จุฬาลงกรณ์มหาวิทยาลัย
ทุนวิจัย
กองทุนรัชดาภิเษกสมโภช

รายงานวิจัย

การพัฒนาแบคทีเรียเซลลูโลส เพื่อการใช้แทนที่ผิวหนังแบบชั่วคราว

โดย

เหมือนเดือน พิศาลพงศ์
นีรชา สารชวนะกิจ

ธันวาคม 2549

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30 ธันวาคม 2549

กิตติกรรมประกาศ

คณะผู้วิจัยขอแสดงความขอบคุณ กองทุนรัชดาภิเษกสมโภช จุฬาลงกรณ์มหาวิทยาลัย ปีงบประมาณ 2549สำหรับการสนับสนุนงานวิจัยนี้ นอกจากนี้ขอขอบคุณ อาจารย์ ปราโมทย์ ธรรมรัตน์ สถาบัน วิจัยและพัฒนาผลิตภัณฑ์อาหาร มหาวิทยาลัย เกษตรศาสตร์ สำหรับ สายพันธุ์จุลินทรีย์ และ ร.ศ. ดร. ศิริพร ดำรงค์ศักดิ์กุล ห้องปฏิบัติการวิจัย วิศวกรรมพอลิเมอร์ ภาควิชาวิศวกรรมเคมี จุฬาลงกรณ์มหาวิทยาลัย สำหรับการช่วยเหลือ ในการวิเคราะห์สมบัติความต้านทานแรงดึง ของแผ่นฟิลม์

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ชื่อโครงการวิจัย การพัฒนาแบคทีเรียเซลลูโลสเพื่อการใช้แทนที่ผิวหนังแบบชั่วคราว

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เดือนและปีที่ทำวิจัยเสร็จ 30 ธันวาคม 2549

<u>บทคัดย่อ</u>

แผ่นฟิลม์บางของแบคทีเรียเซลลูโลส (BC) จากการสังเคราะห์โดยจุลินทรีย์ในน้ำมะพร้าว ได้ถูกนำมาพัฒนาทดสอบคุณลักษณะและทดสอบผลต่อการเจริญเติบโตของเซลล์ผิวหนังคน Keratinocytes และ Fibroblasts โดยพบว่าค่าเฉลี่ยของขนาดเส้นผ่าศูนย์กลางรูพรุนและพื้นที่ผิวรวมของแผ่นฟิลม์แห้งเท่ากับ 224 อังสะตอม (°A) และ 12.62 ตารางเมตร ต่อ กรัม (m2/g) ตามลำดับ โดยที่ฟิลม์หนา 0.12 มิลลิเมตร ค่าคุณสมบัติการต้านทานของแรงดึง (Tensile strength) และค่า Break Strain โดยเฉลี่ย ของแผ่นฟิลม์แห้งคือ 5.21 MPa และ 3.75% ในขณะที่ แผ่นฟิลม์เปียกมีค่าที่ 1.56 MPa และ 8.00% ตามลำดับ ค่าความดูดซึมน้ำของแผ่นฟิลม์แห้งเท่ากับ 5.09 กรัม ต่อ กรัมฟิลม์แห้ง สำหรับการนำไปใช้สำหรับการรักษาแผลที่ผิวหนังจากการศึกษาผลกระทบโดยใช้เซลล์ผิวหนังคน Keratinocytes และ Fibroblasts รายงานวิจัยนี้เป็นรายงานแรกที่ได้แสดงให้เห็นผลของ แผ่นฟิลม์ BC ในการสนับสนุน การเจริญเติบโต การแพร่ และการเคลื่อนที่ ของเซลล์ Keratinocytes ของคน ในขณะเดียวกัน ไม่พบว่าแผ่น BC สนับสนุนการการแพร่ และการเคลื่อนที่ ของเซลล์ Fibroblasts ของคน จากการวิเคราะห์ ของ E-cadherin และ α-3 chain ของ lamin แสดง Phenotype ของ Keratinocytes ของคน บนแผ่น BC

Project Title Development of Bacterial Cellulose for Temporary Skin Substitute

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Abstract

Thin films of bacterial cellulose (BC) from *nata de coco* culture system were developed, characterized and investigated for the growth of human keratinocytes and fibroblasts. The average pore diameter and total surface area of the dried BC films estimated by BET were 224 °A and 12.62 m^2/g, respectively. With the film thickness of 0.12 mm, the average tensile strength and break strain of the dried films were 5.21 MPa and 3.75 % while those of the wet films were 1.56 MPa and 8.00 %, respectively. The water absorption capacity of air-dried film was 5.09 g water/g dried films. For the uses in the therapy of skin wounds, the potential biological mechanism of action of BC film was evaluated by using human keratinocytes and fibroblasts. Our results were the first direct demonstration that BC film supported the growth, spreading and migration of human keratinocytes, but not those of human fibroblasts. Expressions of E-cadherin and alpha-3 chain of laminin confirmed the phenotype of human keratinocyte on BC film.

