CHAPTER II
BACKGROUND AND LITERATURE REVIEW

2.1 Wound Healing Process

Wound healing is continuous and complex process, involving the interaction of biological system an immunological with coordinated interaction. It is divided into four phases: 1). Homeostasis 2). Inflammatory 3). Proliferate 4). Remodeling (Velnar, *et al.*, 2009).

2.1.1 Homeostasis

This phase occur immediately after injury. The aim of this phase is to limit blood loss by platelet aggregation and clot formation. The blood clot formation consists of fibronectin, fibrin, vitronectin and thrombospondin. The important of clot formation is also in term of providing as matrix for cell migration in the subsequent phases of the haemostatic and inflammatory phases (Velnar, *et al.*, 2009).

2.1.2 Inflammatory

This inflammatory phase produce immune barrier against invading of microorganisms. Neutrophil infiltrate in the wound site to prevent infection by phagocytosis. The function of phagocytosis activity is to destroy and remove bacteria, foreign particles, and damaged tissue by releasing proteolytic enzymes and oxygen derived free radicals species. The excess of ROS cause the harmful on cells and tissue from oxidative damage by involving NADPH mechanism which generate from neutrophil accumulation in wound area (Singh, *et al.*, 2006). Moreover, the inflammatory response regulate cells and provide an abundant reservoir of potent tissue growth factors, particularly TGF-β, as well as other mediators (TGF-α, heparin binding epidermal growth factor, fibroblast growth factor (FGF), collagenase), activating keratinocytes, fibroblasts and endothelial cells (Velnar, *et al.*, 2009).

2.1.3 Proliferative

The proliferative phase involves the fibroblast migration and deposition of newly synthesized extracellular matrix to replace the loss tissue. First, the formation of granulation occurs in this stage. Fibroblast synthesized collagen, act

as foundation for intracellular matrix formation in the wound and many new capillaries are formed. After that, the open wound was closed by wound contracture. Myofibroblasts composed of actin and myosin which generate contractile force resulting in smaller wound size (Stojadinovic, *et al.*, 2008). Next, migration of epithelial cells from wound edge, cells migrate and attach to the provisional matrix below. The advancing epithelial cells meet resulting in migration stops and the basement membrane starts to form (Velnar, *et al.*, 2009).

2.1.4 Remodeling

This phase is final step of wound healing process. This phase is responsible for controlling the equilibrium of synthesis and breakdown collagen. The tensile strength of wound derives from collagen collection. Collagen fibers contribute to the approximately 80% of the original strength compared with unwounded tissue (Velnar, *et al.*, 2009).

2.2 Bacterial Cellulose

2.2.1 Cellulose

Cellulose is the most abundant organic compound on the Earth, and it is a biopolymer present in nature as a structural component of the plants and algae cell walls. The molecular formula of cellulose is $(C_6H_{10}O_5)_n$ which is a linear polymer composed of repeating β(1→4) linked D-glucose units, as shown in Figure 2.1.

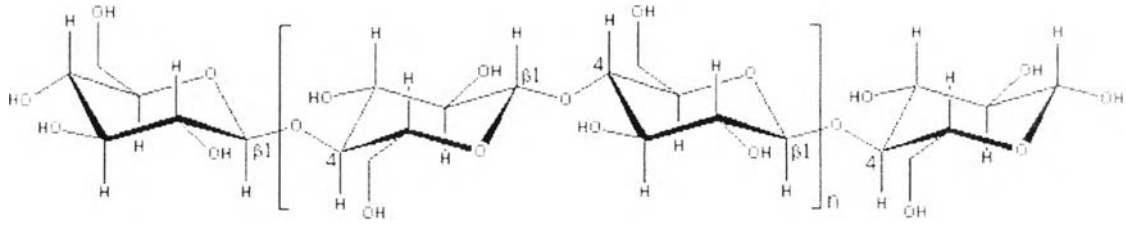


Figure 2.1 The structural unit of cellulose (Chaplin, 2009).

The β(1→4) bonds contribute a linear alignment to the molecules of cellulose because they allow the formation of two intra-molecular hydrogen bonds within every glucose residue: one bond links the O(6) to the O (2)H of the next residue and the other bond links O (3)H to O(5). The pattern of intermolecular hydrogen bonding links the different chains of cellulose from O(3) to O(6)H (Sjoestrom, 1993).

There are two different types of crystalline structures of cellulose: Cellulose I and II — Major binding forces in Cellulose I are van der Waals while Cellulose II crystal possesses two hydrogen bondings between the layers.

