ผลกระทบของการเติมเจลว่านหางจระเข้ต่อกระบวนการสังเคราะห์ แผ่นฟิล์มแบคทีเรียเซลลูโลส โดย Acetobacter Xylinum

นาย องอาจ สายบัวทอง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรมหาบัณฑิต
สาขาวิชาวิศวกรรมเคมี ภาควิชาวิศวกรรมเคมี
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ปีการศึกษา 2550

EFFECT OF ADDITION OF ALOE VERA GEL ON SYNTHESIS OF BACTERIAL CELLULOSE FILM BY *ACETOBACTER XYLINUM*

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering Program in Chemical Engineering
Department of Chemical Engineering
Faculty of Engineering
Chulalongkorn University
Academic Year 2007

Thesis Title	EFFECT OF ADDITION OF ALOE VERA GEL ON SYNTHESIS OF BACTERIAL CELLULOSE FILM BY *ACETOBACTER XYLINUM*
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องอาจ สายบัวทอง: ผลกระทบของการเติมเจลว่านหางจระเข้ต่อกระบวนการสังเคราะห์ แผ่นฟิล์มแบคทีเรียเซลลูโลส โดย Acetobacter Xylinum
(EFFECT OF ADDITION OF ALOE VERA GEL ON SYNTHESIS OF BACTERIAL CELLULOSE FILM BY *ACETOBACTER XYLINUM*)
อ. ที่ปรึกษา: รศ. ดร. เหมือนเดือน พิศาลพงศ์, 70 หน้า

จากคุณสมบัติเฉพาะตัวทางชีววิทยาของว่านหางจระเข้และแบคทีเรียเซลลูโลส งานวิจัยนี้ จึงได้ทำการศึกษาผลกระทบของการเติมเจลว่านหางจระเข้ต่อกระบวนการสังเคราะห์แผ่นฟิล์มแบคทีเรียเซลลูโลส โดย Acetobacter Xylinum โดยเจลว่านหางจระเข้ได้ถูกผสมลงในอาหารเลี้ยงเชื้อของแบคทีเรียเซลลูโลส เพื่อปรับปรุงคุณสมบัติทางกายภาพและทางชีวภาพของแผ่นฟิล์มที่สร้างขึ้น ซึ่งเจลว่านหางจระเข้ที่ทำการศึกษานั้นอยู่ในช่วง 0-50 เปอร์เซ็นต์ปริมาตรของว่านหางจระเข้ต่อปริมาตรในอาหารเลี้ยงเชื้อ โดยแผ่นฟิล์มแบคทีเรียเซลลูโลส-ว่านหางจระเข้ที่สังเคราะห์ขึ้น จะถูกนำไปศึกษาถึงคุณสมบัติทางกลการบวมน้ำ โครงสร้างที่เป็นรูพรุน ดัชนีความเป็นผลึก และการเจริญเติบโตของเซลล์ผิวหนังคนบนแผ่นฟิล์มชีวภาพสังเคราะห์ จากการศึกษาพบว่าที่ 30 เปอร์เซ็นต์ปริมาตรของว่านหางจระเข้ต่อปริมาตรในอาหารเลี้ยงเชื้อให้คุณสมบัติเชิงกลที่ดีที่สุด โดยตัวชิ้นงานที่สังเคราะห์นั้น มีความเหนียวและแน่นขึ้น หลังจากที่เติมว่านหางจระเข้ลงไป นอกจากนี้ การเกิดปฏิสัมพันธ์ระหว่างโมเลกุลเซลลูโลสของแบคทีเรียเซลลูโลส กับเจลว่านหางจระเข้สามารถอธิบายได้จากผลการทดสอบด้วยอินฟราเรดทางโครงสร้างโมเลกุล นอกจากนี้ยังพบว่า คุณสมบัติทางกลและความสามารถของการบวมน้ำของแผ่นฟิล์มแบคทีเรียเซลลูโลส-ว่านหางจระเข้มีค่าสูงกว่าแผ่นฟิล์มแบคทีเรียเซลลูโลส และลักษณะรูพรุนของแผ่นฟิล์มแบคทีเรียเซลลูโลส-ว่านหางจระเข้มีขนาดเล็กกว่าแผ่นฟิล์มเซลลูโลส ในขณะที่พื้นที่ผิวนั้นเพิ่มขึ้นไม่มากนัก และจากศึกษาค่าดัชนีความเป็นผลึกของแผ่นฟิล์มแบคทีเรียเซลลูโลส-ว่านหางจระเข้ที่ ความเข้มข้น 30 เปอร์เซ็นต์ มีค่าเท่ากับ 82.77 และนอกจากนี้ จากการศึกษายังพบว่าแผ่นฟิล์มแบคทีเรียเซลลูโลส-ว่านหางจระเข้ที่สังเคราะห์ได้นั้นไม่เป็นพิษ และส่งเสริมการเจริญเติบโตของเซลล์ผิวหนังมนุษย์

ภาควิชา วิศวกรรมเคมี ลายมือชื่อนิสิต องอาจ สายบัวทอง
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ปีการศึกษา 2550

4970670921: MAJOR CHEMICAL ENGINEERING
KEY WORD: BACTERIAL CELLULOSE / CELLULOSE / ALOE VERA / WOUND DRESSING

ONG-ARD SAIBUATONG: EFFECT OF ADDITION OF ALOE VERA GEL ON SYNTHESIS OF BACTERIAL CELLULOSE FILM BY *ACETOBACTER XYLINUM*
THESIS ADVISOR: ASSOC.PROF.MUENDUEN PHISALAPHONG, Ph.D., 70 pp.

In this thesis, the effects of addition of aloe vera gel on synthesis of bacterial cellulose film by *Acetobacter Xylinum* was studied due to the unique properties of both aloe vera gel and bacterial cellulose (BC), which might improve the physical and biological properties of the developed film. The mechanical property, equilibrium water content, porous structure, crystallinity index, and the growth of human skin cells on the biosynthesized film with the supplement of aloe vera gel at 0-50 percent-range (v/v) in the culture medium were investigated. From the study, the 30 percent addition of the BC-aloe vera gel showed the best combination for the film formation. The BC-aloe vera film was found to be more strengthen than the BC film. In addition, the interaction between BC fibrils and aloe vera gel could be illustrated from FTIR analysis. Furthermore, both of the mechanical properties and equilibrium water content of the BC-aloe vera film were found to be higher than those of the BC film. The average pore sizes of the BC-aloe vera were smaller than those of the BC film, but the surface area did not much increase from the latter. The crystallinity index of the BC-aloe vera film content at 30 percent was improved to 82.77. Additionally, the BC-aloe vera film had no toxicity and supported the growth and proliferation of human skin cells.

Department: Chemical Engineering Student's signature Ong-ard Saibuatong
Field of study: Chemical Engineering Advisor's signature .....
Academic year: 2007

ACKNOWLEDGEMENTS

I would like to express my gratitude to all those who gave me the possibility to undertaken this thesis, and whose help and advice have been very important. Firstly, I would like to thank Associate Professor Dr. Muenduen Phisalaphong, my thesis advisor, for all of her excellent advice and suggestions to elude many problems that I encountered during the experiment.

In addition, I would also grateful to Assistant Professor Dr. Montree Wongsri as the chairman, Assistant Professor Dr. Bunjerd Jongsomjit, and Dr. Sopee Sanguandelkul as member of thesis committee.

Also, I would like to express my gratitude to Mr. Jeerun Khingkaew, a Ph.D. student of Chemical Engineering, Chulalongkorn University, for his great kind help supporting me not only the aloe vera for the experiment, but also many additional suggestions and important hints.

I have furthermore to thank the biochemical engineering laboratory of Chulalongkorn University, for necessary supports on place, materials, chemicals, and also the equipment during the experimental period.

Finally, I would like to extend my special thanks to my family that always stays me aside all the time and gives me a spirit to reasonably face with many problems throughout the thesis period. Really thanks for all of you.

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CHAPTER I

INTRODUCTION

Cellulose as a linear polysaccharide is the most abundant regrowing organic material with outstanding properties and a variety of useful applications. It is found as a structural component, often bound to other polymers (pectin, lignin, hemicellulose, etc.) in the cell wall of plants, algae and also of some lower animals and bacterial generas. Interestingly, only a few bacterial species, taxonomically related to the genus *Acetobacter xylinum*, extracellularly secrete the synthesized cellulose as fibres. Bacterial cellulose (BC), a fine fiber network, secreted by *Acetobacter xylinum* has unique properties including high water holding capacity, high crystallinity, hydrophilicity, purity, and high tensile strength.

BC has recently been studied for the use as artificial skin and blood vessels (Klemm *et al.*, 2001), scaffold for tissue engineering of cartilage (Svensson *et al*., 2005) and wound-dressing (Czaja *et al.*, 2006). The innovative wound dressing still has been continuously developed in a wide range of good candidate materials such as alginate, polyurethane, chitosan, and aloe vera.

Aloe vera has been shown to have multiple beneficial properties during wound healing, including the abilities to: (1) penetrate tissue; (2) anesthetize tissue; (3) preclude bacterial, fungal, and viral growth, (4) act as an anti-inflammatory agent; and (5) dilate capillary beds and enhance blood flow (Grindlay and Reynolds, 1986). Moreover, aloe vera may be used to inhibit fibroplasia in wound healing, and to promote both tissue growth and differentiation in tissue culture (Davis *et al*.,1994; Heggers *et al*.,1996).

Therefore, this study aims to develop BC-aloe vera film from microbial synthesis under static conditions by *Acetobacter xylinum* in coconut-water. Microstructure and mechanical properties of the BC films were then characterized. Furthermore, the growth of human skin HaCat on BC films was examined. The

present study would provide indications for the preparation of the BC-aloe vera film for using in therapy of skin wound.

Objectives

1. To develop BC-aloe vera film from biosynthesis by *Acetobacter xylinum*.
2. To investigate the effect of aloe vera content on the characteristics of BC-aloe vera film.

Research Scopes

1. Prepare BC-aloe vera film from biosynthesis under static conditions by *Acetobacter xylinum*.
2. Examine effects of addition of aloe vera gel in the range of 0 – 50% vol/vol in the culture medium.
3. Characterizing the developed BC-aloe vera film by:
    a. Scanning Electron Micrographs (SEM) for preliminarily investigating morphology.
    b. Fourier Transform Infrared (FT-IR) spectrometer for identifying the chemical structure.
    c. Universal testing machine for determining stress-strain curve.
    d. X-Ray Diffraction (XRD) for finding crystallinity index.
    e. Brunauer-Emmett-Teller (BET) for identifying the pore size, porosity, and pore size distribution.
4 In vitro study of human skin on the developed film in 24 well culture plate.

Overview

This present work is organized as follows:

Chapter I present an introduction of this study.

Chapter II contains background theory of cellulose, BC, and aloe vera.

Chapter III is consisted of the literature review: medical application of BC and aloe vera for wound dressing.

Chapter IV states the details of the experimental procedures and techniques of this research.

Chapter V reviews the experimental results of the characterization of BC and BC-aloe vera films.

Chapter VI contains the overall conclusion obtained from this research. Future work and recommendations are also stated.

Finally, the additional data of the experiments which had emerged from this study are included in appendixes at the end of this thesis.

CHAPTER II

THEORY

2.1 Cellulose

Cellulose is the main component of higher plant cell walls. Cellulose is considered as one of the most abundant organic compounds on earth. It is also formed by some algae, fungi, bacteria, and marine animals. Physically, it is a linear, insoluble polymer of D-glucose unit joined by glycosidic linkages as polysaccharides. Cellulose molecules form long chains in polycrystalline fibrous bundles that contain crystalline as well as amorphous regions. (OMRI, 2001)

Powdered cellulose - a purified white powder is odorless and consists of fibrous particles in various grades and degrees of fineness ranging from a dense, free flowing powder to a coarse, fluffy, non-flowing material. It is insoluble in water, in dilute acids and most organic solvents and slightly soluble in sodium hydroxide. Normally, fibers vary in length from 0.5 to 4mm and in width from 0.005 to 0.35 mm (OMRI, 2001). Cellulose molecule forms straight, almost fully extended chain as shown in Figure 2.1. The cellulose chains are organized in a crystalline or semi-crystalline lattice, thus giving rise to micro fibrils with high tensile strength. Chemical formula of cellulose is $(C_6H_{10}O_5)_n$. In general, the advantages of cellulose include high specific strength and good thermal stability.

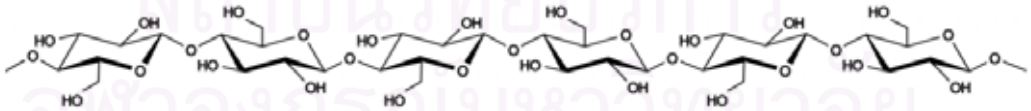


Figure 2.1 Structure of Cellulose (Gardner, 1974)

2.2 Bacterial Cellulose (BC)

Bacterial cellulose produced by *Acetobacter xylinum* (Gluconacetobacter xylinus) was found to be superior to many accounts as compared with the plant one. *A. xylinum* is a simple Gram-negative bacterium which has an ability to synthesize a large quantity of high-quality cellulose organized as twisting ribbons of microfibrillar bundles (Czaja *et al*., 2006). It can be produced from many different substrates such as Nata de pina and Nata de coco, synthesized by using *A. xylinum* with pineapple water and coconut water as medium, respectively.

Bacterial cellulose (BC) displays unique properties, including high mechanical strength, high water absorption capacity, high crystallinity, and has an ultra-fine and highly pure fibre network structure. It is extremely hydrophilic, absorbing 60 to 700 times its weight in water. Plant-derived cellulose, wood or cotton, must be physically disintegrated to make them hydrophilic (Brown *et al.,* 1991). Bacterial cellulose retains its long fibrils and exceptional strength because it is formed in a hydrophilic matrix and needs no treatment.

BC traditionally originates as a white gelatinous pellicle on the surface of the liquid medium in a static culture. These bacteria produce cellulose nanofibrils of 3-8 nm diameters. Together, the mesh of these fibrils forms a gelatinous membrane. The size of BC fibrils is about 100 times smaller than that of plant cellulose as shown in Figure 2.2 (Czaja *et al*., 2006). This unique nano-structure results in a larger surface area. It is extremely hydrophilic, absorbing 60 to 700 times its weight in water (Suwanmajo *et al.,* 2006).

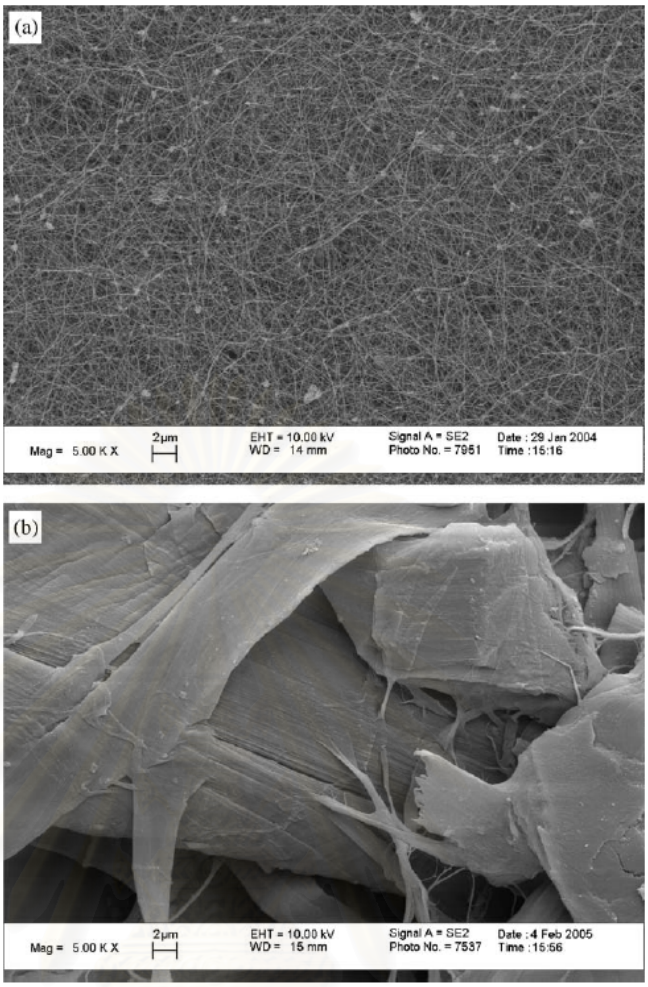


Figure 2.2 A comparison of microfibrillar organization between BC (a) and wood pulp (b).

2.3 Aloe Vera

Aloe vera (Aloe barbadensis Miller) is a perennial succulent belonging to the Liliaceal family. It is a cactus-like plant that grows in hot, dry climates. In nature, it may be damaged physically by ultraviolet (UV) irradiation or by insects. Perhaps its survival in a harsh environment encourages people to believe that aloe vera has wound-healing and antibiotic effects. It is, therefore, less than fortuitous that aloe vera has been reported to possess immunomodulatory, antiinflammatory, UV protective, antiprotozoal, and wound- and burn-healing promoting properties (Reynolds *et al.*, 1999). However, previous treatments of such diseases and conditions with aloe vera gel have been empirical rather than theoretical. Therefore, the clarification of the modes of action of the biochemical components of aloe vera is important in the

determination of the most efficient way of using such active species effectively and developing their applications. It is essential to establish the relationships between the components of aloe vera and their pharmacologic effects. Many attempts have been made to isolate single, active components to examine their effects and clarify their functional mechanisms (Choi and Chung, 2003).