Table of Contents

List of Tables

List of Figures

Content

1. Introduction

In the view of their biocompatibility, natural polymers like polysaccharides have been extensively used as biomaterials for many applications. Cellulose, the basic material of all plant substance, is the most abundant polysaccharide found in nature. Cellulose derived from plant is known as unpurified cellulose associated with many kinds of natural fiber whereas bacterial cellulose (BC) is nearly-purified cellulose. BC was extracellularly synthesized into nano-sized fibrils by the bacteria *Acetobactor xylinum.*

BC displayed advantages superior to the counterpart from plants with its physical and chemical properties. Unlike plant cellulose, BC has the unique properties such as high mechanical strength, high crystallinity, high hydrophilicity and ultra-fine network structure. There have been several applications of BC in medical fields such as artificial skin for humans with extensive burns (Fontana et al., 1990), artificial blood vessels for microsurgery (Klemm, 2001), scaffold for tissue engineering of cartilage (Svensson et al., 2005) and wound-dressing (Czaja et al, 2006). Especially in the wound dressing application, BC has been widely utilized in burn wound therapy system. The excellent dressing should be maintained the wound in a wet condition, inexpensive, and its antigen properties must be avoided. BC being used as wound dressing is eligible, because of its outstanding properties. BC also show high water content, good sorption of liquids, non-allergenic, and can be safely sterilized without any change of its characteristics. BC could also be applied as skin substitute in treating extensive burns.

In the present study, thin films of BC were developed by using static culture of *Acetobacter xylinum* in coconut-water, which was a waste from coconut milk production plants. Microstructure and mechanical properties of the BC films were partially characterized. Furthermore, the growth of human skin keratinocytes and fibroblasts on BC films were examined. The present study provided indications for the potentail biological mechanism of action of BC film for the uses in therapy of skin wounds.

2. Survey of related literature

The gram-negative bacterium *Acetobacter xylinum* is an efficient producer of extracellular β-glucan chains as cellulose. In an aerobic condition, a single cell of *Acetobacter* acts as a nano-spinneret and produces a bundle of sub-microscopic fibrils. Together, the entangled mesh of these fibrils forms a gelatinous membrane of bacterial cellulose (BC) (*1*). From previous studies, *Acetobacter* forms cellulose ribbons with cross-sectional dimension of 800 x 40 °A2 and the crystalline regions are normal cellulose I (*2, 3*).

BC has unique properties including high purity, high water holding capacity, high degree of crystallinity, high tensile strength and tissue biocompatibility (*4-7*). Its properties draw a lot of attention for application as potential biomaterials in medical areas. BC does not contain any components of animal origin or any foreign species proteins, therefore it does not cause allergic reaction. Fontana et al. (*8*) reported successful application of BC as a temporary skin substitute on exudating or bloody tissues. An excellent healing process without any hypergranulation effect was observed when BC in dried and swollen forms were applied as dressing materials on wounds of horses (*9*). Moreover, the structure and properties of BC induced a rapid adhesion and an optimal coating with endogeneous cells in

the living body (*10*). Microvessel interpositions in a rat experiment showed a high potential of BActerial SYnthesized Cellulose (BASYC®) in a tubular form as an artificial blood vessel for micronerve surgery (*10*). Bifill® and Gengifex®, commercial BC, were successfully applied as temporary substitutes for human skin (*10-11*). Examination of the growth of bovine chondrocytes on BC film showed that this substrate supported bovine chondrocyte proliferation at about 50% of that observed for type II collagen (*7*).

3. Procedure

3.1. Microbial strains and Bacterial cellulose (BC) formation

3.1.1. Microbial strains

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

3.1.2. Culture media and method for BC formation

Cell cultivation was performed in a simply and inexpensive way following the procedure developed by Pramote Thammarad. The medium for the inoculum was coconut–water supplemented with 5.0% sucrose and 0.5% ammonium sulphate $(NH_4)_2SO_4$ (chemical grade, Merck) and 1.0 % acetic acid. The medium was sterilized at 121°C for 15 min. Precultures were prepared by transferring 50 ml of a stock culture to 1000 ml in 1500 ml bottle and incubated statically at 30°C for 4 days. After the surface pellicle was removed,

the preculture broth was added to the main culture in a ratio of 100 ml to 2 l of medium. The activated medium was inoculated in a tray (surface/volume ratio = 0.4 cm^{-1}), covered with a piece of porous paper and kept at 30ºC for 24 - 48 hours. The developed BC film was first purified by washing with DI water and then was treated with 1% NaOH at 35 °C for 24 hours to remove bacterial cells followed by a rinse with DI water until pH came to 7. Afterwards, the BC film was air dried at room temperature (30°C) and stored in plastic film at room temperature.

3.2. Characterization

3.2.1. Morphology

For surface morphology, scanning electron micrographs (SEM) were taken on a JEOL JSM-5410LV scanning electron microscope. The BC films were frozen in liquid nitrogen, immediately snapped and vacuum dried. Then, the films were sputtered with gold and photographed.

The pore size, porosity and pore size distribution of the BC film were determined by BET (Brunauer, Emmett and Teller) model Micromeritics Pore Sizer 9320. The analysis was performed at ambient temperature.

3.2.2 Rejection Coefficient

BC film was tested for rejection coefficient using aqueous solutions of 1 wt% salts (sodium chloride and calcium chloride), 1 wt% mono saccharide, 1 wt% di saccharides and 0.1 wt% dextrans MW 10,000- 2,000,000. The experiments were performed at the average

membrane pressure of 9 kg/cm^2 and at the flow velocity of 0.85 m/min. The rejection coefficient (R) was calculated as follow:

$$R = 1 - \frac{c_p}{c_f}$$

where, c_p = permeate concentration and c_f = feed concentration.

