skin development and repair. Organogenesis 14(1): 46-63.

Hughes, E. (2001). Skin: its structure, function and related pathology. Dermatology Nursing: a Practical Guide. Churchill Livingstone, Edinburgh.

Hur, H. (2008). Chemical ingredients of *Cordyceps militaris*. Mycobiology 36: 233-235.

Iadecola, C. (2017). The neurovascular unit coming of age: a journey through neurovascular coupling in health and disease. Neuron 96(1): 17-42.

Ijaz, I., E. Gilani, A. Nazir and A. Bukhari (2020). Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. Green Chemistry Letters and Reviews 13(3): 223-245.

Jadhav, S. P., H. Chawra, K. R. Jadhav and D. D. Sonawane (2020). Nanotechnology in Pharmaceutical Science: A Concise Review. SGVU J Pharm Res Educ 5(2): 520-532.

Jang, H.-J., K. E. Yang, I.-H. Hwang, Y. H. Huh, D. J. Kim, H.-S. Yoo, S. J. Park and I.-S. Jang (2019). "Cordycepin inhibits human ovarian cancer by inducing autophagy and apoptosis through Dickkopf-related protein 1/β-catenin signaling." American Journal of Translational Research 11(11): 6890.

Kaokaen, P., A. Jaiboonma, N. Chaicharoenaudomrung, P. Kunhorm, K. Janebodin, P. Noisa and P. Jitprasertwong (2021). Cordycepin-loaded Nanoparticles from Cassava Starch Promote the Proliferation of Submandibular Gland Cells and Inhibit the Growth of Oral Squamous Carcinoma Cells. Nutr Cancer 73(10): 2014-2029.

Khan, I., M. Khan, M. N. Umar and D. H. Oh (2015). Nanobiotechnology and its applications in drug delivery system: a review. IET Nanobiotechnol 9(6): 396-400.

Kim, J., J. Y. Shin, Y.-H. Choi, S. Y. Lee, M. H. Jin, C. D. Kim, N.-G. Kang and S. Lee (2021). Adenosine and Cordycepin Accelerate Tissue Remodeling Process through Adenosine Receptor Mediated Wnt/β-Catenin Pathway Stimulation by Regulating GSK3b Activity. International journal of molecular sciences 22(11): 5571.

Kolarsick, P. A., M. A. Kolarsick and C. Goodwin (2011). Anatomy and physiology of the skin. Journal of the Dermatology Nurses' Association 3(4): 203-213.

Lawton, S. (2019). Skin 1: the structure and functions of the skin. Nurs. Times 115: 30-33.

Lee, H., Y. Hong and M. Kim (2021). Structural and Functional Changes and Possible Molecular Mechanisms in Aged Skin. Int J Mol Sci 22(22): 12489.

Lei, J., Y. Wei, P. Song, Y. Li, T. Zhang, Q. Feng and G. Xu (2018). Cordycepin inhibits LPS-induced acute lung injury by inhibiting inflammation and oxidative stress. Eur J Pharmacol 818: 110-114.

Mahmoudi, M., H. Hofmann, B. Rothen-Rutishauser and A. Petri-Fink (2012). Assessing the in vitro and in vivo toxicity of superparamagnetic iron oxide nanoparticles. Chem Rev 112(4): 2323-2338.

Mathew-Steiner, S. S., S. Roy and C. K. Sen (2021). Collagen in Wound Healing. Bioengineering (Basel) 8(5): 63.

Meena, H., M. Pandey, P. Negi and Z. Ahmed (2010). Estimation of cordycepin by improved HPLC method in the natural and cultured mycelia of high medicinal value Himalayan entomogenous fungus Cordyceps sinensis. Electronic Journal of Environmental, Agricultural & Food Chemistry 9(10).

Mitchell, J. P. and R. J. Carmody (2018). NF-kappaB and the Transcriptional Control of Inflammation. Int Rev Cell Mol Biol 335: 41-84.

Olatunji, O. J., Y. Feng, O. O. Olatunji, J. Tang, Z. Ouyang and Z. Su (2016). Cordycepin protects PC12 cells against 6-hydroxydopamine induced neurotoxicity via its antioxidant properties. Biomed Pharmacother 81: 7-14.

Park, E. S., D. H. Kang, M. K. Yang, J. C. Kang, Y. C. Jang, J. S. Park, S. K. Kim and H. S. Shin (2014). Cordycepin, 3'-deoxyadenosine, prevents rat hearts from ischemia/reperfusion injury via activation of Akt/GSK-3beta/p70S6K signaling pathway and HO-1 expression. Cardiovasc Toxicol 14(1): 1-9.

Park, J. M., J. S. Lee, K. R. Lee, S. J. Ha and E. K. Hong (2014). Cordyceps militaris extract protects human dermal fibroblasts against oxidative stress-induced apoptosis and premature senescence. Nutrients 6(9): 3711-3726.

Price, P. J., W. A. Suk, R. L. Peters, C. E. Martin, T. M. Bellew and R. J. Huebner (1975). Cordycepin inhibition of 3-methylcholanthrene-induced transformation in vitro. Proc Soc Exp Biol Med 150(3): 650-653.

Rajeswari, L., S. Moorthy and K. Rajasekhran (2011). Preparation of cassava starch nanoparticles and their application as a carrier system for curcumin delivery. International Journal of Nanotechnology and Applications 5(3): 193-201.

Ren, H., F. Zhao, Q. Zhang, X. Huang and Z. Wang (2022). Autophagy and skin wound healing. Burns Trauma 10: tkac003.