Components of Aloe Vera

Table 2.1 summarizes the components of aloe vera, which are primarily glycoproteins, anthraquinones, saccharides, and low–molecular-weight substances. Polysaccharides are largely glucomannans of various compositions; some are acetylated while others are not. Galactose and galactouronic acid polymers are also frequently found. Different investigators have reported different polysaccharide structures, which may be due to different geographical origins or to the use of different varieties or subspecies. Acetylated mannan has a range of interesting biologic activities as described below. Recently glycoproteins with cell proliferation-promoting activity have been reported. (Yagi A *et al.*, 1997)

Aloe-specific anthraquinones are also present including aloin, aloe-emodin, barbaloin, isobarbaloin, and others. In addition to these, low–molecular-weight substances are reported,such as aloesin, ß-sitosterol, diethylhexylphthalate, vitamins, and beta-carotene. Apart from technical differences and inconsistencies, it appears that the types and levels of components present in aloe gel vary according to geographic origin or variety, therefore, the identification of the active components of aloe vera is important for the effective use of the plant.

Anthraquinones	aloe-emodin aloetic acid aloin anthranol barbaloin isobarbaloin emodin ester of cinnamic acid
Saccharides	cellulose glucose mannose aldopentose acetylated mannan (acemannan) glucomannan acetylated glucomannan galactogalacturan glucogalactomannan galactoglucoarabinomannan
Vitamins	B1 B2 B6 C ß-carotene choline folic acid ∞-tocopherol
Enzymes	amylase carboxypeptidase catalase cyclooxydase lipase oxidase
Low-molecular-weight substances	arachidonic acid cholesterol gibberellin lectin-like substance lignins salicylic acid ß-sitosterol steroids triglycerides uric acid

Table 2.1 Major Components of Aloe Vera (Choi and Chung, 2003).

Studies have found that there are 75 ingredients contained in the Aloe leaf. These ingredients have a variety of medical benefits. They are divided into the following categories (Atherton P., 1997):

• **Ligin** – This cellulose substance is found in the gel has no known medical properties except it posses the property of penetrating the human skin.

• **Saponins** – These forms soapy lathers when mixed and agitated with water. They have been used in detergents, foaming agents and contain antiseptic properties.

• **Anthraquinones** – There are 12 of these contained in the sap of Aloe Vera: Aloin, Isobarbaloin, Anthracene, Emodin, Ester of Cinnamonic acid, Chrysophanic acid, Barbaloin, Anthranol, Aloetic acid, Aloe Emodin, Ethereal oil and Resistannol. These act as natural laxatives, painkillers and analgesics, and they contain powerful antibacterial, antifungal and virucidal properties.

• **Minerals** – Aloe Vera contains the following minerals:

- Calcium (essential for proper bone and teeth density)
- Manganese (a component of enzymes necessary for the activation of other enzymes)
- Sodium (control the body fluids not become high acidic or high alkaline)
- Copper (enable iron to work as oxygen carriers in the red blood cells)
- Magnesium (used by nerves and muscle membranes to help conduct electrical impulses)
- Potassium (regulate the acidic or alkaline levels of body fluid)
- Zinc (contribute to the metabolism of proteins, carbohydrates and fats)
- Chromium (necessary for the proper function of insulin, which in turn control the sugar levels in the blood)
- Iron (control the transportation of oxygen around the body via the red blood cells)

• **Vitamins** – Aloe Vera contains numerous vitamins:

- Vitamins A, C, & E (crucial antioxidants that combat dangerous free radicals in the body)
- Vitamin B & Choline (concerned with the production of energy, amino acid metabolism and developing muscle mass)
- Vitamin B12 (responsible for the production of red blood cells)
- Folic acid (help develop new blood cells)

• **Amino Acids** – Amino Acids are the building blocks of protein, which manufacture and repair muscle tissue. The human body requires 22 amino acids and needs 8 essential ones. Aloe Vera provides 20 of 22 required amino acids and 7 of 8 essential ones.

• **Enzymes** – Some of the most important enzymes in Aloe Vera are: Peroxidase, Aliiase, Catalase, Lipase, Cellulase, Carboxypeptidase, Amylase and Alkaline Phosphatase. Enzymes help to break down food and assist in digestion. Some enzymes help break down fats while others break down starches and sugars.

• **Sugars** – Aloe Vera contains both monosaccharides, such as glucose and fructose, and polysaccharides. Polysaccharides are the most important types of sugars. They aid in proper digestion, maintain cholesterol levels, improve liver functions and promote the strengthening of bones.

• **Sterols** – Sterols are important anti-inflammatory agents. The ones found in Aloe Vera are: Cholesterol, Sitosterol, Campesterol and Lupeol. These sterols contain antiseptic and analgesic properties. They also have pain killing properties similar to aspirin.

Aloe vera contains many physiologically active substances that have effective antiinflammatory, immunomodulatory, and wound-healing effects (Schmidt JM. *et al.,* 1991). The active ingredients, whether acting alone or in concert, include glycoproteins, anthraquinones, polysaccharides, and low–molecular-weight species. Moreover, the fact that biologically active components in aloe vera may be labile, varied, or modified explains some of the difficulties that investigators have reported in reproducing results using unfractionated materials from aloe vera. In light of the many pharmacologic activities of the components of aloe vera, each active component has several interacting factors, each of which may be affected by another substance(s).

CHAPTER III

LITERATURE REVIEW

3.1 Medical Application of BC

Bacterial cellulose, produced by Acetobacter species, displays unique properties, including high mechanical strength, high water absorption capacity, high crystallinity with an ultra-fine and highly pure fibre network structure. It is expected to be a new commodity biochemical with diverse applications, if its mass production process could be improved, especially via submerged fermentation technology. It has already found application as a food matrix (nata de coca) and as dietary fibre, as a temporary dressing to heal skin burns, as an acoustic or filter membrane, as ultra-strength paper and as a reticulated fine fibre network with coating, binding, thickening, and suspending characteristics (Vandamme *et al.,* 1997). A wet spinning process for producing textile fibres from bacterial cellulose has also been developed, and applications as a superconducting and optical fibre matrix are under studies.

Fontana *et al.*, (1990) first reported the application of BC as temporary skin substitutes. Artificial skin's BC for burn and skin injuries treatment displays dramatic clinical results such as immediate pain relief, diminished post-surgery discomfort, faster healing, reduced infection rate, and reduced treatment time and cost.

Mayall *et al.*, (1990) used a Biofill skin substitute in the treatment of trophic ulcerations of the limbs, and showed that this material was very effective by shortening the cicatrisation time, reducing the contamination, and saving the cost of treatment.

Kucharzewski *et al.*, (2003) inferred that BC wound dressing was more effective in the treatment of the chronic venous leg ulcers than Unna's boot.

Alvarez *et al.,* (2004) demonstrated the use of BC in the form of a hydrated membrane in the treatment of chronic venous ulcers. BC was more effective than a standard protocol (non-adherent cellulose acetate gauze) in the process of autolytic debridment.

Czaja *et al.*, (2006) studied on Biofills that provide non-woven, shaped objects in medicine such as artificial arteries, vessels, skin, and etc. It has been still utilized for several skin injury treatments such as basal cell carcinoma/skin graft, severe body burns, facial peeling, sutures, dermabrasions, skin lesions, chronic ulcers, and both donor and receptor sites in skin grafts. In addition, the wound healing effects of never-dried BC are fully biocompatible and also successfully protected burn wounds from excessive external fluid loss, thus accelerating the entire process of healing.

Neeracha *et al.,* (2006) studied the growth of human keratinocytes and fibroblasts on bacterial cellulose film. Their results were the first direct demonstration that BC film supported the growth, spreading, and migration of human keratinocytes but not those of human fibroblasts.

3.2 Aloe Vera for Wound Dressing

The whole gel extract of aloe vera has been reported to have various pharmacologic properties: specifically to promote wound, burn, and frost-bite healing, in addition to having antiinflammatory, antifungal, hypoglycemic, and gastroprotective properties (Reynolds *et al.*, 1999). Of those claims, aloe vera's antiinflammatory and wound healing has been the most extensively studied. Wound healing is considered to be composed of three overlapping events: (1) inflammation, (2) new tissue formation, and (3) matrix remodeling (Dunphy *et al.*, 1974). Protein factors related to wound healing have been investigated, such as growth factors, cellmigration related factors, matrix-forming factors, and matrix-degradation factors.

Aloe vera gel extract stimulated fibroblast growth in a synovial model and also enhanced wound tensile strength and collagen turnover in wound tissue (Davis *et al.*, 1992).

Dennis and Ya Fen Zhu (2003) examined glove that delivers aloe vera (AV) gel to the gloved hand in 30 adult females with bilateral occupational dry skin with or without irritant contact dermatitis. Their study showed that Dry-coated AV gloves that provided for gradual delivery of AV gel to skin produced a uniformly positive outcome of improved skin integrity, decreased appearance of fine wrinkling, and decreased erythema in the management of occupational dry skin and irritant contact dermatitis.

Kay *et al.,* (2003) studied the effects of Aloe vera on gap junctional intercellular communication and proliferation of human skin fibroblasts in the presence and absence of basic fibroblast growth factor. It was found that the aloe vera had the ability to stimulate gap junctional intercellular communication and proliferation of human skin fibroblasts in diabetes mellitus.

CHAPTER IV

EXPERIMENTAL

4.1 Materials

4.1.1 Microbial Strains

The *A. xylinum* strain was isolated from *nata de coco*. The stock culture was kindly supplied by Pramote Tammarat, the Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand.

4.1.2 Aloe Vera

Aloe vera leaves were kindly provided by Jeerun Khingkaew, department of Chemical Engineering Chulalongkorn University. Aloe vera juice was prepared by blending aloe vera gel using a moulinex blender (model DAE1, Thailand) under aseptic condition before adding the juce into culture medium.

4.1.3 Other Chemicals

The details of chemicals used in this experiment are shown in Table

Table 4.1 The chemicals used in this experiment

Chemical	**Supplier**
Sucrose	Ajax Finechem
Ammonium sulfate $(NH_4)_2SO_4$	Carlo Erba
Sodium hydroxides (NaOH)	Carlo Erba
Acetic acid	BDH

4.1.4 Equipments.

-Scanning electron microscopy, SEM (JOEL JSM-5410LV, Japan).

-Fourier Transform Infrared (FT-IR) spectrometer (Nicolet SX-170, USA).

-Universal testing machine (LLOYD 2000R, UK).

-X-ray diffraction (Bruker AXS Model D8 Discover,USA).

-Brunauer-Emmett-Teller (BET) surface area analyzer (Model ASAP 2020,USA).

-Autoclave **(**Model Tomy Autoclave SS-325, Ner ima-ku, Tokyo, Japan**).**

4.2 Culture Media and Method

The medium for the inoculum was coconut-water supplemented with 5.0% sucrose, 0.5% ammonium sulfate $(NH_4)_2SO_4$, 1.0% acetic acid, and different aloe vera concentration. The experiment was designed to test effects of supplementation of aloe vera juce (0%, 5%, 10%, 20%, 30%, 40% and 50% V/V). Precultures were prepared by a transfer of 50 ml stock culture to 1000 ml in 1500 ml bottle and incubated statically at 30 ^{o}C for 7 days. After the surface pellicle was removed, the 5% (v/v) preculture broth was added to the main culture a medium with different aloe vera content. The 75 ml of activated medium was inoculated in a Preti-dish and kept at 30 ^{0}C for 7 days.

All sample films were first purified by washing with DI water and then was treated with NaOH at room temperature to remove bacterial cells followed by a rinse with DI water until pH came to 7. Afterward, the BC film was air-dried at room temperature (30 ^{0}C) and stored in plastic film at room temperature.

4.3 Characterization of BC-Aloe Vera Film

The BC-aloe vera films were characterized by Scanning electron micrographs (SEM) for investigating morphology, by Brunauer-Emmett-Teller (BET) for determining the pore size, porosity, and pore size distribution, by universal testing machine for determining stress-strain curve, by Fourier transform infrared (FT-IR) spectrometer for identifying the chemical structure and by X-ray diffraction (XRD) for estimating crystallinity index. Moreover, biological characteristic of the films in vitro study of human skin cells (Keratinocytes) on the developed film in individual wells of falcon twenty-four-well plates was carried out.

4.3.1 Scanning Electron Microscope (SEM)

The examination of the surface properties was performed by scanning electron microscopy (SEM). Scanning electron micrographs were taken with JOEL JSM-5410LV microscope at Scientific and technological research equipment centre, Chulalongkorn University. The BC films were frozen in liquid nitrogen, immediately snapped, and vacuum-dried. Then, the films were sputtered with gold and photographed. The coated specimens were kept in dry place before experiment. SEM was obtained at 15 kV which is considered to be a suitable condition since too high energy can be burn the samples.

4.3.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is used primarily to identify the chemical structure of the sample. FTIR spectra of the membranes were recorded with a Nicolet FT-IR Spectrometer (SX-170) in the region of 4000–500 cm^{-1}, at Polymer Engineering Laboratory, Chulalongkorn University.

4.3.3 Tensile Properties Testing

In this study, the tensile strength of the film was measured by Instron Testing Instron (5567, NY, USA) at Polymer Engineering Laboratory, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University. The test conditions follow ASTM D882. The determination of tensile property was done under BC film was cut into strip-shaped specimens 10 mm in width and 10 cm in length. At least five specimens were used for each blend composition.

4.3.4 BET Surface Analysis

Pore size and surface area of the membranes were measured by a Brunauer-Emmett-Teller (BET) surface area analyzer (Model ASAP 2020). The samples were placed in the sample cell, which was then heated up to 75 °C and held at this temperature for 2 hours. The samples were cooled down to room temperature and ready to measure the surface area. There were three steps to measure the surface area: adsorption step, desorption step and calibration step.

4.3.5 Equilibrium Water Content (EWC)

Equilibrium water content (EWC) was determined by immersing the preweighted of dried membrane in distilled water at room temperature until equilibration. The membrane was then removed from the water. After excess water at the surface of the membrane was blotted out with Kimwipes paper, the weight of the swollen membrane was measured and the procedure was repeated until there was no further weight change. Water content was determined by gravimetric method (Kim *et al*., 1996) and calculated using the following formula:

$$EWC(\%) = \frac{W_h - W_d}{W_d} x100$$

Where W_h and W_d denoted the weight of hydrate and dry membrane, respectively.

4.3.6 The Water Vapor Permeability Measurement

Water vapor transmission rate (WVTR) of the BC film and the cellulose-chitosan film with area of 50.00 cm^2, were measured on water vapor permeation tester; Lyssy L80-4000 (at Thailand institute of scientific and technological research). The test conditions follow ISO 15106-1. The determination of WVTR was done under the following conditions: temperature, 38 °C; % Relative Humidity, 90%. The principle of this electronic tester is similar to that of conventional method. One side of the membrane is exposed to the water vapor. As water solubilizes into the membrane and permeates through the sample material, nitrogen sweeps the opposite side of the film and transports the transmitted water vapor molecules to the calibrated infrared sensor. The response is reported as a transmission rate.

4.3.7 X-Ray Diffraction

X-ray diffraction patterns of the polymers and BC-aloe vera film were determined with a diffactometer (Bruker AXS Model D8 Discover). The operation conditions were as follows: power 40 kV and 30 mA. The crystallinity index (C.I.) was calculated from the reflected intensity data using the Segal et al. method, and calculated using the following formula:

$$C.I. = (I_{020}-I_{am})/ I_{020}$$

Where I_{020} is the maximum intensity of the lattice diffraction, and I_{am} was the intensity at $2\theta = 18^{\circ}$.

4.3.8 Bioactivity

The cytotoxicity and tissue compatibility of BC, aloe vera, and BC-aloe vera film were kindly evaluated by Jirun khewkan, Department of chemical engineering, faculty of engineering and Associate. Prof. Dr.Neeracha Sanchavanakit, Department of Anatomy, Faculty of Dentistry, Chulalongkorn University. Tissue compatibility was evaluated by growth and spreading of keratinocytes and fibroblasts on each material. The test samples were punched into round-shaped samples of 14 mm diameter. The samples were sterilized by autoclaving at 121 °C for 20 min, and transferred aseptically to 24-well culture plates. Proliferations of cells on the films were determined by MTT assay. The experiments were conducted in triplicate. The number of living cells was determined using MTT assay.

CHAPTER V

RESULTS AND DISCUSSIONS

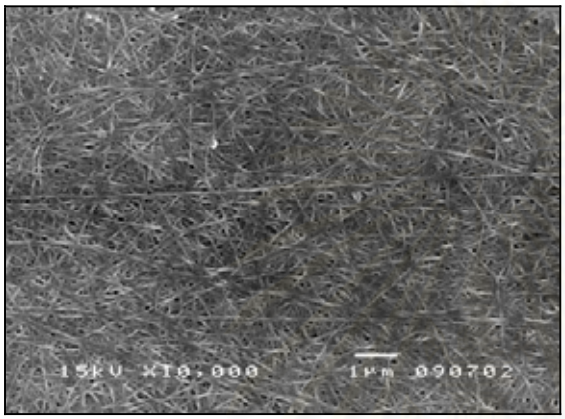
5.1 Cultivating BC-Aloe Vera Film

The bacterial cellulose (BC) was produced in the form of pellicles that float at the surface of the fermentation medium during still fermentation. Due to the unique biological properties of BC and aloe vera, it is interesting to add aloe vera to BC culture medium as it may improve the physical and biological properties of the developed BC-aloe vera film. After cultivating, it was found that the 30% aloe vera adding gave the good homogeneous composition for BC-aloe vera film formation. Also, the addition of aloe vera more than 30% showed the inhibition effect on the BC formation. Due to this, the conditions being studied were at 0%, 5%, 10%, 20%, 30%, 40%, and 50% V/V of the aloe vera gel in the BC culture medium. At these conditions, the investigations via pore morphology, tensile strength, components, chemical structure, and biological properties after adding the the aloe vera were then employed and also compared with the biosynthesized BC film at which none of aloe vera supplementation.