3.2.3. Mechanical analysis

BC film was cut into strip-shaped specimens with 10 mm in width and 10 cm in length. The maximum tensile strength and break strain of BC film in dry and wet states were determined using a LLOYD 2000R universal testing machine. The test conditions followed ASTM D882. The tensile strength and break strain were the average values determined on 10 specimens.

3.3. Human skin cell study

3.3.1. Cells and cell culture

Two types of cells were used in this study: human transformed skin keratinocyte (CRL-2309) and human normal skin fibroblast (CCL-110). Both cell lines were purchased from the American Type Culture Collection (ATCC, VA, USA). Keratinocytes were cultured in keratinocyte-serum free Medium (GIBCO, NY, USA) supplemented with 0.05 mg/ml bovine pituitary extract (BPE) and 35 ng/ml epidermal growth factor (EGF). Fibroblasts were grown in Dulbecco's Modified Eagle's Medium (DMEM, Hyclone, UT,

USA) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin. When the cells reached 80% confluence, they were serially subcultured. For fibroblast, cells from the 10^{th} to 15^{th} subcultured passage were used in the described experiments. Both cultures were incubated at 37°C in humidified atmosphere containing 5% CO_2.

3.3.2. Cell proliferation and morphological analysis

Air-dried BC films 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. The experiments were conducted in triplicate. One ml culture medium was added to each well to equilibrate the samples for 30 min before cell seeding. Proliferation of skin keratinocytes and fibroblasts on BC films were determined by MTT assay as previously described (*12*). Briefly, cells were seeded into 24-well culture plates (Nunc, Rockford, IL, USA) at an initial density of $3x10^4$ cells per well on BC film or polystylene plate control. Cells were incubated at 37°C in humidified atmosphere containing 5% CO_2 for 16 hours. Then, the culture medium was removed and replaced for another 48 hours by keratinocyte complete growth medium and serum free DMEM for the cultures of keratinocytes and fibroblasts, respectively. The number of living cells was determined using MTT assay. Cell morphological imaging was performed using phase contrast microscope-attached Olympus 5050 digital camera.

3.3.3. Cell migration

Cell migration assay was performed using the modified technique of Lee et al. (*13*). The assay was conducted in 24-well culture plates. A sample of BC film was placed on the bottom of each culture well. Cells were seeded at $1x10^4$ cells per well within a metallic ring of 5 mm in diameter on BC film or polystylene plate control, and incubated at 37°C in humidified atmosphere containing 5% CO_2. After 24 hours of incubation, the metallic ring was cautiously removed to allow free movement of cells. All culture medium was removed gently and replaced by keratinocyte complete growth medium and serum free DMEM for keratinocytes and fibroblasts, respectively. For analysis of cell motility, photographs of the cultures at different time points were taken using phase contrast microscope-attached Olympus 5050 digital camera. Percentage increase of areas covered by cells at 7 and 24 hours compared at time zero was analysed using Scion Image analysis program.

3.3.4. MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was based on the reduction of the yellow tetrazolium salt to purple formazan crystals by dehydrogenase enzymes secreted from the mitochondria of metabolically active cells. The amount of purple formazan crystals formed was proportional to the number of viable cells. First, the culture medium was aspirated and replaced with 0.5 g/ml of MTT solution. After that, the plate was incubated for 1 hour at 37°C. The solution was then aspirated and 900 µl of DMSO containing 125 µl of glycine buffer (0.1M Glycine, 0.1M NaCl, pH10) was added to dissolve the formazan crystals. Finally, after 10 minutes of rotary agitation, the

absorbance of the DMSO solution at 540 nm was measured using a Genesis10 UV-visible spectrophotometer (Thermo Spectronic, NY, USA) (*12*).

3.3.5. RT-PCR analysis

Total RNA was isolated from cell culture at 48 hours using Tri-reagent (MRC, OH, USA) according to the manufacturer's instruction. The RT-PCR was performed with 1 g of total RNA using AMV reverse transcriptase (Promega, WI, USA), Taq DNA polymerase (Qiagen GmbH, Germany) and three pairs of oligonucleotides (Proligo LLC, CO, USA). Forward and reverse primers for human alpha-3 chain of laminin, E-cadherin and GAPDH were designed as follow: alpha-3 chain of laminin primer [forward 5' -GAAACTTCAGACATGCCAGCA-3', reverse 5' -CCTGTGTACCCAGCTTTGCA-3'], E-cadherin [forward 5'-CAACAGCTGTGATCACAGTCA-3', reverse 5' -CAGTTGGCAGTGTCTCTCCA-3'], and GAPDH [forward 5' -TGAAGGTCGGAGTCAACGGAT-3', reverse 5' -TCACACCCATGACGAACATGG-3']. RT-PCR products were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide staining with ultraviolet light illumination (UVItec, Cambridge, UK). The expected sizes of the RT-PCR products of alpha-3 chain of laminin, E-cadherin and GAPDH are 500bp, 500bp and 300bp, respectively.