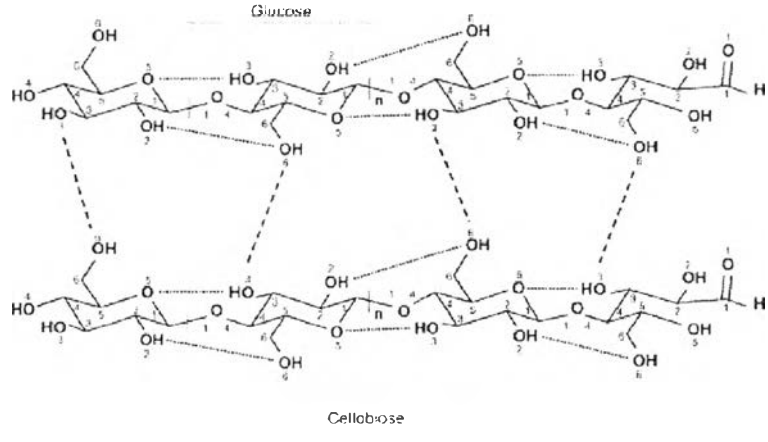


Figure 2.2 Representation of inter- and intra-chain hydrogen bonding network (Sconti, 2010).

The native cellulose (Cellulose I) is found in the complexity of two crystal forms — I_α and I_β. A different content of I_α or I_β in cellulose has been originated from its sources. The I_β-rich cellulose is found in plants, whereas the I_α-rich cellulose is produced by bacteria and algae (Horii *et al.*, 1987).

2.2.2 Synthesis Pathways of Cellulose

Cellulose is synthesized by four different pathways, depending on its origins, as shown in Figure 2.3. The first one is the cellulose from plants, which also contains lignin and hemicelluloses. The second way is cellulose synthesized by different types of microorganisms. The third way is the synthesis via enzymatic

polymerization starting from cellobiosyl fluoride monomer. The last way is the chemosynthesis from glucose by ring-opening polymerization of benzylated and pivaloylated derivatives (Klemm *et al.*, 2001).

Although cellulose is synthesized by bacteria, not all of these bacterial species synthesize cellulose as extracellular fibers in the form of pellicle (Jonas and Farah, 1998) as shown in Table 2.1.

2.2.3 Cellulose Synthesis by *Acetobacter Xylinum*

A. xylinum, a Gram-negative, rod-shaped, non-pathogenic, and aerobic bacterium, is interested in many studies due to the large quantity of cellulose product (Ross *et al.*, 1991). In 1886, Brown found the gelatinous translucent mass on the surface of liquid carbohydrate media for the first time in vinegar plant. In nature, these bacteria are found in rotten fruits and vegetables (Jesus *et al.*, 1971). The microorganisms generate cellulose because the aerobic bacteria produce pellicle to maintain their position close to surface of culture solution. Another reason that bacteria produce cellulose pellicle at the surface is to protect the cells from UV-light, while nutrients can still be provided by diffusion (Williams and Cannon, 1989). The optimal pH range for cellulose production is from 4 to 7 and the optimal growth temperature is in the range of 25 °C to 30 °C (Jonas and Farah, 1998). Surma-Slusarska *et al.* (2008) found that the optimum condition for cellulose production was 7 days at 30°C. Glucose and mannitol were the good carbon source, resulting in high yielding of bacterial cellulose production. Furthermore, bacterial cellulose exhibited a high thermal stability before degrading around 300 °C.

The chemical structure of cellulose synthesized by *A.xylinum* is the same as that of the plant cellulose. However, bacterial cellulose does not contain lignin, pectin, and hemicelluloses. Moreover, the characteristics of bacterial cellulose also differ from plant cellulose due to its high crystallinity, high water absorption, and high mechanical strength in the wet state, ultra-fine network structure (nanoscale fiber), and mould ability (Klemm *et al.*, 2001).