5.2 Surface Morphology

In this section, a Scanning Electron Microscopy (SEM) technique has been used to study the morphology of the BC and BC-aloe vera films. The SEM investigation of the films showed the difference between dried film and swollen film in water. In definition, the BC refers to the BC film with no aloe vera gel adding while the BC-aloe vela gel refers to the BC with the addition of the aloe vera gel. From the static culture, the analyzed SEM images are shown in Figures 5.1 (a) to (g) at 0%, 5%, 10%, 20%, 30%, 40% and 50% V/V of aloe vera gel in culture medium, respectively. As refer to Czaja *et al.*, 2006, the BC generally showed well-organized fibril network. And from this study, the addition of the aloe vera seemed providing

well-bonded into the BC fibril network. Also, the pore size of the BC-aloe vera film was found to be decreased when the percent of the aloe vera increased.



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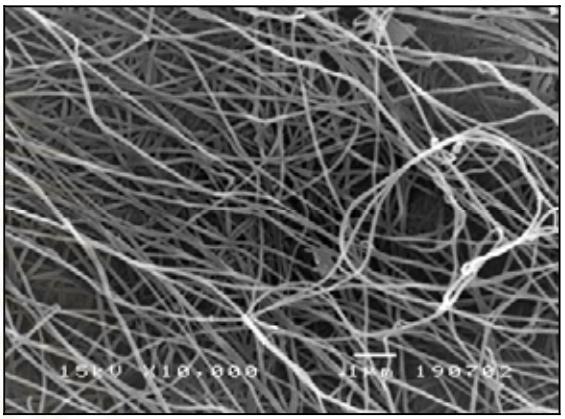

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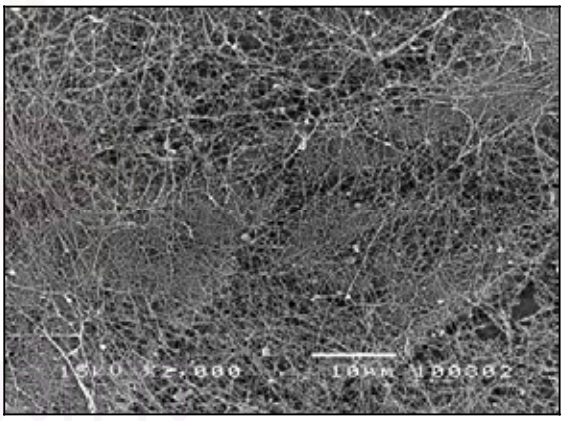

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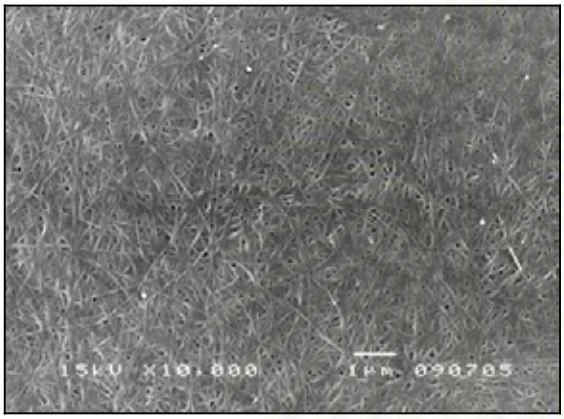
Figure 5.1 (a) SEM images of surface morphology of dried films (upper left) and reswollen film (upper/lower right) at 0% aloe vera.

Figure 5.1 (a) presents SEM images of individual BC without the addition of aloe vera gel. The upper SEM images show the surface morphology of dried (left) and reswollen (right) films at 10,000 magnifications, respectively. The lower one is the reswollen film at 2,000 magnifications. As shown from the above figures, its surface structure was found to be a well-organized network of nano fibril with the diameter of 3-8 nm.



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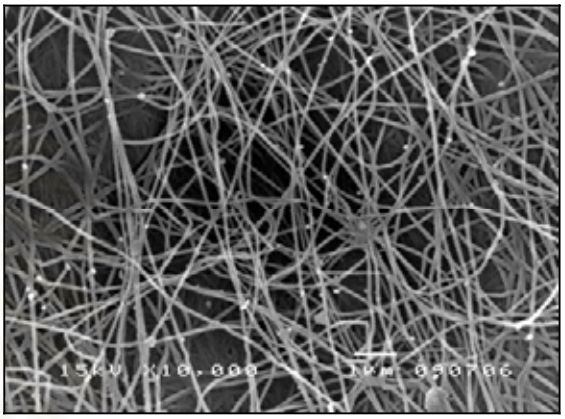

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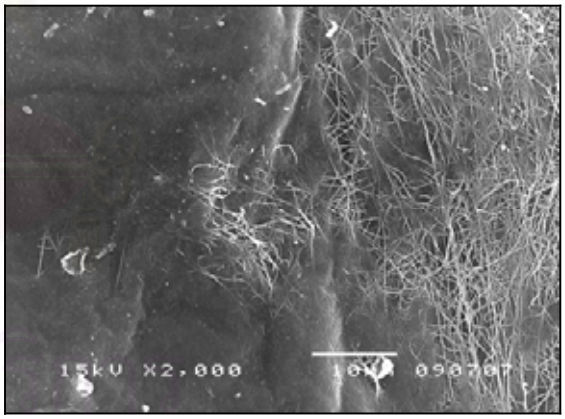

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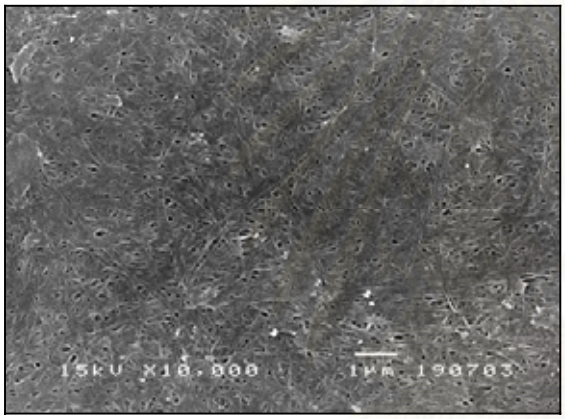
Figure 5.1 (b) SEM images of surface morphology of dried films (upper left) and reswollen film (upper/lower right) at 5% aloe vera.

As alternatively observation on the surface morphology of the BC with the addition of 5% aloe vera, the surface of the nano fibril network was partially coated with the aloe vera gel. This combination causes the reduction in the film pore size From the SEM images at different addition percentage of aloe vera as shown in Figures 5.1 (b) to (g); the increase of the aloe vera gel addition lowered the pore size of the biosynthesis films. The blends with aloe vera gel in the content of 30% v/v showed the best homogeneous structure. Excessive gel coating on the fibril network surface was observed with the supplement of aloe vera gel in the contents of 40 and 50 % (v/v).



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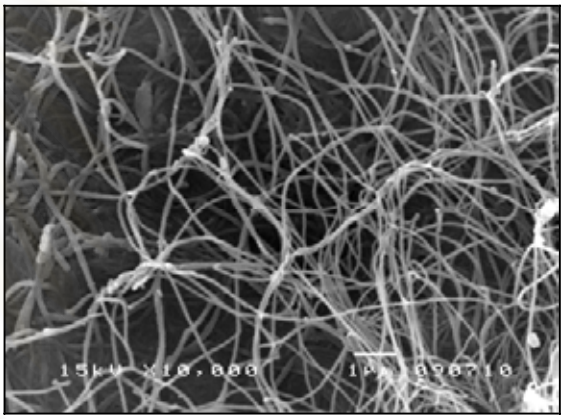

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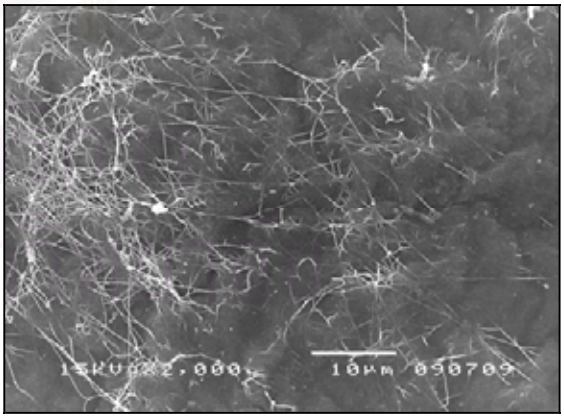

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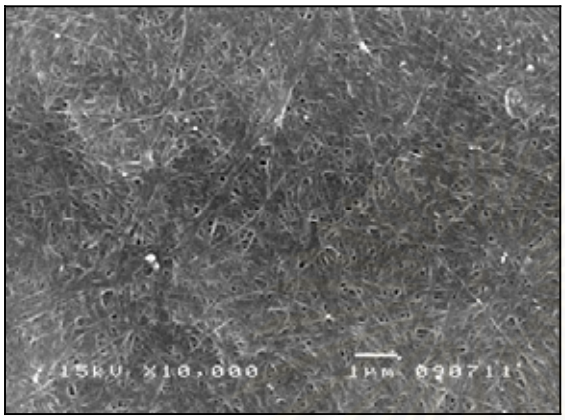
Figure 5.1 (c) SEM images of surface morphology of dried films (upper left) and reswollen film (upper/lower right) at 10% aloe vera.



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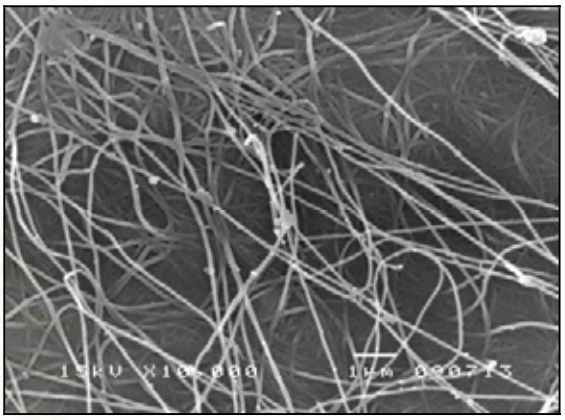

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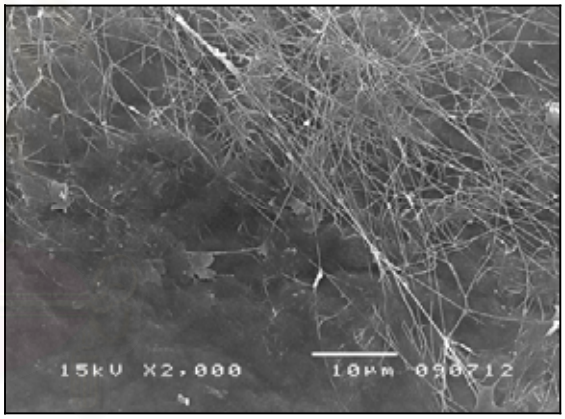

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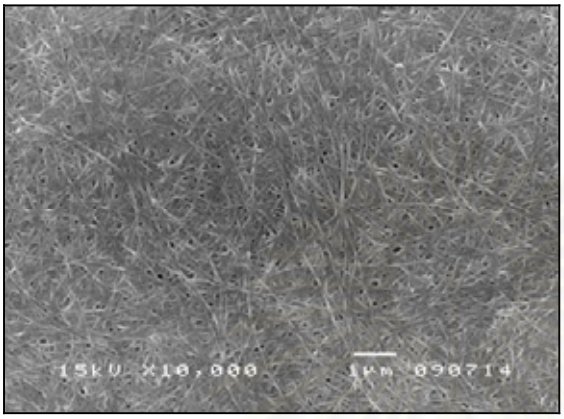
Figure 5.1 (d) SEM images of surface morphology of dried films (upper left) and reswollen film (upper/lower right) at 20% aloe vera.



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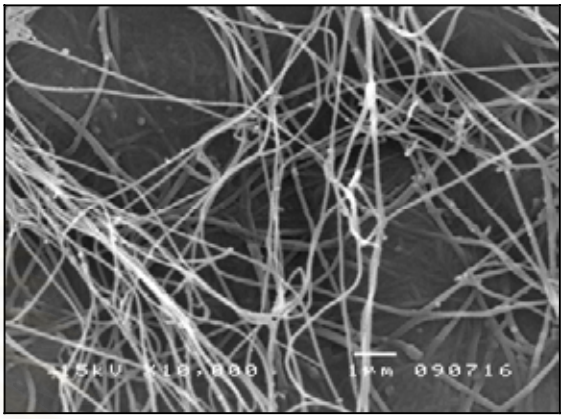

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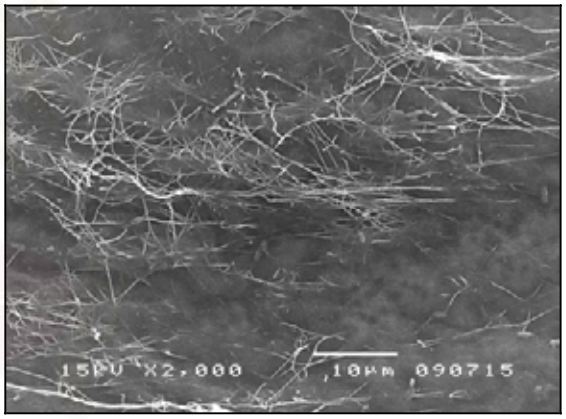

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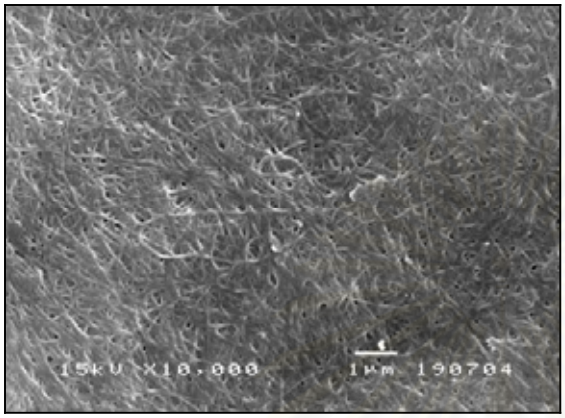
Figure 5.1 (e) SEM images of surface morphology of dried films (upper left) and reswollen film (upper/lower right) at 30% aloe vera.



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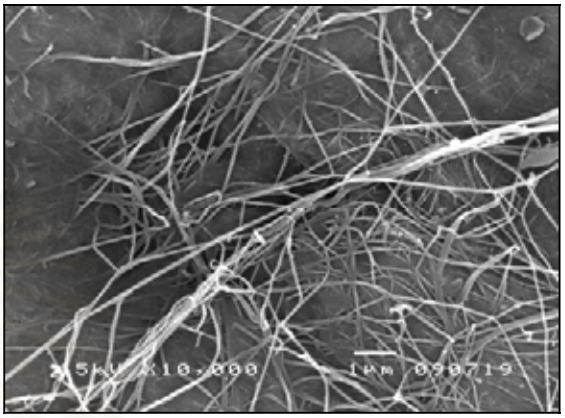

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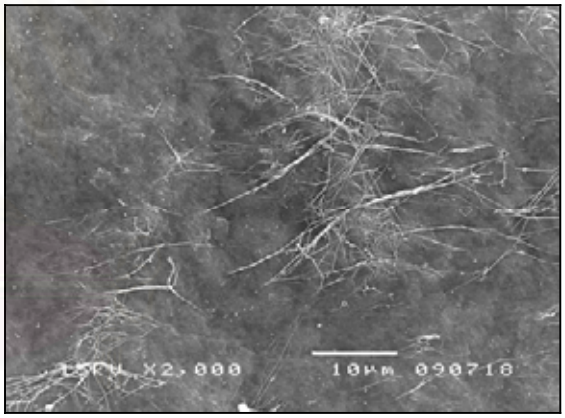

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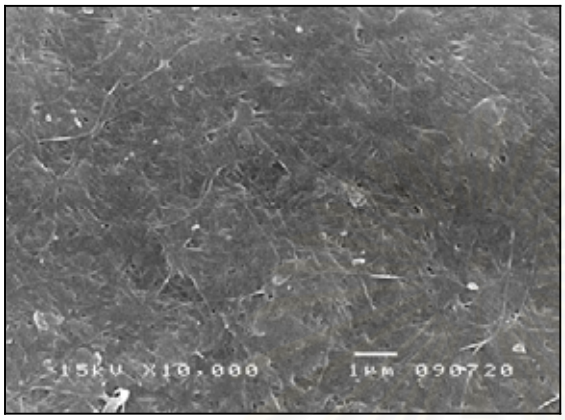
Figure 5.1 (f) SEM images of surface morphology of dried films (upper left) and reswollen film (upper/lower right) at 40% aloe vera.