Rinnerthaler, M., J. Bischof, M. K. Streubel, A. Trost and K. Richter (2015). Oxidative stress in aging human skin. Biomolecules 5(2): 545-589.

Roco, M. C. (2003). Nanotechnology: convergence with modern biology and medicine. Curr Opin Biotechnol 14(3): 337-346.

Sahoo, S. K., S. Parveen and J. J. Panda (2007). The present and future of nanotechnology in human health care. Nanomedicine 3(1): 20-31.

Shih, L., K.-L. Tsai and C. Hsieh (2007). Effects of culture conditions on the mycelial growth and bioactive metabolite production in submerged culture of Cordyceps militaris. Biochemical engineering journal 33(3): 193-201.

Siengdee, P., S. Klinhom, C. Thitaram and K. Nganvongpanit (2018). Isolation and culture of primary adult skin fibroblasts from the Asian elephant (Elephas maximus). PeerJ 6: e4302.

Silvestre, F. J., M. P. Minguez and J. M. Sune-Negre (2009). Clinical evaluation of a new artificial saliva in spray form for patients with dry mouth. Med Oral Patol Oral Cir Bucal 14(1): E8-E11.

Sivamaruthi, B. S., P. kumar Nallasamy, N. Suganthy, P. Kesika and C. Chaiyasut (2022). Pharmaceutical and biomedical applications of starch-based drug delivery system: A review. Journal of Drug Delivery Science and Technology: 103890.

Soltani, M., R. A. Malek, N. A. Elmarzugi, M. F. Mahomoodally, D. Uy, O. M. Leng and H. A. El-Enshasy (2018). Cordycepin: A biotherapeutic molecule from medicinal mushroom. Biology of macrofungi, Springer: 319-349.

Thangapazham, R. L., T. N. Darling and J. Meyerle (2014). Alteration of skin properties with autologous dermal fibroblasts. Int J Mol Sci 15(5): 8407-8427.

Tharanathan, R. N. (2005). Starch--value addition by modification. Crit Rev Food Sci Nutr 45(5): 371-384.

Tuli, H. S., S. Sandhu, D. Kashyap and A. K. Sharma (2014). Optimization of extraction conditions and antimicrobial potential of a bioactive metabolite, cordycepin from Cordyceps militaris 3936. World Journal of Pharmacy and Pharmaceutical Sciences 3(4): 1525-1535.

Tuli, H. S., S. S. Sandhu and A. K. Sharma (2014). Pharmacological and therapeutic potential of Cordyceps with special reference to Cordycepin. 3 Biotech 4(1): 1-12.

Tuli, H. S., A. K. Sharma, S. S. Sandhu and D. Kashyap (2013). Cordycepin: a bioactive metabolite with therapeutic potential. Life Sci 93(23): 863-869.

Wang, F., P. Yin, Y. Lu, Z. Zhou, C. Jiang, Y. Liu and X. Yu (2015). Cordycepin prevents oxidative stress-induced inhibition of osteogenesis. Oncotarget 6(34): 35496-35508.

Wei, Y., J. Zhou, H. Yu and X. Jin (2019). AKT phosphorylation sites of Ser473 and Thr308 regulate AKT degradation. Biosci Biotechnol Biochem 83(3): 429-435.

Wilkinson, H. N. and M. J. Hardman (2020). Wound healing: cellular mechanisms and pathological outcomes. Open Biol 10(9): 200223.

Wilkinson, H. N. and M. J. Hardman (2020). Wound healing: cellular mechanisms and pathological outcomes. Open Biol 10(9): 200223.

Yang, F. Q., K. Feng, J. Zhao and S. P. Li (2009). Analysis of sterols and fatty acids in natural and cultured Cordyceps by one-step derivatization followed with gas chromatography-mass spectrometry. J Pharm Biomed Anal 49(5): 1172-1178.

Yang, F. Q., D. Q. Li, K. Feng, D. J. Hu and S. P. Li (2010). Determination of nucleotides, nucleosides and their transformation products in Cordyceps by ion-pairing reversed-phase liquid chromatography-mass spectrometry. Journal of Chromatography A 1217(34): 5501-5510.

Yang, J., Y. Zhou and J. Shi (2020). Cordycepin protects against acute pancreatitis by modulating NF-kappaB and NLRP3 inflammasome activation via AMPK. Life Sci 251: 117645.

Yoon, S. Y., S. J. Park and Y. J. Park (2018). The anticancer properties of cordycepin and their underlying mechanisms. International journal of molecular sciences 19(10): 3027.

Yousef, H., M. Alhajj and S. Sharma (2017). Anatomy, skin (integument), epidermis.

Zaidman, B. Z., M. Yassin, J. Mahajna and S. P. Wasser (2005). Medicinal mushroom modulators of molecular targets as cancer therapeutics. Applied Microbiology and Biotechnology 67(4): 453-468.

Zhang, D. W., H. Deng, W. Qi, G. Y. Zhao and X. R. Cao (2015). Osteoprotective effect of cordycepin on estrogen deficiency-induced osteoporosis in vitro and in vivo. Biomed Res Int 2015: 423869.

Zhang, J., X. Wang, V. Vikash, Q. Ye, D. Wu, Y. Liu and W. Dong (2016). ROS and ROS-Mediated Cellular Signaling. Oxid Med Cell Longev 2016: 4350965.

Zhang, S., Z. Chu, C. Yin, C. Zhang, G. Lin and Q. Li (2013). Controllable drug release and simultaneously carrier decomposition of SiO2-drug composite nanoparticles. J Am Chem Soc 135(15): 5709-5716.

Zhu, F. (2020). Underutilized and unconventional starches: Why should we care? Trends in Food Science & Technology 100: 363-3

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