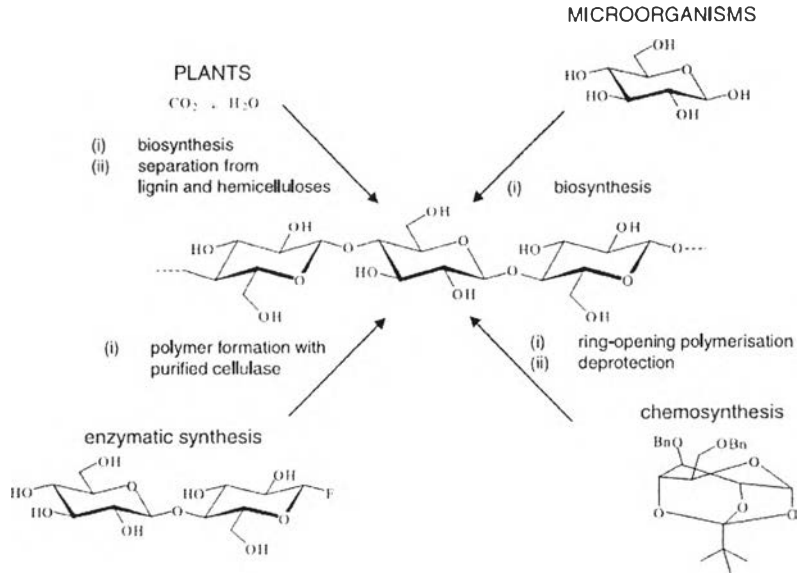


Figure 2.3 Synthesis pathways of cellulose (Klemm *et al.*, 2001).

Table 2.1 Bacterial cellulose producers (Jonas and Farah, 1998)

Genus	Cellulose structure
Acetobacter	extracellular pellicle composed of ribbons
Achromobacter	fibrils
Aerobacter	fibrils
Agrobacterium	short fibrils
Alcaligenes	fibrils
Pseudomonas	no distinct fibrils
Rhizobium	short fibrils
Sarcina	amorphous cellulose
Zoogloea	not well defined

There are many fundamental enzyme mediated steps of the cellulose formation: the glucose transformation to glucose-6-phosphate, then to glucose-1-phosphate, and finally the addition of UDP-glucose to the end of a growing cellulose polymer chain by cellulose synthase, as shown in Figure 2.4. The essential enzyme in the synthesis process is cellulose synthase (UDP-glucose: 1,4-b-d-glycosyl

transferase; EC 2.4.1.12) .In addition, the c-di-GMP phosphodiesterases A and B (PDE-A and PDE-B) acts as a key regulatory element which controls activation and inactivation of cellulose synthase (Vandamme *et al.*, 1998; Klemm *et al.*, 2001).

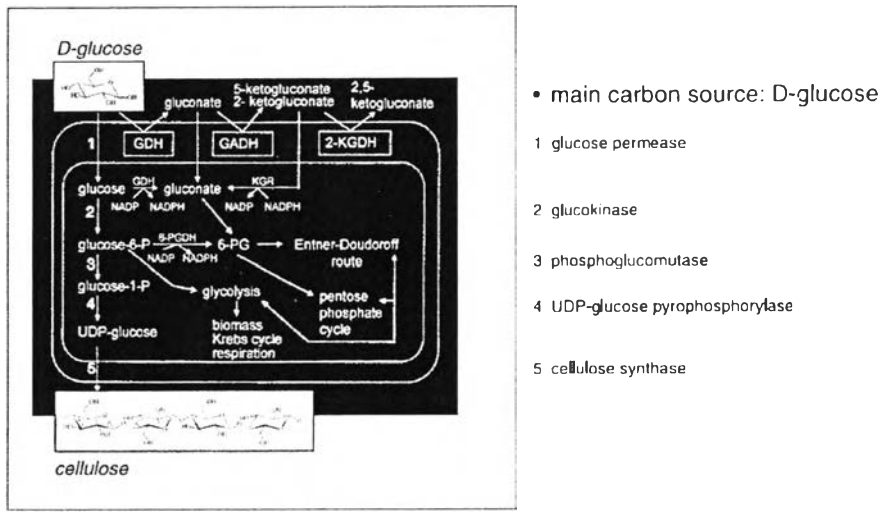


Figure 2.4 Pathways of carbon metabolism in *A. xylinum* (Klemm *et al.*, 2001).

The synthesis of cellulose fibers by *A.xylinum* occurs between the outer and the cytoplasma membrane. The cellulose synthesizing complexes (terminal complexes, TC) arrange as a linear row with cellulose assembly at the pores on the surface of bacterium. The first step of cellulose formation composes of 6 to 8 glucan chains aggregation. In the second step, these sub-elementary fibrils assemble to form micro fibril and, then, tightly assemble to form a ribbon in the third step, as shown in Figure 2.5. The matrix of the interwoven ribbons constitutes the bacterial cellulose membrane or pellicle. Figure 2.6 demonstrates the excretion of bacterial cellulose ribbon from one bacterial cell. *A. xylinum* cells distribute throughout the network of the cellulose ribbons, as shown in Figure 2.7 (Klemm *et al.*, 2001).