4. Results and discussions

4.1. Production and Characteristics of BC

Experimental study was performed in duplicated to determine the BC production rate. After the activated medium was inoculated in a tray covered with a porous paper and kept at 30°C, cellulose was produced in form of thin, leather-like white pellicle at the air-liquid interface of the culture. The average cellulose production profile was in sigmoidal curve as shown in Fig. 1. For the cultivation of 1 and 2 days, the average cellulose production rates were 0.4 and 0.6×10^{-3} g/day/cm^2 with the gel thickness of 0.64 and 1.94 millimeter, respectively. However, for the cultivation of 7 days, the cellulose production rate increased to 1.0×10^{-3} g/day.cm^2 with the gel thickness of 11.21 millimeter, which was comparable to other published results. By using *Acetobacter xylinum*, Masaoka et al. (*14*) and Jonas et al. (*11*) reported BC production rates at 2.4×10^{-3} g/day.cm^2 and 1.0×10^{-3} g/day.cm^2, respectively. Besides the difference from bacterial strains, the rate of cellulose formation strongly depended on air- surface area, carbon and nitrogen sources, pH and temperature.

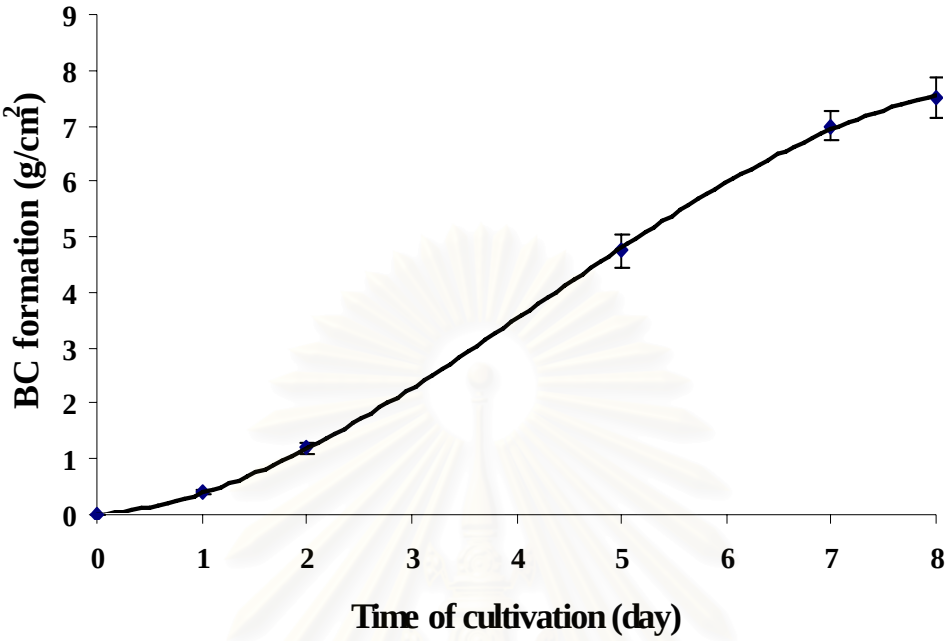
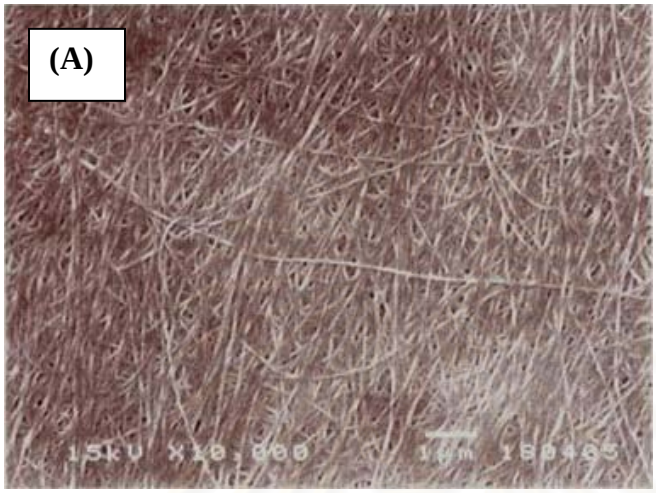


Figure 1 Bacteria cellulose (BC) formation by *Acetobacter xylimum* in *nata de coco* culture system.

Figure 2 demonstrate the dried BC film after the purification. The average water content of the cultivated BC pellicles was 94.8 %. After purification and air drying at room temperature (30°C), the BC pellicles became transparent thin films. The scanning electron micrographs of the air dried film and re-swollen BC film in DI water were shown in Fig. 3. The films displayed ultrafine fiber network structure of micro fibrils below 0.05 µm. With hydrophilic property, after air-drying at room temperature and reswelling with water at 30° C, the apparent pore diameter of the swollen form was 0.2-1.0 µm, whereas the pore diameter of the dried film was less than 0.1 µm. The dimension of the dried BC fibers (0.01-

0.1μm) was comparable to ultrathin membrane (*10*). The BC film demonstrated a remarkable capacity to hold water. The water absorption capacity after air-drying at 30 ºC was 5.09 g water/g dried film (509 %), which was much higher than those of plant celluloses. Drying condition has a strong impact on the water absorption capacity of BC film. In a previous study (*10*), the water retention values of BC (wet state), BC (freeze dried), BC (air dried up to 100°C) and typical plant cellulose (cotton linters) was 1027, 629, 106 and 60%, respectively. The total surface area and average pore size of the dried BC films determined by BET were 12.62 m^2/g and 224 °A, respectively, with pore size distribution from 45 - 600. °A (Figure 4).

Figure 2. Dried BC film after purification.