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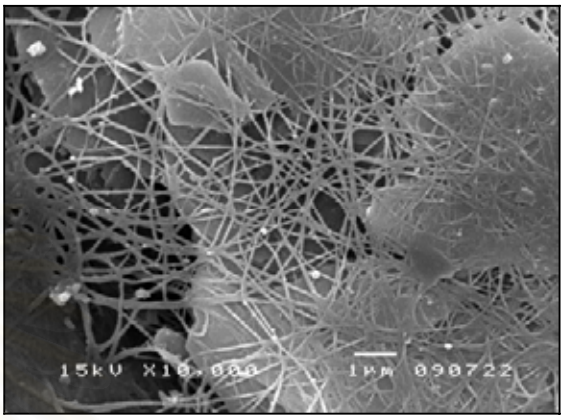

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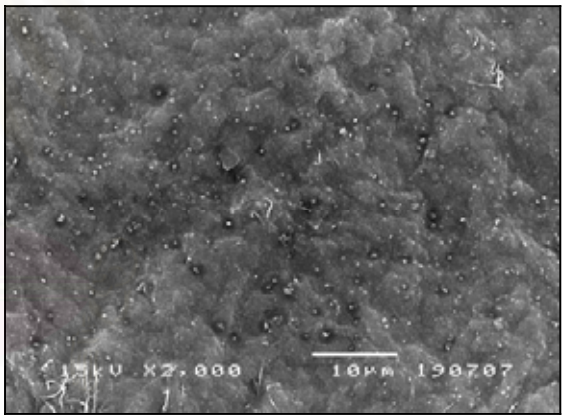

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Figure 5.1 (g) SEM images of surface morphology of dried films (upper left) and reswollen film (upper/lower right) at 50% aloe vera.

5.3 FTIR Analysis

Fourier Transform Infrared (FT-IR) spectroscopy of BC and BC-aloe vera was carried out in order to detect any peak shift that could be attributed to interactions between cellulose and aloe vera. Generally, the FTIR spectroscopy has often been utilized as the useful tools in determining specific functional groups or chemical bonds that exist in a material (Lee *et al*., 1994). As shown in Figures 5.2 and 5.3, the FTIR spectra of all samples were detected at wave number ranging from 1800 to 1500 cm^{-1} and 2800 to 1200 cm^{-1}. The BC-aloe vera films showed the adsorption bands at range around 1642.89 cm^{-1} and around 1594.07 cm^{-1}. The intense absorption in the spectrum of the cellulose is the band at 1642.89 cm^{-1}, which was mostly assigned to glucose carbonyl of cellulose as shown in Figure 5.2 (a). The characteristic absorption of the aloe vera is the band at 1594.07 cm^{-1}, which was assigned to NH_2 of amino groups of aloe vera as shown in Figure 5.2 (h). The bands at 1650-1578 $cm^{-1}$are assigned to C=O stretching (amide I) which overlaps with NH-bending (amide II). The absorption bands at 1565-1540 cm^{-1} are NH deformation (amide II). With the content (v/v) of aloe vera gel of 5, 10, 20, 30, 40 and 50% (v/v), the amino groups of aloe vera (Figure 5.2 (b-h)) were shifted from 1594.07 cm^{-1} to 1555.04, 1562.72, 1563.21, 1573.93:1541.68, 1574.34:1541.96 and 1574.56:1541.77, respectively. The difference between the absorption bands of the films could be attributed to the interaction between the component biopolymers. The results indicated that the intermolecular hydrogen bonding interaction took places between cellulose and aloe vera from biosynthesis culture, leading to a good miscibility film. It should be noted that with the supplement of aloe vera gel 30-50% (v/v), we observed 2 bands at around 1574 and 1542 in place of 1 band at around 1562 of bacteria cellulose film and 1 bands around 1555-1563 of bacteria cellulose with aloe vera gel 5-20% (v/v). The change of the bands could own to different compositions in molecular structure of the films occurred from the reaction or molecular interactions during the biosynthesis. As referred to the SEM analysis, it directly supported to the results that the aloe vera gel attached and bonded to the cellulose fibril corresponded to the shift of the amino group as the percentage of the aloe vera gel addition increased.

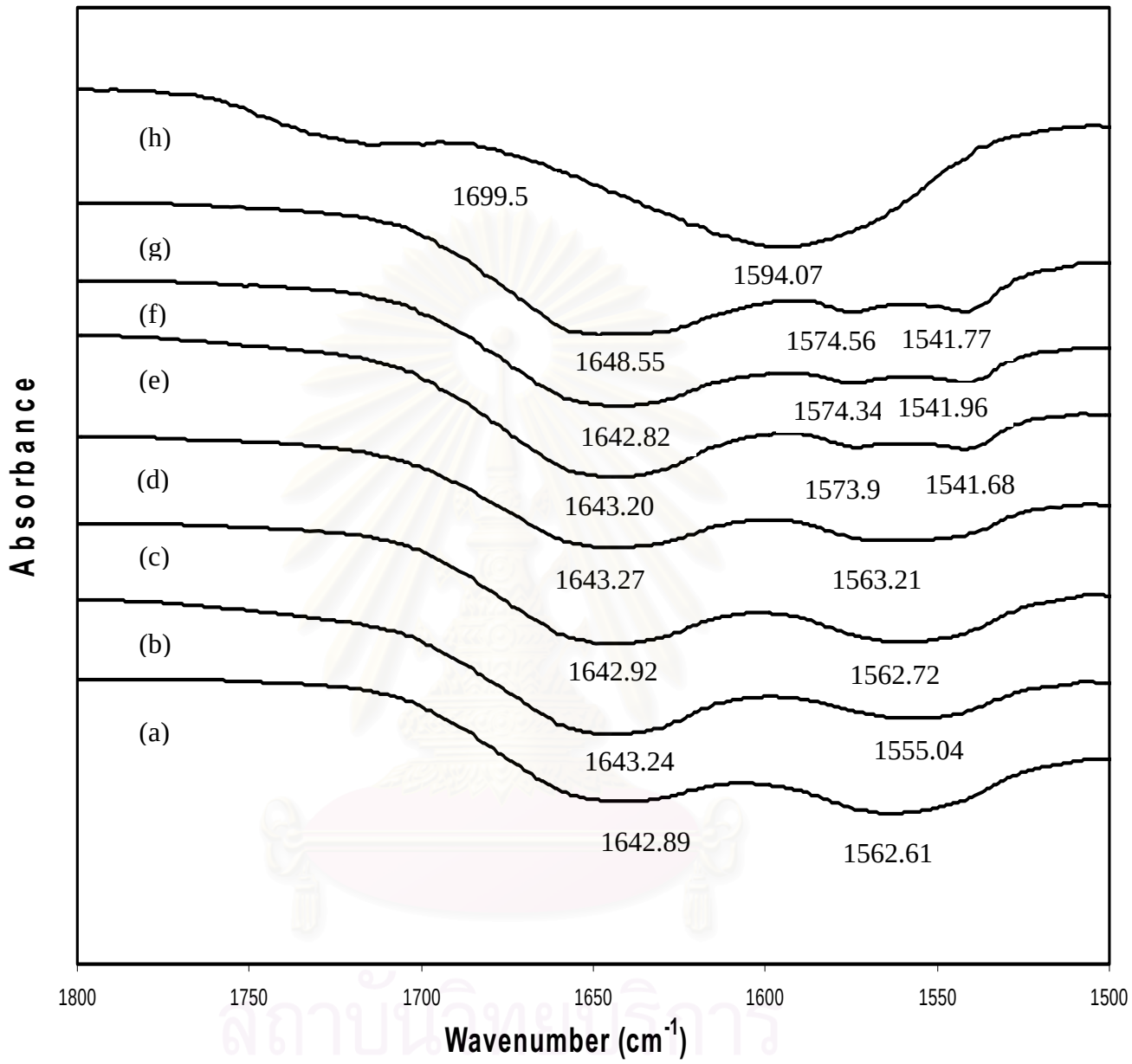


Figure 5.2 The FTIR spectra of (a) BC and (b-g) BC-aloe vera films and (h) aloe vera gel, in wave numbers ranging from 1800 to 1500 cm^{-1}. The supplement of aloe vera gel (% v/v): (b) 5; (c) 10; (d) 20; (e) 30; (f) 40 and (g) 50.

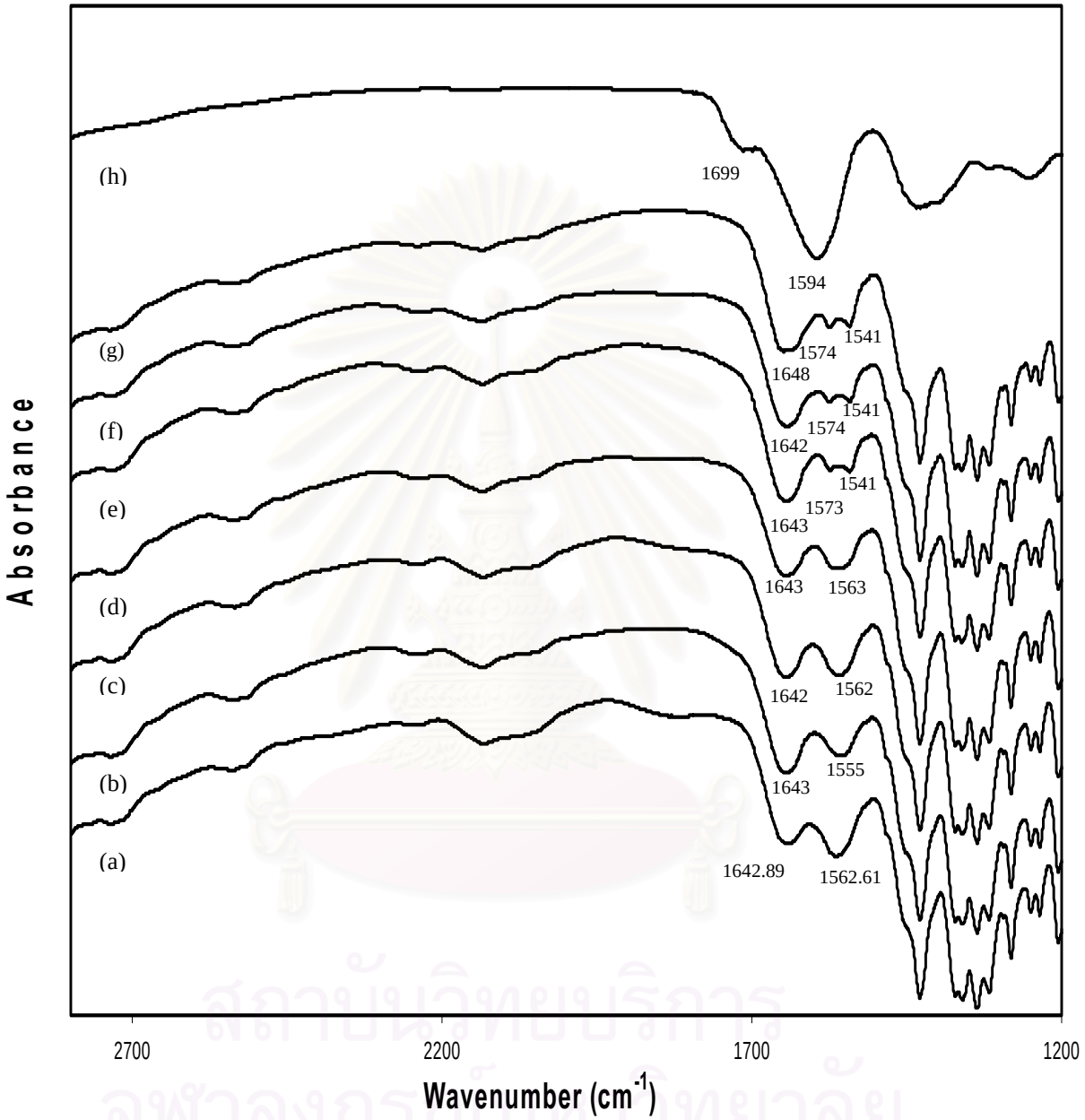


Figure 5.3 The FTIR spectra of (a) BC and (b-g) BC-aloe vera films and (h) aloe vera gel, in wave numbers ranging from 2800 to 1200 cm^{-1}. The supplement of aloe vera gel (% v/v): (b) 5; (c) 10; (d) 20; (e) 30; (f) 40 and (g) 50.

5.4 Mechanical Property

In mechanical analysis, an average thickness of BC and BC-aloe vera films at 0.030 mm was studied via the average tensile strength, Young's modulus, and the elongation at break. The plots showing the change in tensile strength at different percentages of aloe vera addition are presented in Figures 5.4 to 5.6.

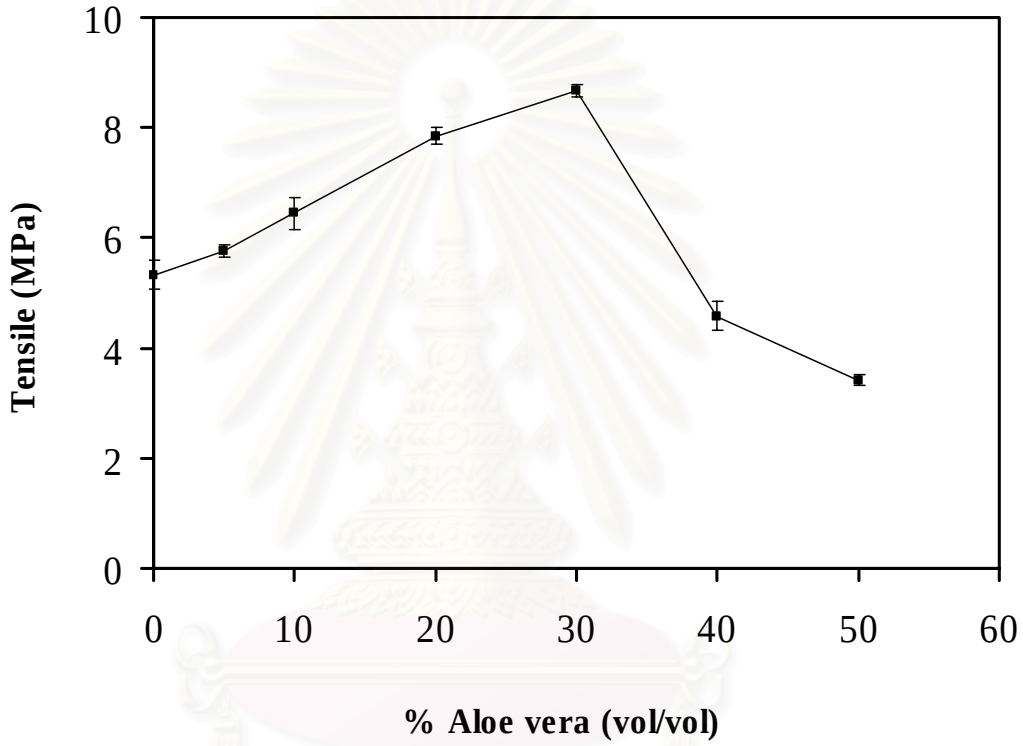


Figure 5.4 Tensile strength of BC-aloe vera film as a function of aloe vera content in culture medium.

Figure 5.4 shows the tensile strength of BC and BC-aloe vera films as a function of aloe vera content. It was found that, the tensile strength increased as the aloe vera gel concentration increased in the range of 0 - 30% (v/v) aloe vera gel content. However, the tensile strength considerably decreased when aloe vera content was more than 30% (v/v). Therefore, aloe vera and bacteria cellulose fibril were well compatible. With the supplement of aloe vera gel up to 30 % (v/v), it was found that the gel well merged into the fibril network resulting in the improved mechanical

strength films. The tensile strength increases from 5.32 to 8.67 MPa as the aloe vera content was increased from 0% to 30% (v/v). However, excessive supplement of the gel (more than 30 %) inhibited the film biosynthesis. Moreover, it was found that, the too high ratio of aloe vera gel: cellulose fibril caused miscellaneous structure of the films. As a result, the tensile strengths decreased from 8.67 MPa to 3.42 MPa after the aloe vera content was increased from 30% to 50%. This indicated that the increment of the tensile strength of the BC-aloe vera films depended on the change of the amount of the aloe vera content. At the suitable additional range, aloe vera gel could well corporate with the bacteria cellulose fiber. On the other hand, the films with the aloe vera content higher than 30% exhibited lower tensile strength according to the miscellaneous structure of the films.

In addition, the cellulose fiber was found to become more elastic after being combined with aloe vera gel. This improved the mechanical properties at which the fiber in the composite membrane could be able to withstand stronger pulled-force than the individual cellulose fiber. Furthermore, as the aloe vera network was formed between the cellulose fibers in the composite membrane, the pull force on the cellulose fiber would be distributed on the aloe vera network. Moreover, the coated composite bacteria cellulose-aloe vera gel film structure resulting in a denser structure with the smaller pore diameter than the BC film which improved the mechanical strength of the films.

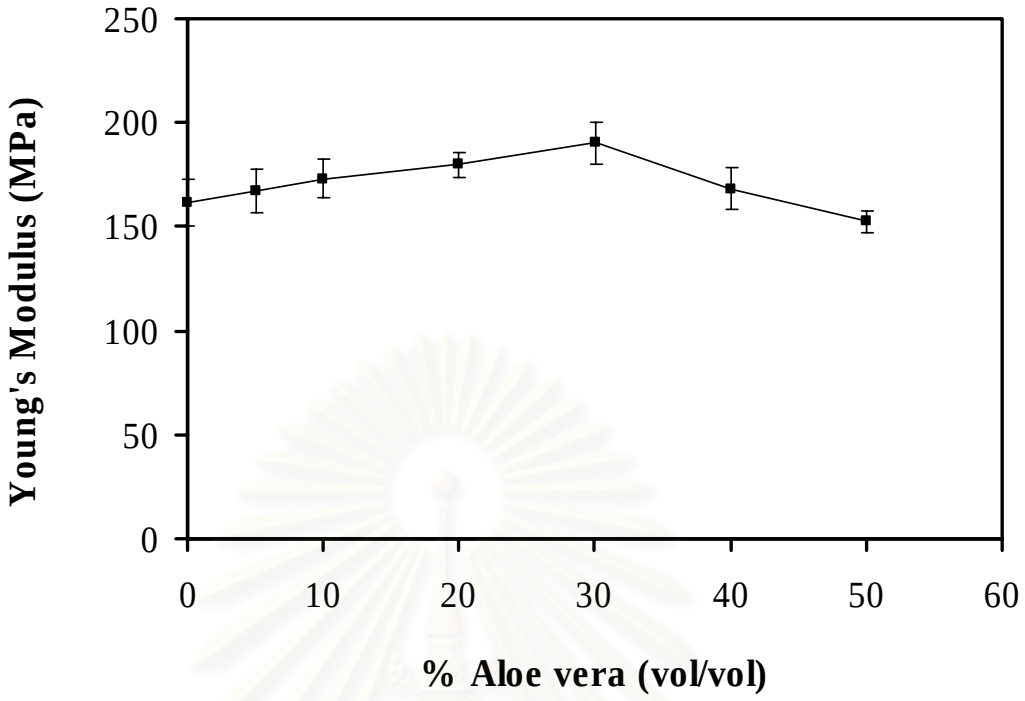


Figure 5.5 Young's modulus of BC-aloe vera film as a function of aloe vera content in culture medium.