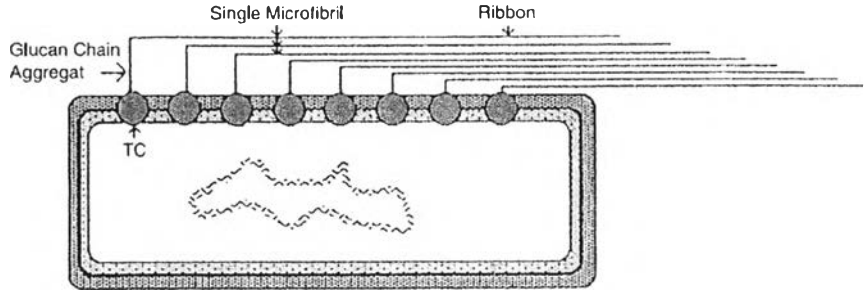


Figure 2.5 Formation of bacterial cellulose (Klemm *et al.*, 2001).

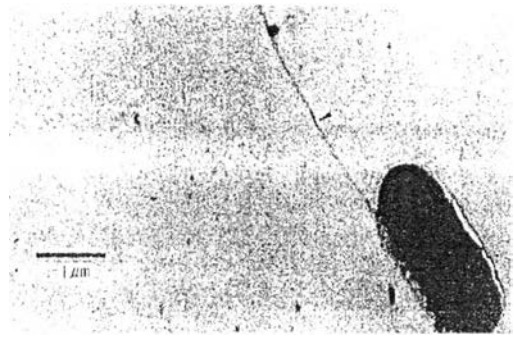


Figure 2.6 TEM image of bacterial cellulose ribbon produced by a bacterial cell (Klemm *et al.*, 2001).

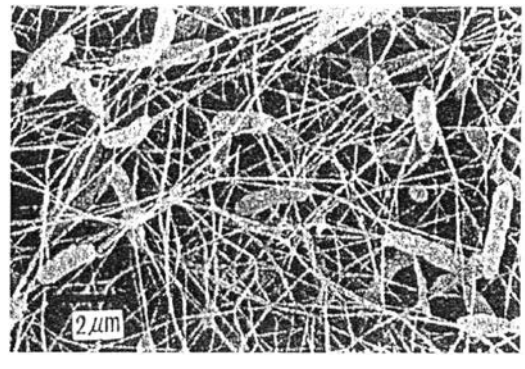


Figure 2.7 SEM image of a bacterial cellulose network including the bacterial cells (Klemm *et al.*, 2001).

2.2.4 Structure of Bacterial Cellulose

Numerous researches have revealed that bacterial cellulose is chemically identical to plant cellulose, but they are different in macromolecular structure and properties, as shown in Figure 2.8. In addition, dimensions of bacterial cellulose fibrils are in the width range of 20 nm to 50 nm with the length of at least 10 μm. The ultrafine ribbon network structure is stabilized by extensive hydrogen bondings (Yamanaka *et al.*, 1989). The degree of polymerization is in the range of 13000 to 14000 for plant cellulose and 2000 to 6000 for bacterial cellulose (Jonas and Farah, 1978).

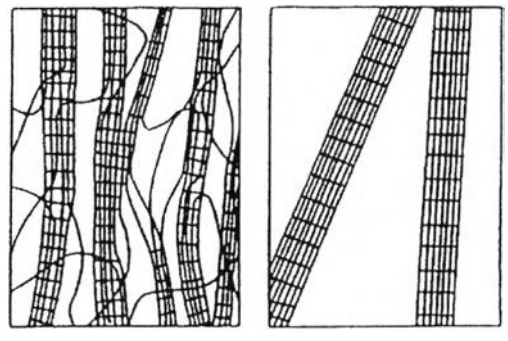


Figure 2.8 Schematic model of bacterial cellulose microfibrils (right) compared to the "fringed micelles" of plant cellulose fibrils (left) (Iguchi *et al.*, 2000).

The production of bacterial cellulose has quite successfully under a static culture, resulting in a pellicle formation on the surface of static culture, as shown in Figure 2.9 (a), but there are labor intensive (low productivity) (Yang *et al.*, 1997). Meanwhile, bacteria in agitated culture produce well-dispersed slurry as irregular mass, as shown in Figure 2.9 (b) (Hestrin and Schram, 1954). The agitated culture has not been successful due to its low yield (Byrom, 1991). Another problem on agitated culture is the culture instability, leading to a loss of cellulose-producing bacterial cells because of non-producing mutants (Valla and Kjosbakken, 1982). However, some researchers suggested that the agitated culture might be suitable for economical scale production (Yoshinaga *et al.* 1997).