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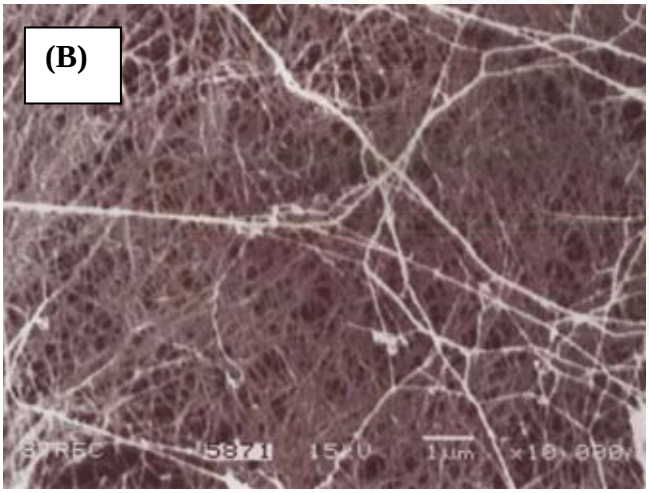

x 10,000

Figure 3 SEM images of surface morphology of dried BC film (A) and reswollen BC film (B).

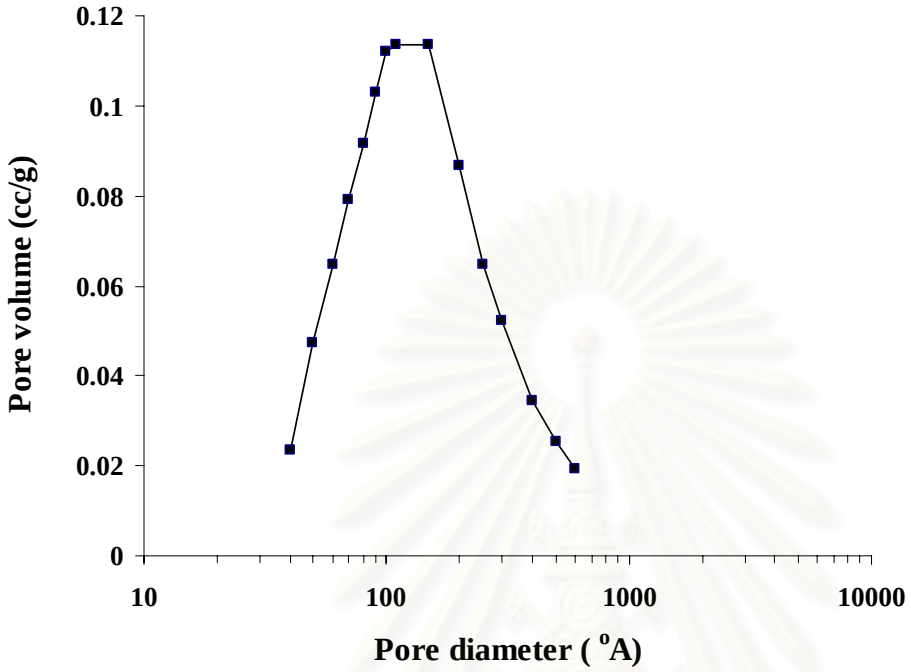

Figure 4. Pore size distribution of the dried BC film from desorption pore volume plot by BET.

The permeability experiments of aqueous solutions of 1 wt% salts (sodium chloride and calcium chloride), 1 wt% mono saccharide, 1 wt% di saccharides and 0.1 wt% dextrans MW 10,000- 2,000,000 were performed at the average membrane pressure of 9 kg/cm^2 and at the flow velocity of 0.85 m/min. The rejection coefficients of 0.0 – 0.1, 0.0- 0.2 and 0.0-1.0 were observed in the separation of salts, mono and di saccharides and dextrans respectively. Rejection increased with the molecular weight of solute and the membrane thickness. Figure 5 demonstrated the membrane rejection coefficient in the separation of dextrans with MW 10,000 -2,000,000 at the BC film thickness varied from 0.005 - 0.120 mm.

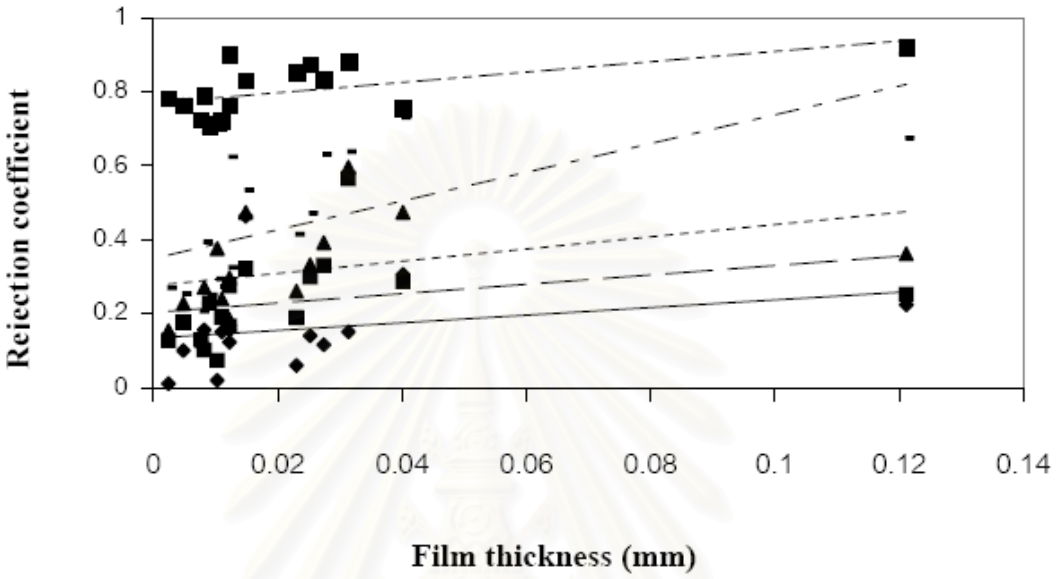

Figure 5. Membrane rejection coefficient at the BC film thickness

varied from 0.005 - 0.120 mm in the separation of dextrans:

(1) ♦ MW 10,000, ■ (2) MW 71,400, ▲ (3) 237500, ▬ (4) 505,000 and

(5) ■ Blue dextran MW 2,000,000.