Figure 5.5 illustrates the change of the Young's modulus when increasing the aloe vera gel concentration. At the average thickness of 0.030 mm, the Young's modulus increased with the content of aloe vera gel supplement correspondingly to the effect on tensile strength. Supplementation of aloe vera gel from 0% to 30% increased the Young's modulus from 161.80 to 190.20 MPa. However, the tensile strength decreased from 190.20 MPa to 152.4 MPa as the aloe vera content was increased from 30% to 50%. The maximum average values of Young's modulus of the films was 190 MPa or an increase by about 1.2 folds at 30 %(v/v) aloe vera gel supplement. The increase in the Young's modulus indicated that the BC-aloe vera film had more both strengthened and strained. This also indicated that the increment of the young's modulus of the BC-aloe vera films depended on the amount of the aloe vera content in the similar way as it was observed previously on the tensile strength.

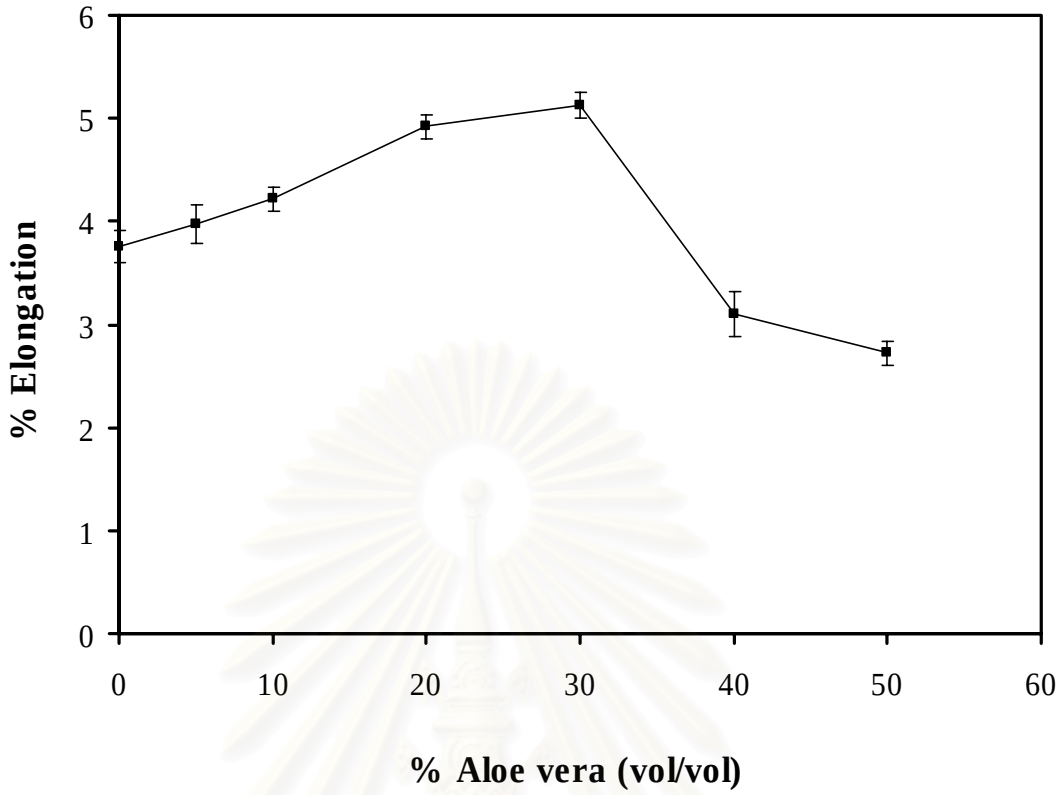


Figure 5.6 The elongation at break of the BC-aloe vera films as a function of aloe vera content in culture medium.

Figure 5.6 shows the effect of the change in percentage of elongation at break when the percentage of aloe vera content increased. At the average thickness of 0.030 mm, the elongation at break increased when the aloe vera concentration increased from 0% to 30%. However, the elongation at break decreased if the aloe vera concentration was higher than 30%. The maximum average value was 4.92 MPa at 30 % (v/v) addition of aloe vera. Later on, the elongation at break increases from 3.75 to 4.92 MPa and 2.72 MPa when aloe vera content was increased from 0% to 30% and 50 % (v/v), respectively. This implies that increasing the elongation at break of the BC-aloe vera films depending on the amount of aloe vera content because the aloe vera could incorporate with cellulose fiber.

In summary, the aloe vera supplement provided good impacts on the mechanical properties of BC-aloe vera films due to the values of the change of tensile strength, Young's modulus, and the elongation at break. The results indicated that the

supplement of aloe vera gel in the medium during the biosynthesis could improve the strength and elastic of the developed BC films by 1.6 and 1.3 folds, respectively. This observation is very important for medical view, for example, an elastic dressing fitting the wound will more provide good protection against external infections.

5.5 Equilibrium Water Content (EWC)

To compare the ability to hold water of the BC-aloe vera film and the BC film, the study via the Equilibrium Water Content (EWC) was employed.

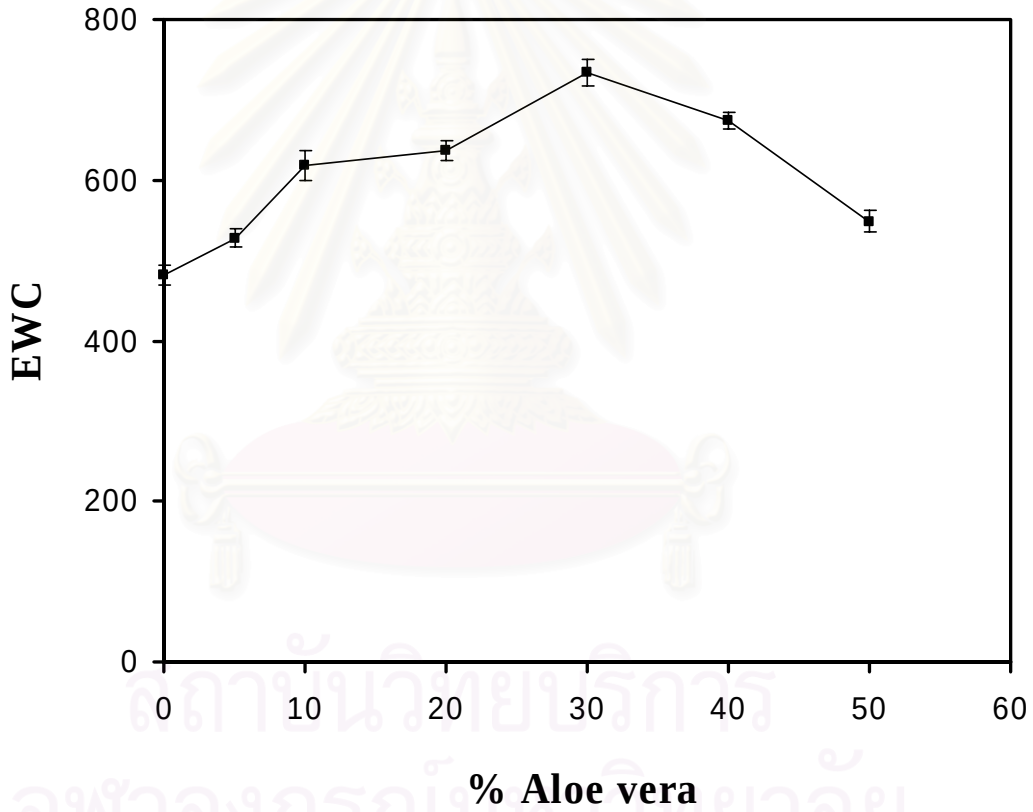


Figure 5.7 The equilibrium water content (EWC) of the BC-aloe vera films as a function of aloe vera content in culture medium.

As can be seen from Figure 5.7, the equilibrium water content (EWC) of the BC film was 481%. The EWC increased when increasing aloe vera gel concentrations from 0 -30 % (v/v). However, the EWC was reduced when the aloe vera content was

higher than 30%. Supplementation of 30 % aloe vera gel during the biosynthesis of the films showed the maximum increment in EWC at 735% or about 1.5 times in comparison to that of the BC film. The increase of the EWC for the BC-aloe vera films depended on the amount of aloe vera content. Since aloe vera gel is hydrophilic and can be well incorporated into the cellulose network structure, the conjugation of cellulose fibril and aloe vera gel increased the EWC of the films. On the other hand, the EWC was inferior when the aloe vera gel content was higher than 30% (v/v). The weaken structure of the film according to the miscellaneous combination between BC fibril and aloe vera gel at the excessive gel content should be the reason for the drop of the EWC.

5.6 XRD (X-Ray Diffraction)

Generally, X-Ray Diffraction (XRD) is used for structure measurement of polymers. The XRD patterns of BC and BC-aloe vera films were shown in Figure 5.8 and Table 5.1. The film of XRD pattern of the BC demonstrated that the peaks observed at 14.56°, 16.87° and 22.74° were attributed to the BC cultured in static circumstance. The broad diffraction peaks observed for the BC because the BC was not a completely crystalline material (Hong *et al*., 2006). The diffracting grams of the addition of the aloe vera gel in the range of 0% to 50% v/v in the culture medium showed nearly no obvious differences from that of the BC.

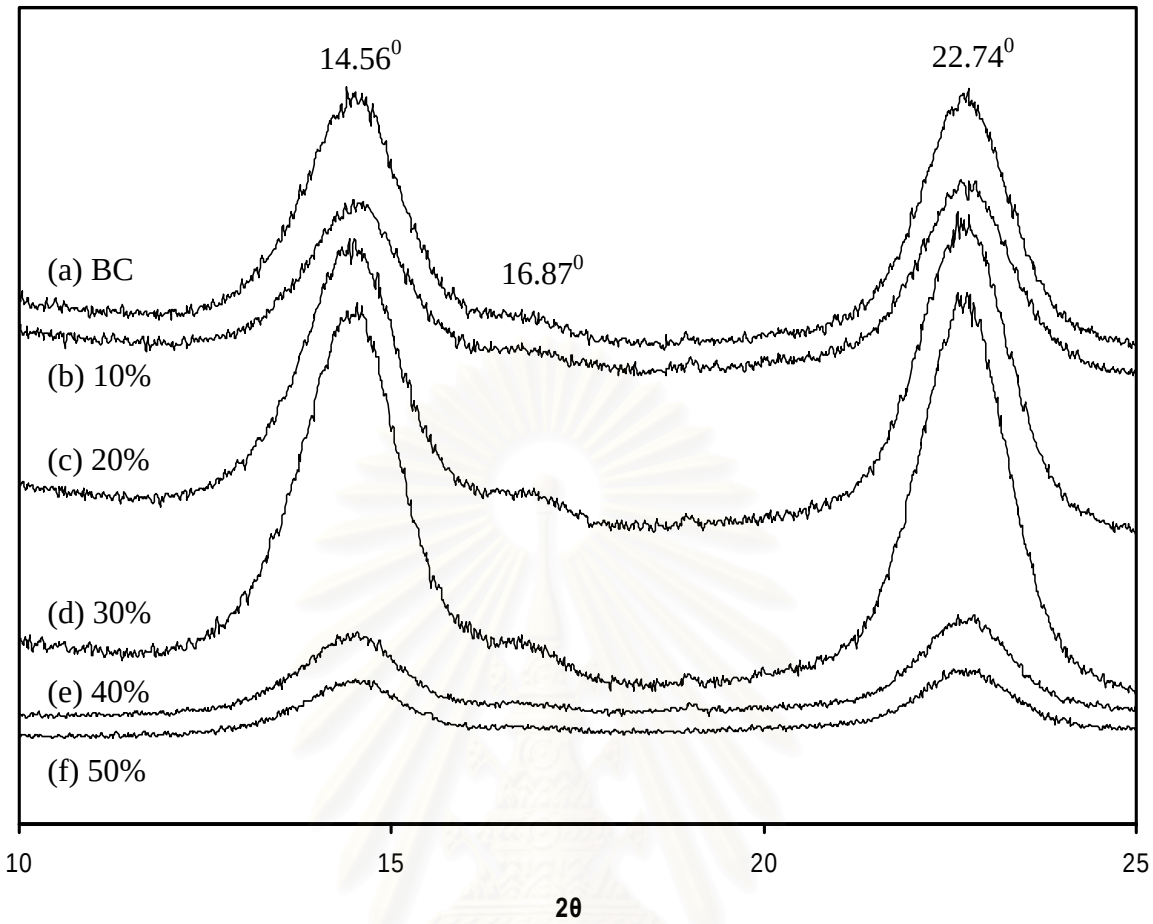


Figure 5.8 X-ray patterns of BC and BC-aloe vera films; (a) BC; (b) BC-10% aloe vera; (c) BC-20% aloe vera; (d) BC-30% aloe vera; (e) BC-40% aloe vera; and (f) BC-50% aloe vera.

Table 5.1 CI, Reflective-Angle, *d*-Spacing Values of BC and BC-aloe vera (10% - 50%) membrane.

Membrane	CI	2θ [*d*(110)]	2θ [*d*(110)]	2θ [*d*(020)]
0%	78.45	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)
10%	79.16	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)
20%	80.25	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)
30%	82.77	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)
40%	69.75	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)
50%	68.52	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)

According to the Table 5.1, a crystalline index of individual BC was 78.45. As furthermore investigate, these crystalline indices would be increased until the adding concentration of the aloe vera reaches 30%. Then, they would be decreased. This indicates that the films of BC – aloe vera formed with the addition of aloe vera gel in the range of 0% to 30% (v/v) was arranged better than those formed with the aloe vera contents higher than 30%.

5.7 Porosity

Surface area and porosity are important characteristics, capable of affecting the quality and utility of many materials. Normally, BET method is one of the most powerful techniques to estimate surface area of material by physical adsorption of gas molecules (Brunauer, Emmett and Teller, 1938).

Table 5.2 Surface area and pore diameter of the BC and BC-aloe vera analyzed by BET analyzer.

Sample	**Pore Diameter (A^0)**	**Surface Area (m^2/g)**
BC (dry form)	224	12.6
At 10% Aloe vera(dry form)	53	14.2
At 30% Aloe vera(dry form)	41	15.7
At 50% Aloe vera(dry form)	38	19.5
BC (reswollen form)	612	55.2
At 10% Aloe vera(reswollen form)	154	59.1
At 30% Aloe vera(reswollen form)	150	62.4
At 50% Aloe vera(reswollen form)	138	65.2

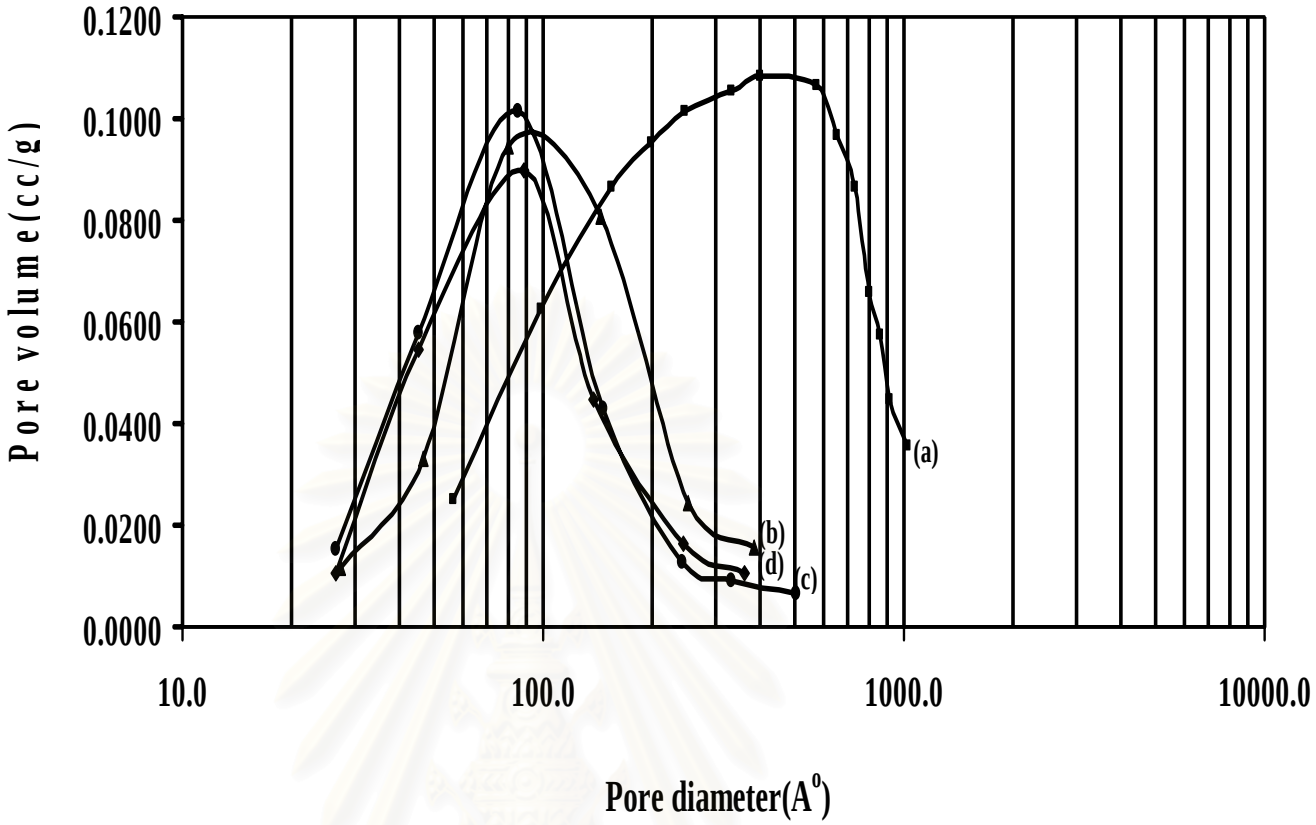


Figure 5.9 The typical pore size distribution of BC and BC-aloe vera films(reswollen films); (a) bacterial cellulose, (b) BC-aloe vera film at 10% aloe vera, (c) BC-aloe vera film at 30% aloe vera, (d) BC-aloe vera film at 50% aloe vera