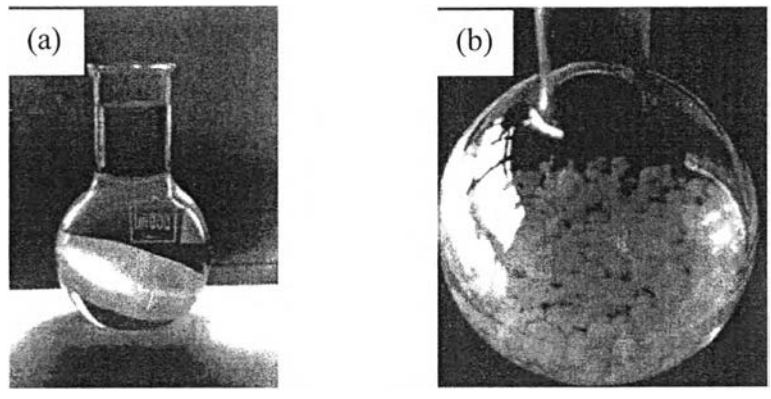


Figure 2.9 Bacterial cellulose pellicle formed in a) static culture and b) agitated culture (Bielecki *et al.*, 2002).

The I_α and I_β contents as well as crystallinity percentage of bacterial cellulose produced under two different culture conditions as determined from Fourier Transform Infrared Spectroscopy (FT-IR) and X-ray Diffraction (XRD) measurement are shown in Table 2.2 (Czaja, 2004). Compared to the agitated culture condition, bacterial cellulose produced under a stationary condition has a higher percentage of crystalline and a higher I_α content (Czaja, 2004), perhaps because the agitated culture condition interferes the crystallization process of bacterial cellulose, leading to the formation of a smaller crystalline (Watanabe *et al.*, 1998). As a result, the formation process of cellulose I_β may be preferentially induced (Watanabe *et al.*, 1998).

Table 2.2 The I_α, I_β, and crystallinity percentages of bacterial cellulose produced under two different culture conditions (Czaja, 2004)

Cellulose sample	I_α	I_β	Percent crystallinity (%)
Stationary	76	24	89
Agitated	71	29	84

2.2.5 The Physical and Mechanical Properties of Bacterial Cellulose

The physical and mechanical properties of bacterial cellulose arise from its unique three dimensional ultrafine network structure. Preliminary study reported that the Young's modulus of bacterial cellulose was greater than 15 Gpa, in any direction across the plane of sheet. It was considered that the high mechanical strength derived from the high density of interfibrillar hydrogen-bonds, due to the very fine fibrils and large contact area. In addition, there was an insignificant effect of cultivation time and amount of cellulose content on mechanical properties. Furthermore, the bacteria cellulose effectively enhanced the reinforcement of the ordinary cotton lint pulp (Yamanaka *et al.*, 1989). The unique structure also allowed the bacteria cellulose to absorb a large amount of water (up to 200 times of its dry mass) because of a large surface area. Moreover, bacterial cellulose in a wet state was reported to possess great elasticity, high strength, high conformability, and transparency (Klemm *et al.*, 2001; Czaja *et al.*,2006).

2.2.6 Bacterial Cellulose in Wound Dressing Applications

Wound repair is a dynamic process that associates with a complex interaction of various cell types, extracellular matrix molecules, soluble mediators, and cytokines. Typically, the process of wound healing is divided into four phases: homeostasis, inflammation, granulation tissue, and remodeling (Eming *et al.*, 2002).

Wound dressings are classified into traditional and modern wound dressings, such as hydrocolloids, alginates, hydrogels, etc. Modern wound dressings have been developed to provide moist environment which facilitates wound healing process (Boateng, *et al.*, 2008). In 1962, Winter found that the re-epithelization was accelerated if the wound was kept moist. In addition, maintaining a moist wound environment can enhance eshar, clot removal, re-epithelialization, and collagen synthesis, which enhance proteolytic environment and also promote the growth factor over the dry wounds (Chen *et al.*, 1992). Thus, moist wound dressing has been developed as an improvement of the traditional wound dressing. For highly water vapor permeable polyether urethane (PEU) wound dressing, it increases the amounts of fibrinogen and fibronectin and is associated with accelerated epithilization during wound healing process (Jonkman *et al.*, 1990).