From mechanical analysis of the dried BC film with thickness of 0.12 millimeter, the average tensile strength and break strain were 5.21 MPa and 3.75 %, respectively. However, after the film was reswollen in DI water, the average tensile strength and break strain became 1.56 KPa and 8.00 %, respectively. From a report by Nichi et al. (*15*), Young's modulus and tensile strength of BC films were much higher than wood fibers and some synthetic materials such as polyethylene and vinyl chloride films at the same thickness. This

consequence was from high crystallinity, high planar orientation of ribbons pressed into a sheet, ultrafine structure and complex network of ribbons of BC film (*10, 16*). Table 1 gave a summary of the BC film characteristics observed in this study.

Table 1 Characteristics of the bacterial cellulose (BC) film in dry and swollen from.

Properties	Dry Form	Swollen Form
Tensile strength[a] (MPa)	5.21	1.56
Break strain[a] (%)	3.75	8.00
Pore diameter (μm)	< 0.1	0.2 -1.0
Water absorption capacity	5.09 g water / g dried film	

[a] Film thickness in dry form = 0.12 mm

4.2 Cell study

Previous studies have demonstrated that BC have no cytotoxic effect on a variety of cells (7, *17*). It has been developed and used for several medical applications, especially for wound dressing. However why BC worked so well in these applications has not been fully understand. This study was the first report that directly demonstrated the responses of human skin keratinocytes and fibroblasts, the major components of the skin, on BC film.

First, we evaluated the effect of BC film on cell proliferation. For both keratinocytes and fibroblasts, percentage of the living cells after seeding on BC film for 24 and 48 hours was comparable to the cells cultured on the polystylene culture plate (Figure 4A and 4B, respectively). However, the patterns of cell distribution between keratinocytes and fibroblasts were different. Phase contrast microscopy demonstrated that keratinocytes spread over the surface of BC film at 24 hours (Figure 5) and became a confluent monolayer at 48 hours (data not shown), whereas proliferative fibroblasts formed clumps in various sizes among a number of isolated cells at 24 hours (Figure 5) and did not reach confluency (data not shown).

From our observation, after fibroblasts were seeded, all of them attached, but did not fully spread on BC. However, they could proliferate, so after a period of culture, groups of proliferative fibroblasts formed clumps which grew larger and detached from BC film over-time (data not shown). A possible explanation for this phenomenon is that the adhesion of fibroblasts to BC film was less than that between fibroblasts themselves in conjunction with the contractile nature of interconnected fibroblasts. Previous studies demonstrated that a

single fibroblast is capable of generating force in the immediate surrounding matrix *in vitro* (*18, 19*). Therefore, if the contractile force between interconnected network of cells was stronger than the adhesion force between fibroblasts and BC film, groups of cells would roll up and would be unable to reach confluence on BC film.

Our results indicated that BC film had no toxicity and supported cell proliferation in both cell types, however; the pattern of cell distribution and stability on BC film were poorer in fibroblast culture. These results demonstrated that keratinocytes responded to BC film better by exhibiting normal cell proliferation and spreading.

Among various factors that played a role in cell attachment and speading were cohesion among keratinocytes and adhesion of keratinocytes to BC film. There were several types of junctional structures such as desmosomes and adherens junctions, which were consisted of several proteins crucial for intercellular adhesion of oral and skin keratinocytes (*20*). E-cadherin was one of several adhesion molecules and was usually used as a characteristic marker for normal differentiation stage of keratinocytes (*21-23*).

Laminin was another functional marker of keratinocytes. It was the major noncollagenous component of the basement membrane glycoprotein with a cruciform-shaped molecule comprising three non-identical chains, alpha, beta and gamma- chains (*24-26*). Laminin had several isoforms. It was reported that laminin isoforms with alpha-3 chain such as laminin 5 were primarily required to maintain differentiation stage, stable anchorage and spreading of keratinocytes in adult skin. The fact that the lack of laminin alpha-3 caused skin blistering suggested the important role of this molecule in keratinocyte homeostasis (*20, 27-28*).

To test for these keratinocyte specific markers, RT-PCR was performed to analyse the expressions of E-cadherin and laminin alpha-3. Both genes were expressed by keratinocytes growing on BC film in the similar level as those on the polystylene plate (Figure 6). The results indicated that BC film was able to support and maintain the normal characteristics of keratinocytes.

Cell migration on BC film was also examined. Free movement of cells was allowed after the removal of a metallic ring. The area covered by keratinocytes was gradually increased from time zero to 7 and 24 hours. Rate of cell migration on BC film was comparable to that on the polystylene plate, especially as early as 7 hours when there was no cell proliferation and the area covered by cells was increased 2-fold by 24 hours (Figure 7). Since fibroblasts tended to detach from BC film, the result was inconsistent and could not be measured (data not shown).