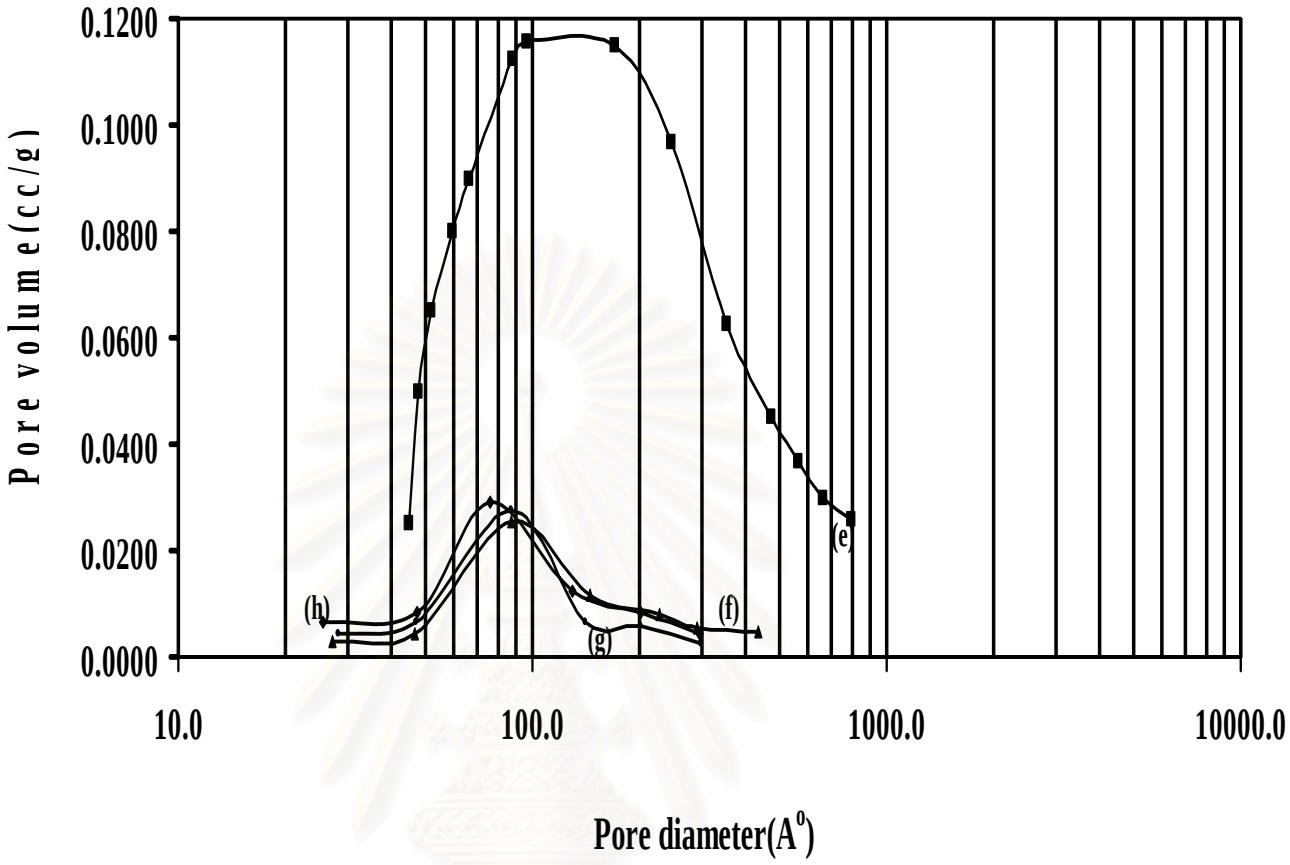


Figure 5.10 The typical pore size distribution of BC and BC-aloe vera films (drying film) ; (e) bacteria cellulose, (f) BC-aloe vera film at 10% aloe vera , (g) BC-aloe vera film at 30% aloe vera, (h) BC-aloe vera film at 50% aloe vera .

The total surface area and average pore size of the BC film determined by BET were 12.62 m^2/g and 224 Å (Sungruangroj *et al.*, 2006), respectively. As shown in Figure 5.9, 5.10 and Table 5.2, the result shows that the BC-aloe vera film had pore sizes much less than that of the BC, while the surface area was slightly increased. The pore sizes of the 10% aloe vera, 30% aloe vera and 50% aloe vera were 53 A°, 41 A° and 38 A°, respectively.

For the reswollen form, the total surface area and the average pore size of the BC film determined by the BET were 5.5 m^2/g and 612 Å, respectively. The pore size of the 10% aloe vera, 30% aloe vera, and 50% aloe vera were 154 A°, 150 A° and 138 A°, respectively. This result well supports the conclusion from SEM micrographs.

5.8 Water Vapor Permeability Test (WVTR)

From the test, the water vapor transmission rate (WVTR) of the BC film was 1,616.5 g/m^2 day, whereas those for the BC-aloe vera film at 10%, 30%, and 50% concentrations were 1,821, 2,029.5, and 1,066 g/m^2 day, respectively. The result directly supported the test on equilibrium water content that showed the increment of EWC in the BC-aloe vera films with the addition of aloe vera gel from 0 to 30% (v/v), and then it was decreased with the supplement of aloe vera gel more than 30% (v/v). Therefore, it could be concluded that the developed BC-aloe vera film was high hydrophilic, which can be further applied in water separation processes.

5.9 Cell Study

In this study, cytotoxic effect of BC and BC-aloe vera films and their effects on proliferation and morphology of human skin cells were studied. The major components of the skin cells, human transformed skin keratinocyte (HaCat), and human normal skin fibroblast (CRL- 2211) were used in this study. Both cell lines were purchased from the American Type Culture Collection (ATCC). The human skin cell cultures were seeded on Polystyrene culture plates (control) and on the film samples, and were then cultivated for 0, 24, and 48 hr. Those different skin cells was compared with the reference cells cultured on the Polystyrene The relative cell numbers in comparison to the initial concentration of the control were shown in Figures 5.11, 5.12, and 5.14, respectively. Also, the morphology of those compared cells was illustrated in Figures 5.13 and 5.15.

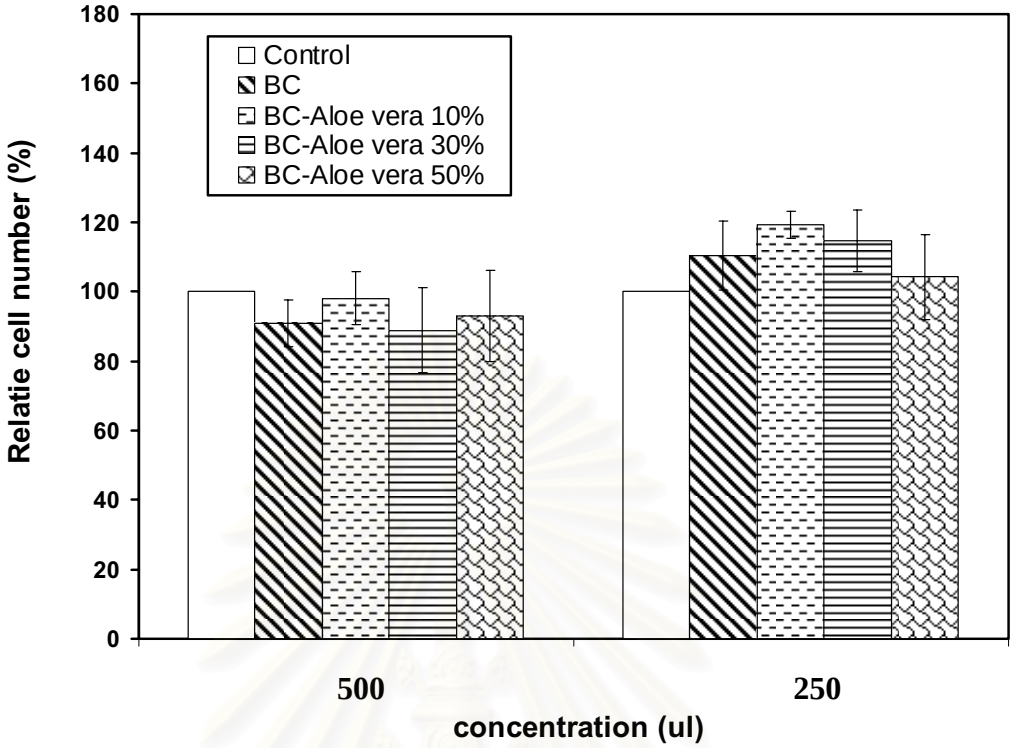


Figure 5.11 Toxicity of BC and BC-aloe vera films at 10%, 30% and 50%.

The experimental result in Figure 5.11 demonstrated that the BC and BC- Aloe vera films had no cytotoxic effect on mouse cell (L929). The relative cell number cultivated on the control plate and the films of BC and BC-Aloe vera for all BC-Aloe vera composition after seeding 250 and 500 μL of the cell culture into 1 milliliter of the culture medium and cultivation for 30 min were compared (Figure 5.11)

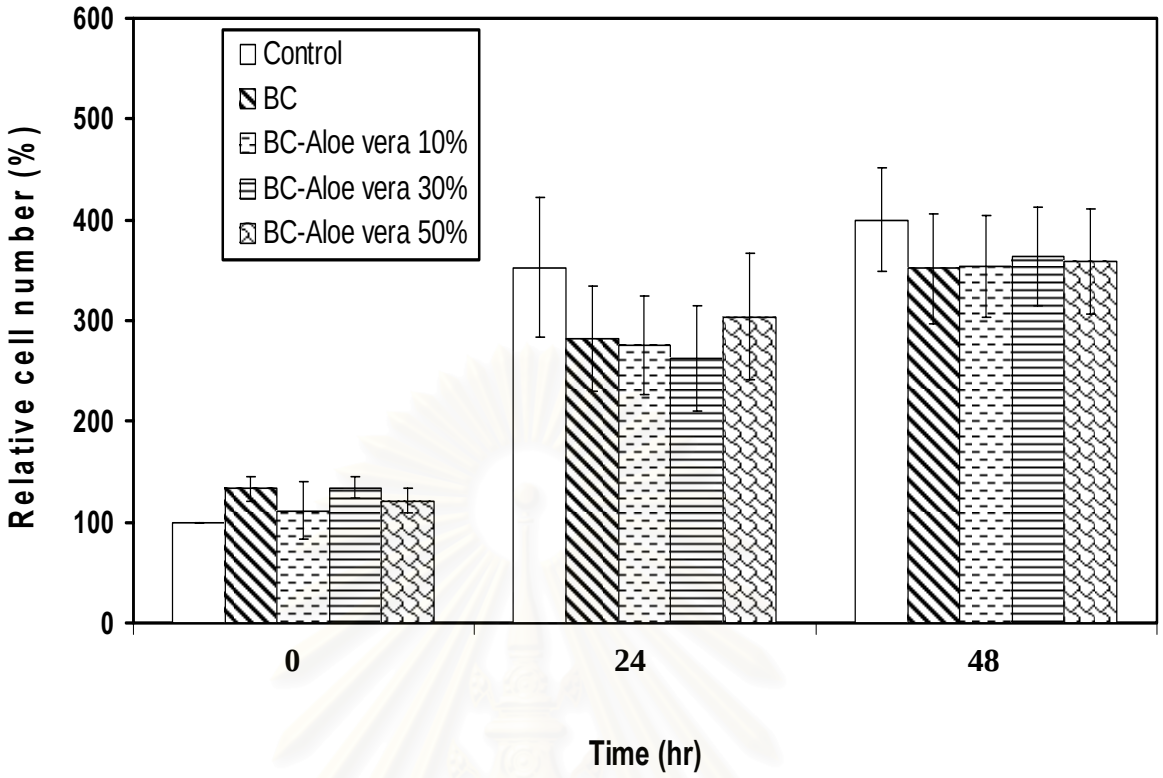
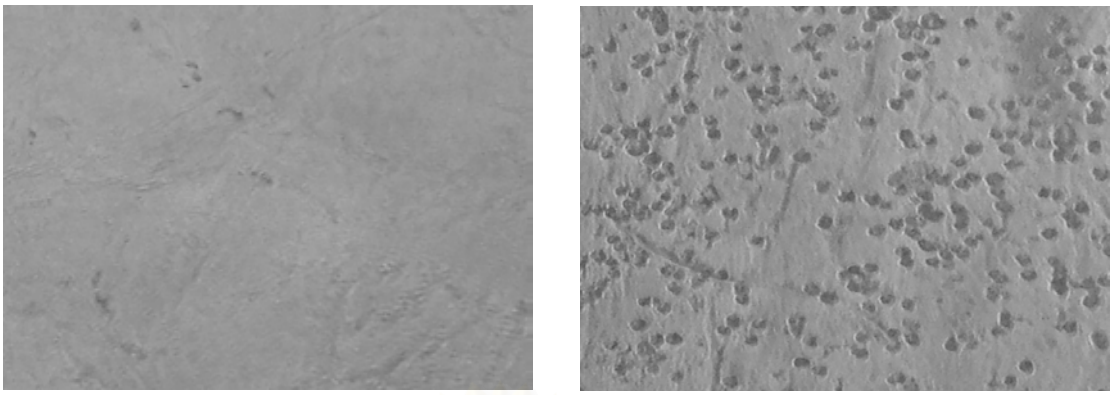
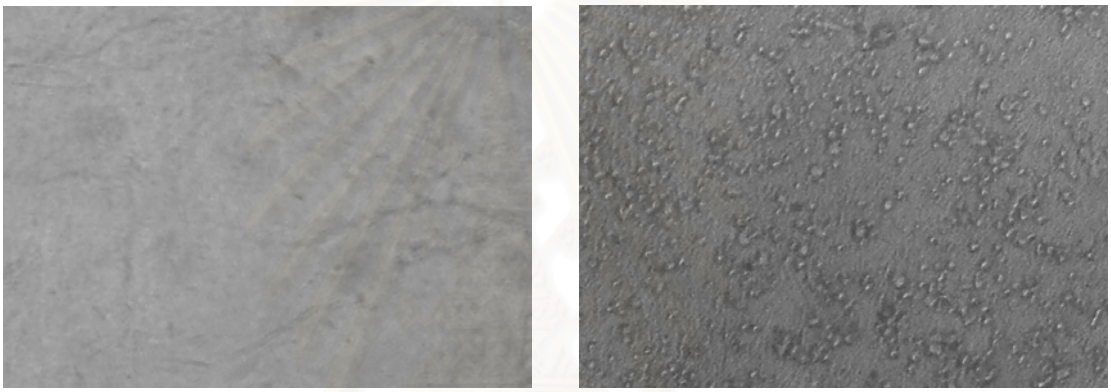


Figure 5.12 Proliferation of HaCat on control, BC, BC-aloe vera at 10%, 30%, and 50%.

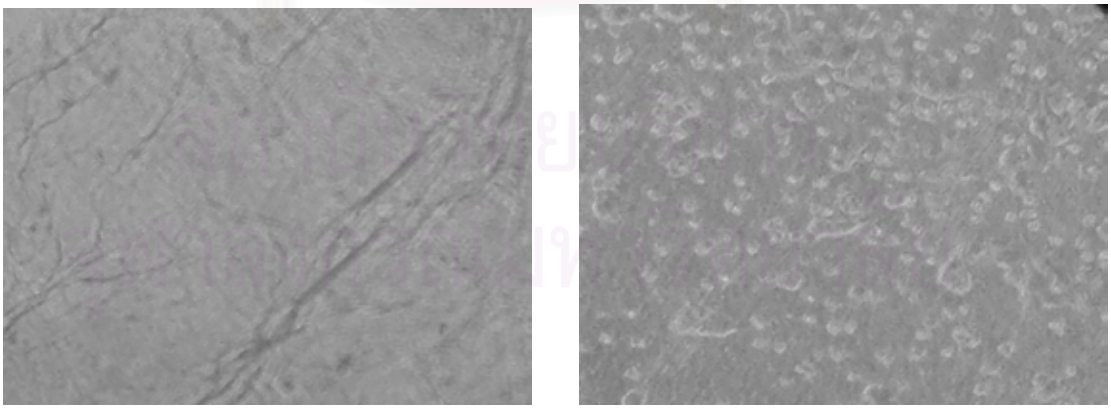
As shown from Figure 5.12, both the BC and BC-aloe vera films supported the proliferation and spread of HaCat. The percentage of relative number of cells after seeding on the films for 24 and 48 h was compared to the cells cultured on the polystylene culture plate (Figure 5.12). The microscopy demonstrated that keratinocytes spread over the surface of BC and BC-Aloe films at 24 h and became a confluent monolayer at 48 h. The morphologies of these films are illustrated in Figure 5.13.