Due to the unique properties of bacterial cellulose, it has shown a great potential use as a wound dressing material, as shown in Table 2.3. Bacterial cellulose possesses a better performance than conventional wound dressings: 1) conforming to the wound surface (excellent molding to all facial contours and a high degree of adherence even through the contoured parts, such as nose, mouth, etc., are observed), 2) maintaining a moist environment within the wound, 3) significantly reducing pain, 4) accelerating re-epithelialization and the formation of granulation tissue, and 5) reducing scar formation (Czaja *et al.*, 2007).

Many studies have reported on the successful of bacterial cellulose as wound dressing. The product called "Biofill" has been used for temporary skin substitutes. It helps to promote healing of many skin injuries treatments, such as basal cell carcinoma, severe body burns, facial peeling, sutures, dermabrasions, skin lesions, chronic ulcers, and skin graft (both donor and receptor sites) (Fontana *et al.*, 1990). Farah *et al.* (1990) described many advantages of Biofill product on the lesion region, such as close adhesion to body, enhancement of the exudates absorption, pain reduction (isolated nerve ending), decreased scar formation, no allergic reaction, and easy store.

Table 2.3 Properties of bacterial cellulose and its relation to the properties of an ideal wound dressing material (Czaja *et al.*, 2007)

Properties of ideal wound care dressing	Properties of bacterial cellulose dressing
Maintain a moist environment at the wound dressing surface	High water holding capacity (typical membrane can hold up to 200 g of its dry mass in water); high water vapor transmission rate
Provide physical barrier against bacterial infections	Nanoporous structure does not allow any external bacteria to penetrate into wound bed
Highly absorbable	Partially dehydrated membrane is able to absorb fluid up to its original capacity
Sterile, easy to use, and inexpensive	Membranes are easy to sterilize (by steam or γ-radiation) and package. The cost of production of 1 cm × 1 cm is $0.02

Available in various shapes and sizes	Ability to be molded *in situ*
Provide easy and close wound coverage, but allow easy and painless removal	High elasticity and conformability
Significantly reduce pain during treatment	The unique bacterial cellulose nanomorphology of never-dried membrane promotes specific interaction with nerve endings
Provide porosity for gaseous and fluid exchange	Highly porous material with pore sizes ranging from several nanometers to micrometers
Nontoxic, nonpyrogenic, and biocompatible	Biocompatible, nonpyrogenic, nontoxic
Provide high conformability and elasticity	High elasticity and conformability
Provide mechanical stability	High mechanical strength (Young's modulus value of several GPa)

Another bacterial cellulose product is "Xcell". Unlike other wound dressing products in the market, Xcell has ability to manage the moisture balance by absorbing excess exudates and donating moisture in wound area. Alvarez *et al.* (2004) reported that Xcell succeeded in the chronic venous ulceration treatment. The combination of bacterial cellulose wound dressing and compression bandage resulted in less wound pain as well as improved autolytic debridement and development of granulation tissue, compared to a standard wound care product, such as hydrogel, calcium alginate, and hydrocolloid. Moreover, Heasley *et al.* (2003) proved that Xcell was effective for the treatment of diabetic foot ulcers.

However, bacterial cellulose dressing has no antibacterial property to prevent the infection of wound. Recently, Maneerung *et al.* (2007) developed the antibacterial bacterial cellulose wound dressing by impregnating silver nanoparticles into bacterial cellulose.

Another important advantage of the bacterial cellulose dressing includes its transparency, which facilitate for the observation in the healing progress.

2.3 Sericin

2.3.1 Background of Sericin

Silk obtained from silkworm *Bombyx mori*, is a natural protein which is mainly composed of sericin and fibroin. Sericin constitutes about 25 % to 30 % of silk proteins, and it glues the fibroin fibers with successive sticky layers that help the formation of a cocoon, as shown in Figure 2.10. During raw silk production, most of sericin is discarded in silk processing wastewater. There are about 50,000 tons of sericin discarded as waste each year. Therefore, if silk sericin is recovered and recycled, it could lead to a significant economic and social benefit (Zhang, 2002).