Taken together, the results demonstrated that apart from promoting keratinocyte proliferation and spreading, BC film also supported keratinocyte differentiation and migration. Therefore, BC film was more compatible to skin keratinocytes than skin fibroblasts.

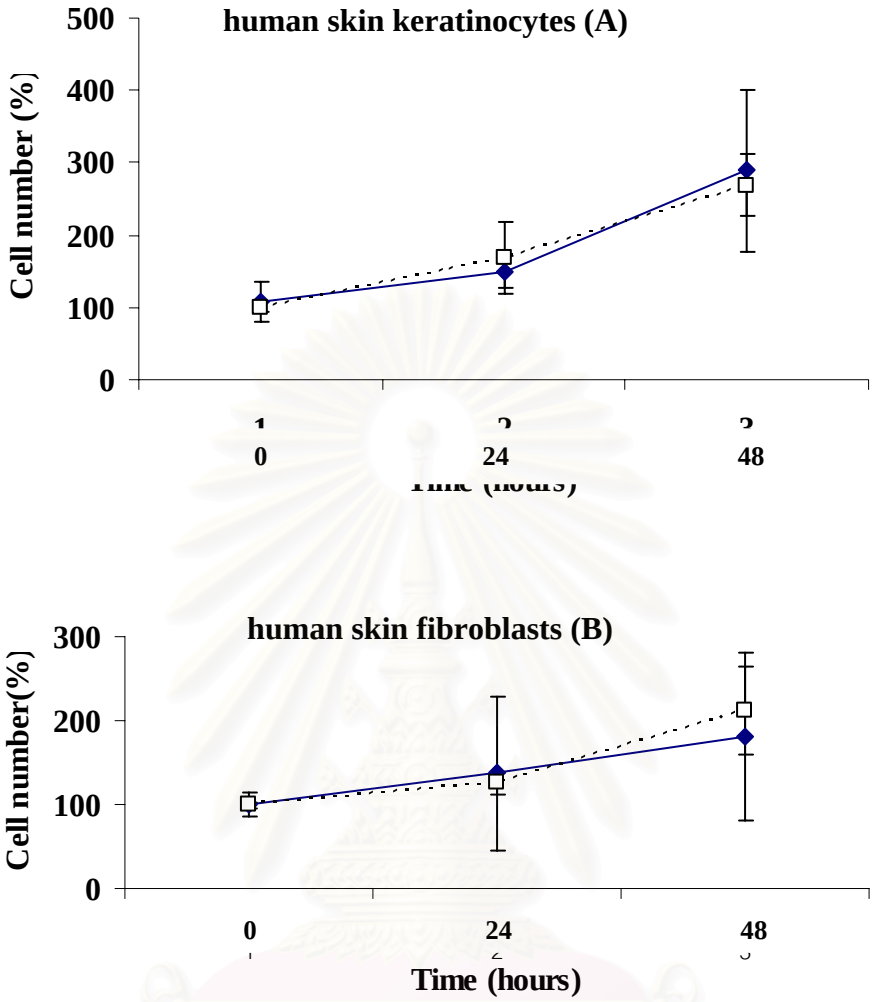

Figure 6. Proliferations of human skin keratinocytes (A) and fibroblasts (B) on the BC film (♦) compared to cultured-treated polystylene (□) Percentage of living cells was assessed at 0, 24 and 48 hours in culture by MTT assay.

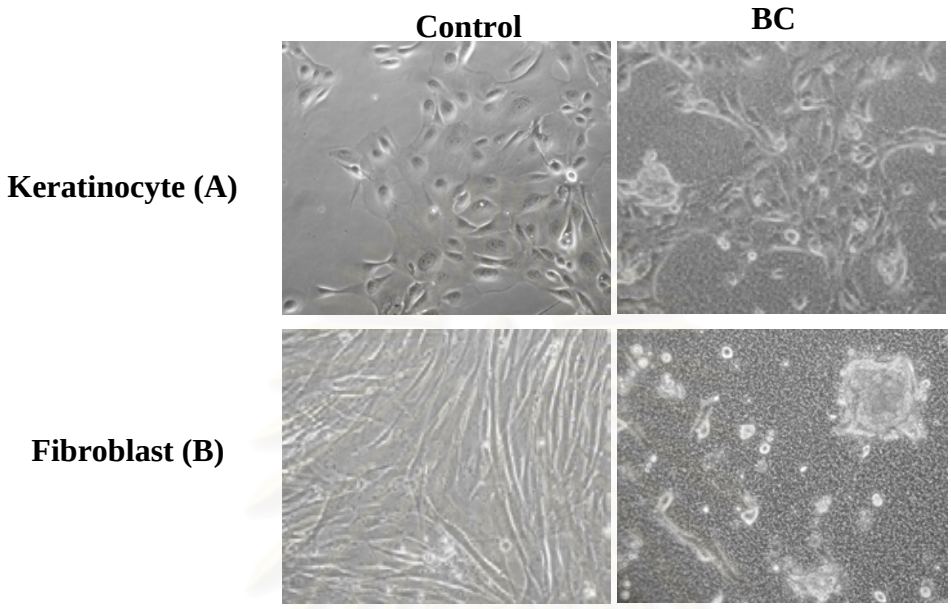

Figure 7. Morphology and distribution of human skin keratinocytes (A) and fibroblasts (B) on the BC film and cultured-treated polystylene plate (control) demonstrated at 48 hours by using phase-contrast microscopy.