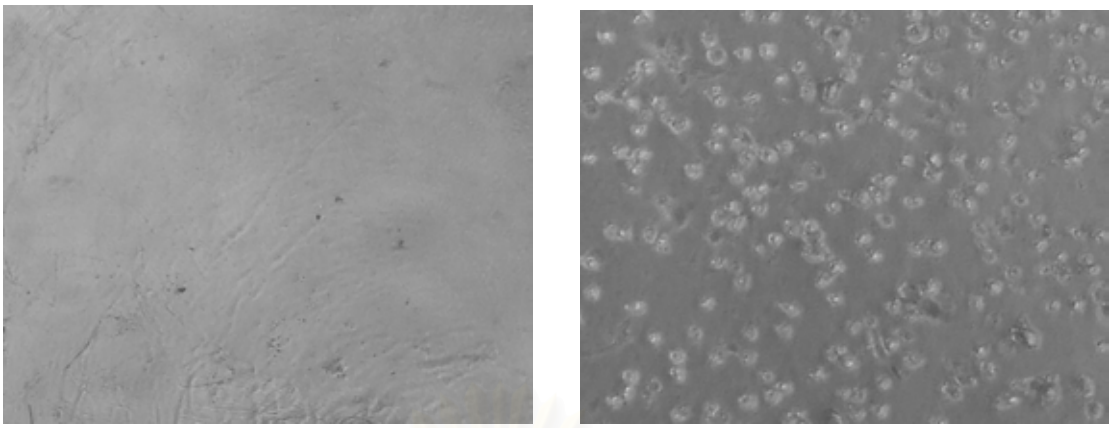
BC film



BC-aloe vera 10%



BC-aloe vera 30%



BC-aloe vera 50%

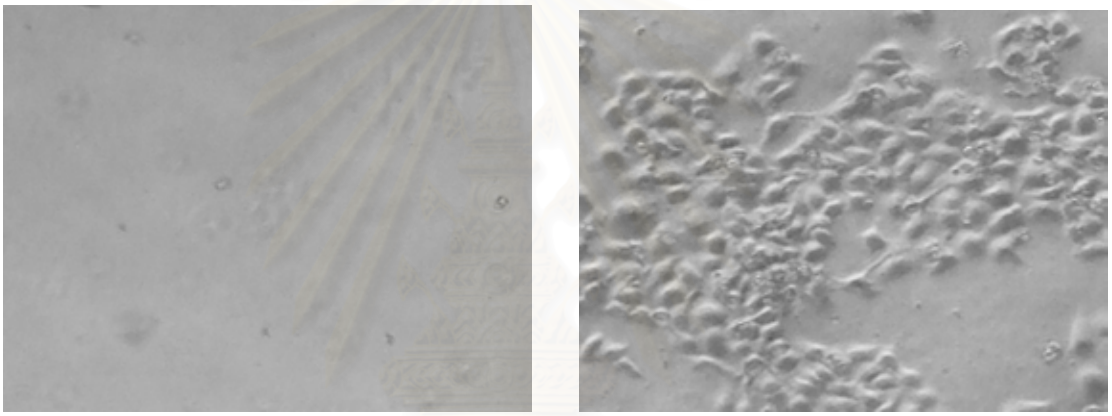


plate control

Figure 5.13 Morphology of before and after cell growth for control, BC, BC-aloe vera at 10%, 30%, and 50% at 48 hours of cultivation.

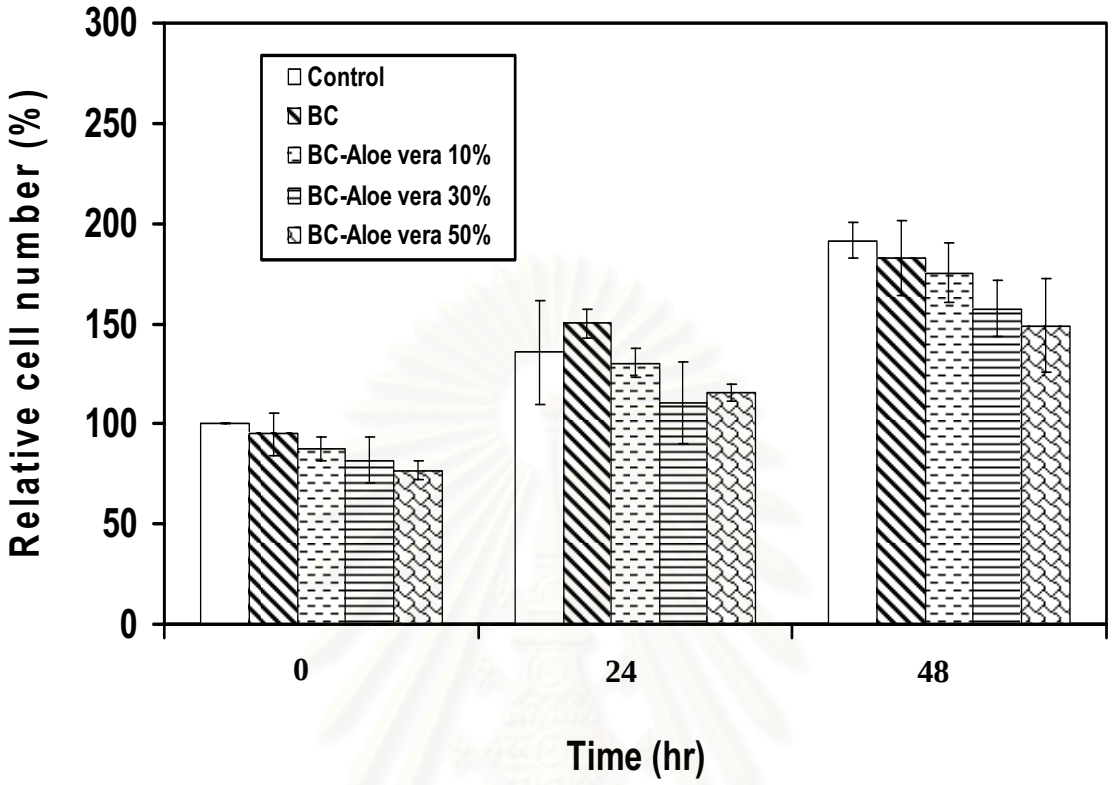
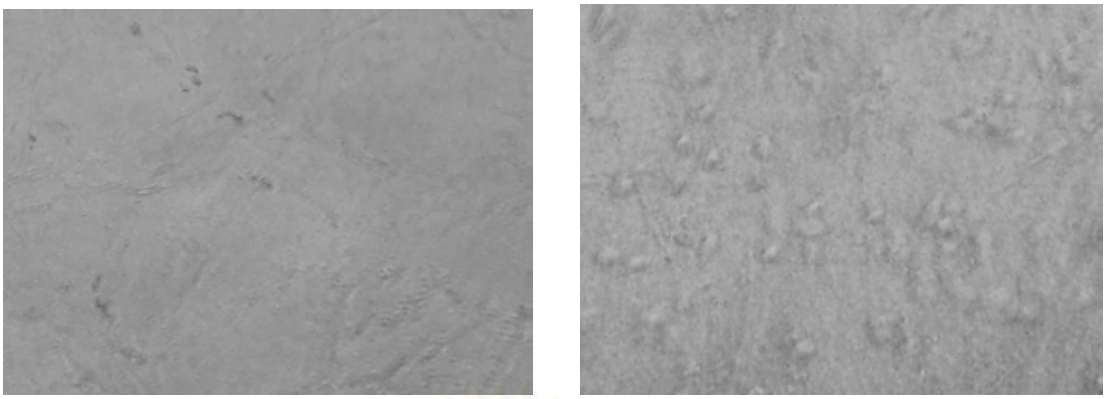
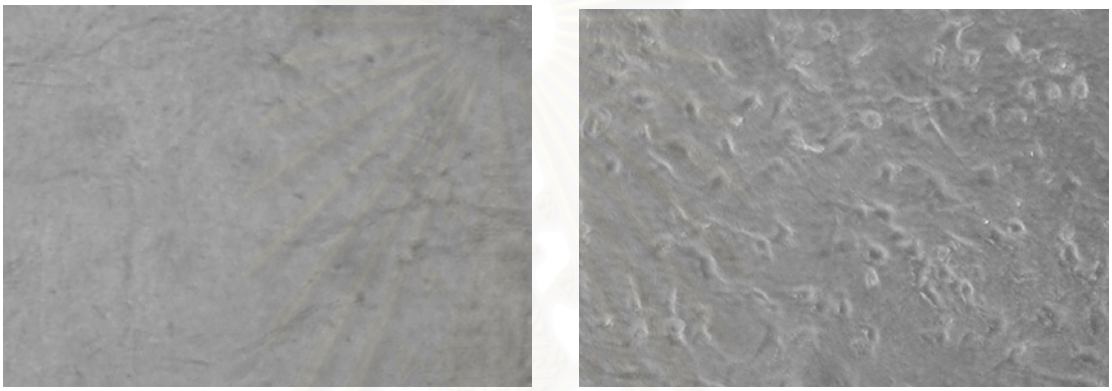


Figure 5.14 Proliferation of Fibroblast on control, BC, BC-aloe vera at 10%, 30%, and 50%.

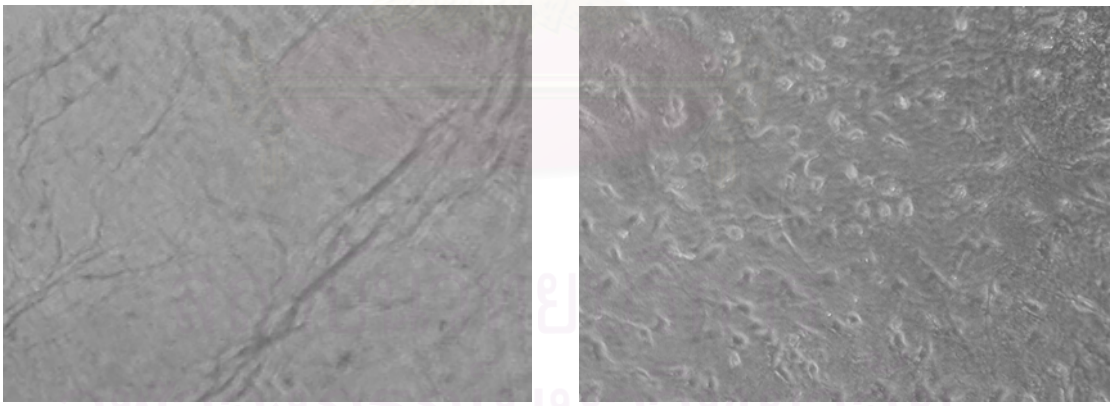
As shown from Figure 5.14, both the BC and BC-aloe vera films supported proliferation of Fibroblast. The relative cell numbers on the control plate and the films were all increased with cultivation time from 0 – 48 hours. Overall, the growth of the cell on the control plate was slightly better than that on the BC and on BC-aloe vera, respectively. However, the patterns of cell distribution and cell morphology on the BC film and the BC-Aloe vera were different. The microscopes in Figure 5.15 demonstrated that Fibroblast could spread over the surface of BC-Aloe vera in the similar pattern as the culture on the control plate film, whereas proliferative fibroblasts on the BC film formed clumps in various sizes among a number of isolated cells and were easily detached from the BC film over time. This observation on the BC film was previously reported by the study of Davis *et al.*, (1992). They found that there was one type of polysaccharides named acemannan contained in aloe vera which affected the better spread and proliferation of fibroblast cell over the BC-Aloe vera's surface than over the BC film.



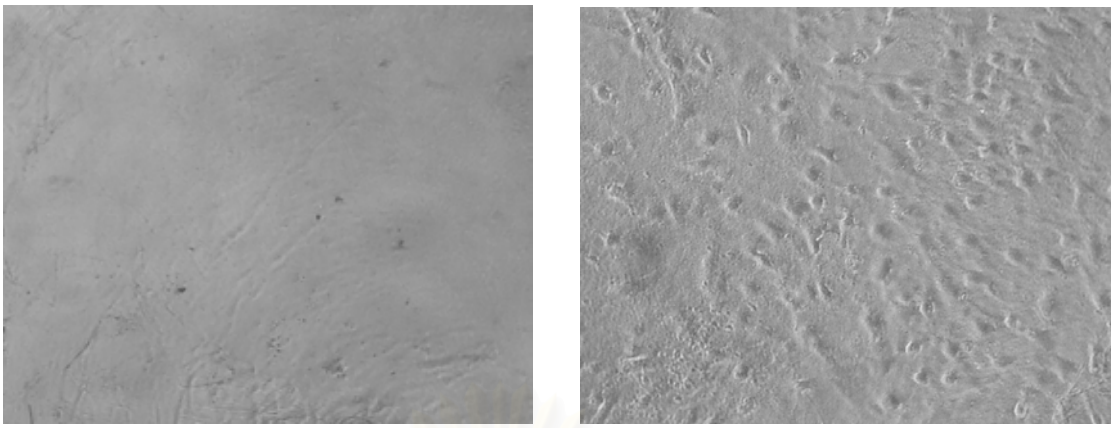
BC film



BC-aloe vera 10%



BC-aloe vera 30%



BC-aloe vera 50%

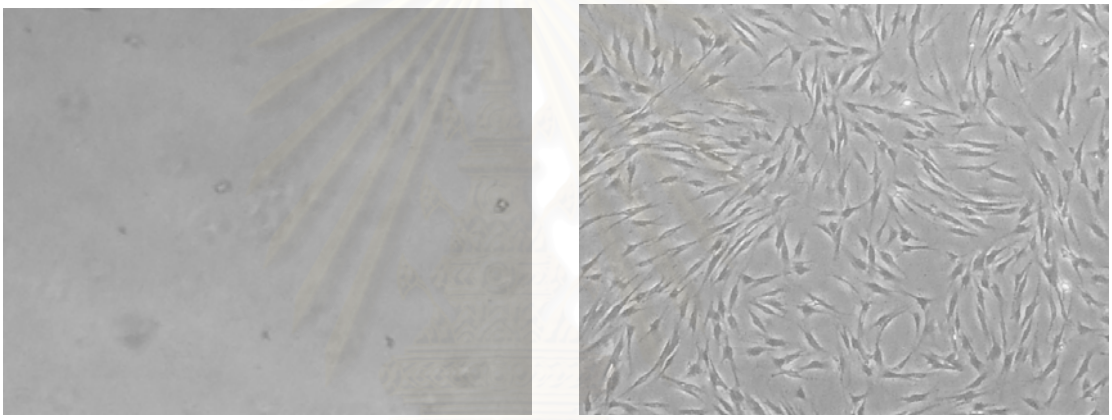


plate control

Figure 5.15 Morphology of before and after cell growth for control, BC, BC-aloe vera at 10%, 30%, and 50% at 48 hours of cultivation.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Improvement of bacteria cellulose by the addition of different concentrations of aloe vera in the culture medium via biosynthesis was found to provide many valuable in medical applications. The increments in mechanical properties, equilibrium water content (EWC), crystalline index, and average surface area were apparently found in this study.

Among the 0%, 5%, 10%, 20%, 30%, 40%, and 50% additions of the aloe vera concentrations, the 30% gave the best in mechanical properties, equilibrium water content (EWC), and crystalline index. Regard as the mechanical properties, the BC-aloe vera film with aloe vera's content more than 30 % (v/v) demonstrated weaken film construction due to the miscellaneous structure of the film from excessive aloe vera gel content. Moreover, the inhibition effect on the film biosynthesis was observed with the aloe vera gel supplement greater than 30%.

In addition, FTIR spectroscopy was used to evaluate the interaction between the BC fiber and aloe vera molecules. This result indicated that the interactions occurred between the hydroxyl groups of the BC fiber and the amino groups of the aloe vera.

For BC film, the tensile strength, Young's modulus, the elongation at break, the equilibrium water content (EWC), the average crystallinity index, and the average pore diameter of BC film were 5.32 MPa, 161.80 MPa, 3.75%, 481%, 78.45, and 224 A^{o}, respectively. For BC-aloe vera film with 30% aloe vera, the tensile strength, Young's modulus , the elongation at break, the equilibrium water content (EWC), the

present average crystalline index, and the average pore diameter of BC-aloe vera film were 8.67 MPa, 190.20 MPa, 4.92%, 735%, 82.77, and 41A^{o}, respectively.

The study of cell growth demonstrated that the BC-aloe vera film had no toxicity effect on mouse cell and supported cell proliferation and spread of the keratinocyte (HaCat) and fibroblast (CRL-2211).

6.2 Recommendations for Future Studies.

Based on this study, further studies for the improvement of bacterial cellulose film are recommended.

1. The study of modifying bacterial cellulose by synthesizing with other natural polymer.

2. The study for more applications such as the use in membrane separation.

REFERENCES

Alvarez, O.M., Patel, M., Booker, J. and Markowitz, L. Effectiveness of a biocellulose wound dressing for the treatment of chronic venous leg ulcers: results of a single center randomized study involving 24 patients. Wounds. 16(7)(2004): 224–233.