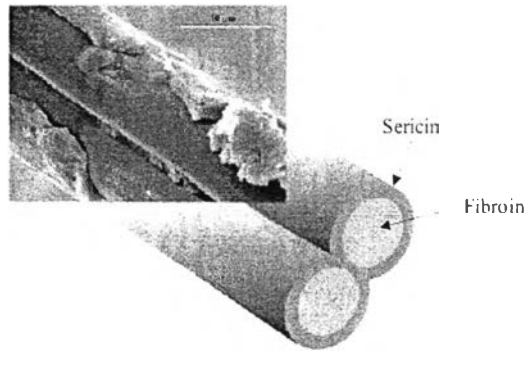


Figure 2.10 Structure of silkworm silk fiber (Rigueiro *et al.*, 2001).

Sericin is a macromolecular protein (Figure 2.11). Its molecular weight ranges widely from about 10 kDa to over 300 kDa. The sericin protein is composed of 18 amino acids. Most of these amino acids have strong polar side groups, such as hydroxyl, carboxyl, and amino groups (Zhang, 2002), as shown in Tables 2.4 and 2.5.

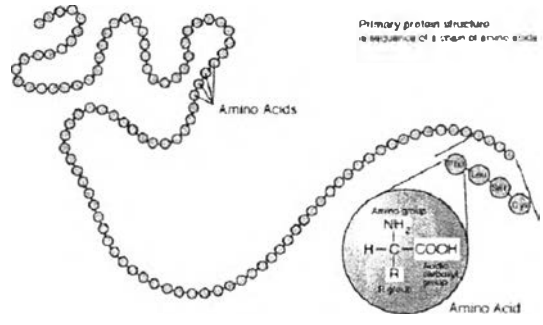


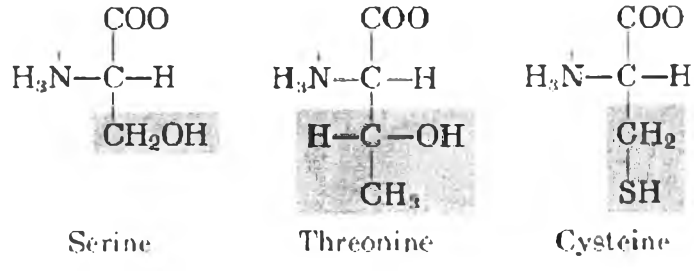
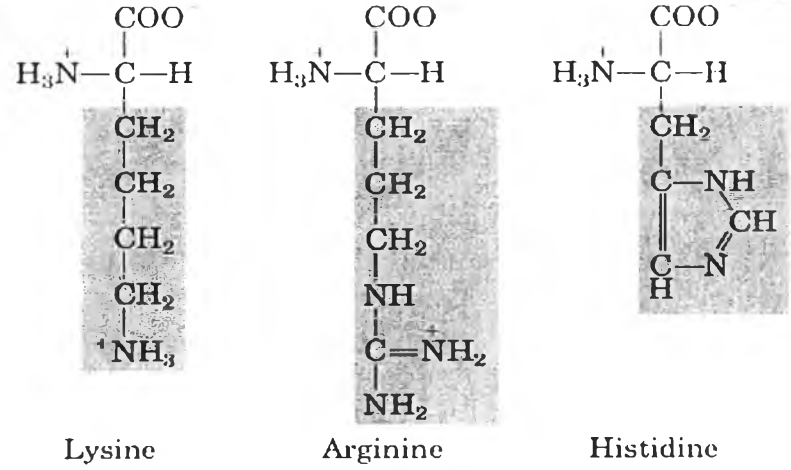
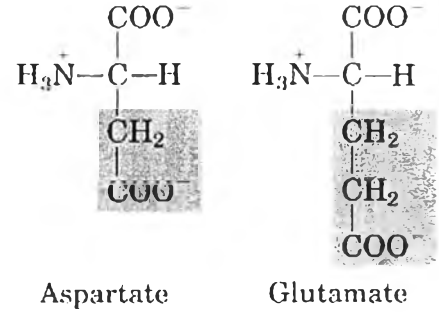
Figure 2.11 Structure of primary protein.