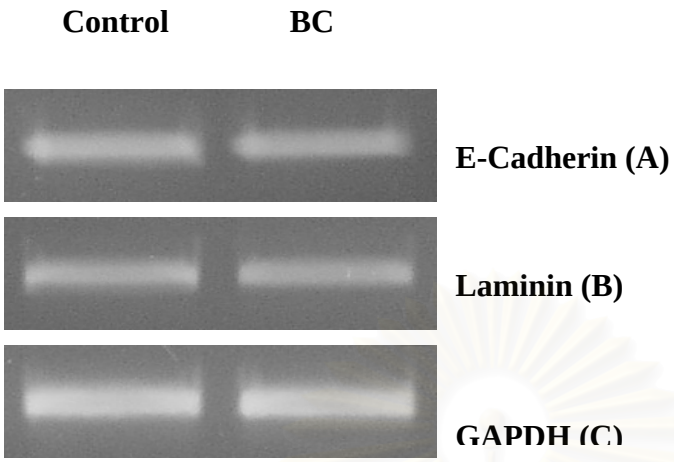

Figure 8. RT-PCR analysis of levels of alpha-3 chain of E-cadherin (A), laminin (B) and GAPDH (C) mRNA expressed by 48 hour culture of human skin keratinocytes on the BC film and cultured-treated polystylene plate.

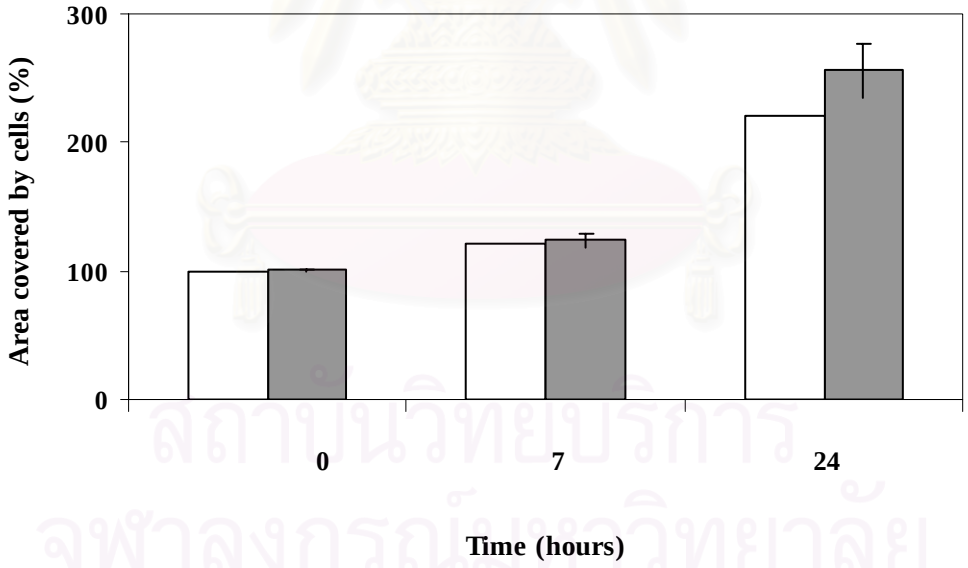

Figure 9. Migration of keratinocyte on cellulose film (■) and on cultured-treated polystylene (□). Area covered by keratinocyte was photographed at 0, 7 and 24 hours and analysed by using Scion Image analysis program.

5. Conclusion

In present study, thin films of bacterial cellulose (BC) by *Acetobacter xylinum* were developed from *nata de coco* culture system. The average pore diameter and surface area of the dried BC film estimated by BET was 224 °A and 12.62 m^2/g, respectively. BC film had remarkable water holding capacity and high tensile strength. The examination of the growth of human skin keratinocytes and fibroblasts on BC film demonstrated that the film supported proliferation for both cell lines comparable to those on the control polystylene plate. However, while keratinocytes could spread out over the surface of BC film, proliferative fibroblasts were loosely attached to the film. Expression E-cadherin and alpha-3 chain of laminin confirmed the phenotype of keratinocyte when growing on BC film. The results also showed that BC film promoted keratinocyte migration.

To our knowledge, this is the first report of the responses of human skin keratinocytes and fibroblasts on BC film. The results suggest that BC film can promote re-epithelialization process. This may be a biological mechanism by which BC film facilitates wound healing. Therefore, BC film hold a high potential for therapeutic application for skin wound, which is suitable for partial thickness dermal loss wound as donor-site skin graft wound or post abrasive laser wound. Non-promoting fibroblast adhesion may promote inhibition of hypertrophic scar. Since degradation of cellulose in the human body might be the question that still needs to be solved, BC may be applied as a long-term soft tissue augmentation material.

6. Suggestion for Further Work

6.1 Modification and improvement of BC in physical and biological properties need further studies.

6.2 Growth and characteristics of other human cells on BC and modified BC films should be carried on to gain information for more applications.

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8. Appendixes

Output from the research

1. Sanchavanakit, N., Sangrungraungroj, W., Kaomongkolgit, R., Banaprasert, T., Pavasant, P. and Phisalaphong, M. Growth of human keratinocytes and fibroblasts on bacterial cellulose film, *Biotechnology Progress* 22, (2006) 1194-1199.