Atherton, P. The Essential Aloe Vera. Newport Pagnell: Mill Enterprises. (1997)

Brown, Jr. R.M. Advances in Cellulose Biosynthesis Polymers from Biobased Materials. New Jersey, 1991.

Choi, S., Chung,M.-H. "A review on the relationship between Aloe Vera components and their biological effects". Seminars in Integrative Medicine, Vol 1, No 1 (March), 2003: 53-62

Czaja,W., Krystynowicz, A. and Bielecki , S. Microbial cellulose the natural power to heal wounds. Biomaterials. 27(2006): 145 – 151.

Davis RH, Stewart GJ, Bregman PJ: Aloe vera and the inflamed synovial pouch model. J Am Podiatr Med Assoc. 82(1992):140-148

Davis RH, DiDonato JJ, Hartman GM, Haas RC. Anti-inflammatory and wound healing activity of a growth substance in aloe vera. J Am Pediatric Med Assoc. 84(1994):77–81.

Dennis P., Ya Fen Zhu," Evaluation of aloe vera gel gloves in the treatment of dry skin associated with occupational exposure" 2003

Dunphy JE: Modern biochemical concepts on the healing of wounds: Wound healing. Baltimore, MD, Williams and Wilkins, 1974, pp 22-31

Fontana, J.D., de Souza, A.M., Fo0ntana, C.K., Torriani, I.L., Moreschi, J.C., Gallotti, B.J., de Souza, S.J., Narcisco, G.P., Bichara, J.A. and Farah, L.F.X. Acetobacter cellulose pellicle as a temporary skin substitute. Applied Biochemistry and Biotechnology. 24/25(1990): 253-263.

Gardner, K. H. and Blackwell, J.,"The structure of native cellulose", Biopolymers. 13(1974): 1975-2001.

Grindlay D, Reynolds T. The Aloe vera phenomenon: A review of the properties and modern used of the leaf parenchyma gel. J Ethnopharmacol. 16(1986): 117–151.

Heggers JP, Kucukcelebi A, Listengarten D, Stabenau J, Ko F, Broemeling LD, Robson MC, Winters WD. Beneficial effect of Aloe on wound healing in an excisional wound model. J Altern Complement Med. 2(1996): 271–277.

Hong, L., Wang, Y.L., Jia, S.R., Huang, Y., Gao, C. and Wan, Y.Z. Hydroxyapatite/bacterial cellulose composites synthesized via a biomimetic route. Materials Letters. 60(2006): 1710 – 1713.

Kay M., Mary lynn J., Jerzy J., Kimberly P., Dale A., Lawrence P., and Anna T.," Effects of Aloe vera on Gap Junctional Intercellular Communication and Proliferation of Human Diabetic and Nondiabetic Skin Fibroblasts "The Journal of Alternative and Complementary Medicine" Volume 9, Number 5, 2003. 711–718

Klemm D, Schumann D, Udhardt U, Marsch S. Bacterial synthesized cellulose artificial blood vessels for microsurgery. Prog Polym Sci. 26(2001):561–603.

Kucharzewski, M., Slezak, A. and Franek, A. Topical treatment of nonhealing venous ulcers by cellulose membrane. Phlebologie. 32(2003): 147 – 151.

Lee, Y.M., Kim, S.H. and Kim, S.J. Preparation and characterization of β - chitin and poly (vinyl alchol) blend. Polymer. 37(1994): 5897 - 5905.

Mayall R.C., Mayall A.C., Mayall L.C., Rocha H.C. and Marques L.C. Tratamento das ulceras troficas dos membros com um novo substitute da pele. Rev Bras Cir. 80(4) (1990):257–283.

National Organic Standards Board Technical Advisory Panel Review Compiled by Organic Materials Review Institute for the USDA National Organic Program. (2001)

Neeracha S., Wunwisa S., Ruchadaporn K., Tanom B., Prasit P., and Muenduen P., "Growth of Human Keratinocytes and Fibroblasts on Bacterial Cellulose Film." Biotechnol. Prog. 22(2006):1194-1199

Reynolds T, Dweck AC: Aloe vera leaf gel: a review update. J Ethnopharmacol 68(1999):3-37

Sangrungraungroj, W. Development of cellulose membrane from nata-de-coco for material separation. Master's thesis, Department of chemical engineering, Faculty of Engineering, Chulalongkorn University, 2003.

Schmidt JM, Greenspoon JS: Aloe vera dermal wound gel is associated with a delay in wound healing. Obstet Gynecol 78(1991):115-117

Suwanmajo, T. Development of nanostructure membrane from regeneragerd bacterial cellulose. Master's thesis, Department of chemical engineering, Faculty of Engineering, Chulalongkorn University, 2006.

Vandamme E. J.,De Baets S., Vanbaelen A., Joris K., and De Wulf P."Improved production of bacterial cellulose and its application potential" polymer Degrnddon and Sfabihfy 59 (1997): 93-99

Yagi A, Egusa T, Arase M, et al: Isolation and characterization of the glycoprotein fraction with a proliferation- promoting activity on human and hamster cells in vitro from Aloe vera gel. Planta Med. 63(1997):18-21

APPENDICES

APPENDIX A

A.1 Supercritical Drying Method

In this study, supercritical drying method was used for the preparation of porous BC and BC-aloe vera film. Firstly, the films were dipped in distilled water for 24 hours. After that, to replace water with ethanol, the swollen films were immersed in 10, 30, 50, and 70 % (w/v) ethanol for 30 mins in each step and in 100 % (w/v) ethanol for 1 hour, respectively. Lastly, the swollen films were dried by using supercritical drying method.

In supercritical drying method, the films were placed in a vessel inside the high-pressure cell with inner diameter 10 cm. The cell was immediately filled with supercritical CO_2 and controlled at temperature = 40 ^{0}C and pressure = 1200 Psi (the critical point of carbon dioxide; P_c =1072 Psi and T_c = 31 ^{0}C). Temperature and pressure were selected such that the CO_2 and ethanol inside the films were fully miscible. Subsequently, the cell was flushed by adding fresh CO_2 at the same conditions of pressure and temperature in order to replace the residual ethanol inside. The adding was performed for 2 hours and then the system was slowly depressurized at a constant rate of 150 psi/min to remove CO_2.

A.2 Cell Study

A.2.1 Measurement of Toxicity

A plate cell L929 was first added into a 24-well plate culture. Each well contained 50,000 cells of the L929 and had 500 µl DMEM with 10% serum for cell seeding. Then, the plate was shook to make the cells distributed over the well. After that, the L929 cell was seeded at 37 °C with 5% CO supply. The cell was next staffed by sucking out the DMEM with 10% serum, and the 500 µl DMEM without serum was subsequently added and seeded at the same condition (37 °C with 5% CO_2 supply). Next, the last added DMEM without serum was again sucked out and then 50 µl of new DMEM without serum after being sterilized for 24 hr was added and later seeded for 24 hr. The sample was finally measured of cell living by MTT Assay.

A.2.2 Measurement of Cell Keratinocytes using Plate Cell HaCat

Firstly, the material was soaked in clean water and then sterilized at 121°C for 20 min in an Autoclave. The sterilized material was next put in the bottom of the 24-well culture plate and covered with stainless steel ring under Aseptic condition. After that, it was added with 500 µl/well DMEM with 10% serum. The plate cell HaCat was then added into the 24-well plate culture of 50,000 cell/well and softly shook to distribute the cell over the well at 37 °C with 5% CO_2. The cell was next staffed by sucking out the DMEM with 10% serum, and the 500 µl DMEM without serum was subsequently added and seeded at the same condition. Lastly, the samples seeded at 0, 24, and 48 hr were measure of cell living by MTT Assay.

Note: HaCat is normal human skin keratinocyte.

A.2.3 Measurement of Cell Keratinocytes using CRL-2211

For this experiment, the procedure is the same as that the measurement of cell keratinocytes using plate cell HaCat except that 30,000 cell/well of plate cell CRL-2211 was added into the 24-well plate culture instead.

Note: CRL-2211 is Leg human skin fibroblast.

- MTT Assay

Firstly, 5 mg/ml MTT solution was prepared in culture medium without Phenol red. Then the culture medium was sucked out and subsequently washed the cells twice with PBS (Phosphate buffer saline) solution. The culture medium without Phenol red was mixed with the MTT that was previously prepared at 6:1 ratio and later shook. Next, 320 µl of mixed solution was added into each well and seeded in incubator at 37 °C with 5% CO_2 for 30 min. The last solution was sucked out and subsequently washed with PBS solution for 2 times, this made the solution color changed. To dissolve that color, 900 µl DMSO (Dimethyl sulfoxide) and 100 µl Glycine buffer were dropped into the solution. The final solution was measured of absorbance at 540 nm and compared the amount of cell with standard curve.

- 10% Serum Culture Medium

This consists of:

1. DMEM (dulbecco's Mdified Eagle's medium)
2. Antibiotic, Antimycotics 1% (Penicilline & Streptomycine & Amphotericine B)
3. L-Glutamine 1%
4. Lactalbrumine 0.2%

- Serum Free Culture Medium

This consists of:

1. DMEM (dulbecco's Mdified Eagle's medium)
2. Antibiotic, Antimycotics 1% (Penicilline & Streptomycine & Amphotericine B)
3. L-Glutamine 1%
4. Fetal Calf Serum 10%

APPENDIX B

Table B1 Data of Figure 5.4.

Aloe vera content (%vol)	Tensile strength (MPa)						
	1	2	3	4	5	Average	SD
0	5.63	5.53	5.32	5.02	5.12	5.32	0.26
5	5.69	5.94	5.75	5.74	5.70	5.76	0.10
10	6.10	6.89	6.32	6.45	6.48	6.45	0.28
20	7.85	7.61	7.92	7.84	8.03	7.85	0.15
30	8.50	8.67	8.72	8.83	8.63	8.67	0.12
40	4.86	4.85	4.35	4.37	4.48	4.58	0.25
50	3.48	3.45	3.26	3.54	3.37	3.42	0.11

Table B2 Data of Figure 5.5.

Aloe vera content (%vol)	Young's Modulus (MPa)						
	1	2	3	4	5	Average	SD
0	168	153	175	148	165	161.80	11.08
5	170	175	154	159	178	167.60	10.32
10	169	177	168	187	164	173	9.14
20	182	179	170	185	184	180	10.04
30	195	188	194	200	174	190.02	10.01
40	162	178	155	171	176	168.40	9.71
50	148	161	153	149	151	152.40	5.18

Table B3 Data of Figure 5.6.

Aloe vera content (%vol)	Elongation at break (%)						
	1	2	3	4	5	Average	SD
0	3.78	3.99	3.68	3.55	3.77	3.75	0.16
5	3.71	3.92	3.99	4.21	4.07	3.98	0.18
10	4.21	4.31	4.35	4.05	4.18	4.22	0.12
20	4.86	4.94	4.99	4.76	5.05	4.92	0.11
30	5.01	5.25	5.13	5.24	4.98	5.12	0.13
40	2.88	3.23	2.86	3.25	3.28	3.10	0.21
50	2.54	2.68	2.85	2.79	2.74	2.72	0.12

Table B4 Data of Figure 5.7.

Aloe vera content (%vol)	Equilibrium Water Content (%)						
	1	2	3	4	5	Average	SD
0	479	487	460	490	490	481	12.67
5	545	526	516	530	520	527	11.21
10	605	616	600	625	645	618	17.85
20	652	620	630	640	640	636	12.03
30	756	711	742	730	735	735	16.51
40	668	686	670	661	681	673	10.13
50	547	569	542	532	550	548	13.58

Table B5 Data of Figure 5.11.

- Number of cell growth

Toxicity						
Type of film		**Control**	**BC**	**Aloe Vera**		
				10%	**30%**	**50%**
24 hr (250 µl)	1	0.677	0.719	0.806	0.713	0.615
	2	0.695	0.846	0.804	0.807	0.743
	3	0.648	0.67	0.799	0.795	0.745
	Average	0.673	0.745	0.803	0.772	0.701
	SD	0.0237	0.091	0.004	0.051	0.074
24 hr (500 µl)	1	0.783	0.687	0.73	0.635	0.652
	2	0.811	0.701	0.763	0.668	0.713
	3	0.787	0.776	0.843	0.811	0.851
	Average	0.794	0.721	0.779	0.705	0.739
	SD	0.0151	0.048	0.058	0.904	0.102

Table B6 Data of Figure 5.11.

- Relative cell number (%)

Toxicity						
Type of film		**Control**	**BC**	**Aloe Vera**		
				10%	**30%**	**50%**
24 hr (250 µl)	1	100	106.203	119.054	105.317	90.841
	2	100	121.726	115.683	116.115	106.906
	3	100	103.395	123.302	122.685	114.969
	Average	100	110.442	119.346	114.706	104.239
	SD	0	9.873	3.817	8.769	12.283
24 hr (500 µl)	1	100	87.739	93.231	81.098	83.269
	2	100	86.436	94.081	82.367	87.916
	3	100	98.602	107.115	103.049	108.132
	Average	100	90.926	98.142	88.838	93.105
	SD	0	6.679	7.782	12.323	13.218

Table B7 Data of Figure 5.12

- Number of cell growth

HaCat						
Type of film		**Control**	**BC**	**Aloe Vera**		
				10%	**30%**	**50%**
0 hr	1	0.219	0.26	0.195	0.27	0.236
	2	0.172	0.25	0.248	0.249	0.229
	3	0.197	0.27	0.2	0.264	0.242
	Average	0.196	0.259	0.214	0.261	0.236
	SD	0.0235	0.01	0.029	0.0108	0.0065
24 hr	1	0.607	0.515	0.528	0.444	0.509
	2	0.712	0.582	0.571	0.517	0.593
	3	0.721	0.541	0.503	0.556	0.66
	Average	0.680	0.546	0.534	0.506	0.587
	SD	0.063	0.034	0.034	0.057	0.076
48 hr	1	0.755	0.677	0.68	0.714	0.688
	2	0.77	0.71	0.703	0.719	0.714
	3	0.803	0.655	0.672	0.683	0.682
	Average	0.776	0.681	0.685	0.705	0.695
	SD	0.025	0.028	0.016	0.0195	0.017

Table B8 Data of Figure 5.12

- Relative cell number (%)

HaCat						
Type of film		**Control**	**BC**	**Aloe Vera**		
				10%	**30%**	**50%**
0 hr	1	100	119.634	89.041	123.287	107.726
	2	100	144.186	144.186	144.767	133.139
	3	100	135.025	101.522	134.01	122.842
	Average	100	132.948	111.583	134.021	121.248
	SD	0	12.406	28.916	10.739	12.763
24 hr	1	277.168	235.159	241.095	202.739	232.420
	2	413.953	338.372	331.976	300.581	344.767
	3	365.989	274.619	255.329	282.233	335.025
	Average	352.371	282.717	276.134	261.851	304.071
	SD	69.402	52.08	48.882	52.007	62.242
48 hr	1	344.748	309.132	310.502	326.027	314.155
	2	447.674	412.791	408.721	418.023	415.116
	3	407.614	332.487	341.116	346.705	346.192
	Average	400.012	351.470	353.446	363.584	358.488
	SD	51.882	54.374	50.256	48.265	51.591

Table B9 Data of Figure 5.14

- Number of cell growth

Fibroblast						
Type of film		**Control**	**BC**	**Aloe Vera**		
				10%	**30%**	**50%**
0 hr	1	0.260	0.238	0.238	0.185	0.209
	2	0.246	0.213	0.198	0.197	0.193
	3	0.238	0.254	0.214	0.225	0.170
	Average	0.248	0.235	0.217	0.202	0.191
	SD	0.011	0.021	0.020	0.021	0.020
24 hr	1	0.277	0.368	0.325	0.286	0.310
	2	0.382	0.378	0.314	0.222	0.274
	3	0.345	0.369	0.330	0.312	0.277
	Average	0.335	0.372	0.323	0.273	0.287
	SD	0.053	0.006	0.008	0.046	0.020
48 hr	1	0.483	0.484	0.412	0.451	0.332
	2	0.497	0.490	0.448	0.369	0.428
	3	0.445	0.387	0.442	0.353	0.345
	Average	0.475	0.454	0.434	0.391	0.368
	SD	0.027	0.058	0.019	0.053	0.052

Table B10 Data of Figure 5.14

- Relative cell number (%)

Fibroblast						
Type of film		**Control**	**BC**	**Aloe Vera**		
				10%	**30%**	**50%**
0 hr	1	100	91.538	91.538	71.154	80.385
	2	100	86.585	80.488	80.081	78.455
	3	100	106.723	89.916	94.538	71.429
	Average	100	94.949	87.314	81.924	76.756
	SD	0	10.493	5.967	11.800	4.714
24 hr	1	106.538	141.538	125.000	110.000	119.231
	2	155.285	153.659	127.642	90.244	111.382
	3	144.958	155.042	138.655	131.092	116.387
	Average	135.594	150.080	130.445	110.445	115.666
	SD	25.687	7.429	7.243	20.428	3.974
48 hr	1	185.769	186.154	158.462	173.462	127.692
	2	202.033	199.187	182.114	150.000	173.984
	3	186.975	162.605	185.714	148.319	144.958
	Average	191.592	182.649	175.430	157.260	148.878
	SD	9.062	18.541	14.805	14.056	23.393

APPENDIX C

CONFERENCE

Mr.Ong-ard Saibuatong, Muenduen Phisalaphong, "**Biosynthesis and Characterization of Bacterial Cellulose** ", Extended Abstract for Conference at Chiang Mai, Thailand with the name of " The 17th Thailand Chemical Engineering and Applied Chemistry Conference" (TICHE) 2007 – Fundamental of Chemical Engineering and Applied Chemistry, 29-30 October 2007.

VITA

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