Table 2.4 Amino acid composition of sericin (Wu *et al.*, 2007)

Amino acid	Percentage of total amino acid (%)
Ser	27.3
Asp	18.8
Gly	10.7
Thr	7.5
Glu	7.2
Arg	4.9
Tyr	4.6
Ala	4.3
Val	3.8
Lys	2.1
His	1.7
Leu	1.7
Phe	1.6
Ile	1.3
Pro	1.2
Met	0.5

Cys	0.3
Trp	0.4
Hydrophilic	70 %
Hydrophobic	30%
Aromatic	6.6%

Table 2.5 Classification of amino acid composition of silk sericin (Lehninger, 1971)

Classification	Amino acid
Polar uncharged amino acid	 Serine Threonine Cysteine
Polar amino acids with positively charged side chains	 Lysine Arginine Histidine
Polar amino acids with negatively charged side chains	 Aspartate Glutamate

Sericin is a water-soluble protein which is dissolved in a polar solvent, hydrolyzed in acid or alkaline solutions, and degraded by a protease. Low molecular weight sericin peptides (≤20 kDa) are suitable for used in cosmetics, including skincare and haircare products, health products, and medications. High-molecular weight sericin peptides (≥20 kDa) are mostly used as medical biomaterials, degradable biomaterials, compound polymers, functional biomembranes, hydrogels, and functional fibers and fabrics (Zhang, 2002).

2.3.2 Characteristics of Sericin

2.3.2.1 Gelling Property

Sericin exists in random coil and β-sheet structures. Sericin has random coil structure when dissolve in hot water. The structure of sericin converts from random coil to β sheet structure during cooling, resulting in gel formation (Zhu *et al.*, 1998).

2.3.2.2 Sol-Gel Transition

Sericin can be dissolved in water at 50-60°C, and then return to gel during cooling which showed the sol-gel property (Zhu *et al.*, 1996).

2.3.2.3 Isoelectric Ph

The isoelectric point of sericin is about 4.0 because sericin contains more acidic than basic amino acid residues (Voegeli *et al.*, 1993).

2.3.3 Applications of Sericin

Sericin is proteinous nature which is susceptible with protein in human body. Thus, sericin is applicable in the field of medical, pharmaceutical, and cosmetics (Padamwar and Pawar, 2004). Moreover, as mentioned above, sericin contains a large amount of polar side groups (-OH, -COOH, and $-NH_2$) which is capable of covalently bonding, cross-linking, copolymerization, and blending with other materials to the formation of new biopolymers with improved properties (Kundu *et al.*, 2008).

Tsobouchi *et al.* (2005) found that sericin enhanced the attachment of human skin fibroblasts during skin lesion healing. The sericin coating on polystyrene petridishes enhanced the cell number after cultivated for 72 h to 250 % of the blank control (without sericin). The effect of sericin coating on dishes was similar to

collagen coating. Phase-contrast microscopic illustrated that cells growing on sericin coating exhibited a well-extend fibrous shape, whereas no sericin coating showed a round cell shape. Thus, sericin coating was suitable for using as substratum of cultured human skin fibroblasts.

Aramwit *et al.* (2007) found that sericin had excellent potential as a wound healing agent in rats. The results revealed that the treatment of rat wound with a cream containing 8 %(w/v) sericin showed less inflammation, faster wound size reduction, less healing time, denser collagen, and full recovery of epidermis growth, when compared to the untreated wound. Moreover, sericin cream has a better efficiency in wound healing than betadine and cream base (control).

Teramoto *et al.* (2008) prepared sericin gel film by mixing sericin with ethanol and fabricated by using molding process. The FT-IR results indicated that during gelation process, sericin formed water stable networks of intermolecular β sheet which provided good handling property and flexibility in a wet state. Since sericin contains hydrophilic amino acid, it swelled about 80 % within 3 min. Moreover, cell proliferation testing of sericin-based materials showed no toxicity. It was also described that a lower cell adhesion on sericin provided less damaging of new regenerated tissues while gel film was peeled off. Thus, sericin gel film should be appropriate for wound dressing material.

Aramwit *et al.* (2009) investigated the inflammatory mediators activated by sericin using a rat wound healing model. The result obtained from the colorimetric cell viability assay showed that sericin was non-toxic to cells. After fifteen days of dermatotomy, sericin-treated wound showed better epithelization, higher wound size reduction, faster in wound healing process, compared to the control wounds (normal saline-soaked (NS), base cream). The inflammatory mediators, tumor necrosis factor (TNF-α), and interleukin-1β (IL-1β) of sericin-treated wound were lower than NS and base cream. Thus, sericin had a high potential in wound healing process.

Sarovart *et al.* (2003) successfully enhanced the antioxidant and antimicrobial properties of air filter by coating sericin on nylon and poly(ethylene terephathalate) (PET) fibers. They found that sericin had good antioxidant property

because of the reduction in hydroxyl radical level. The antifungal and antimicrobial efficiency increased with increasing a sericin concentration. Furthermore, the sericin coating showed a smooth surface but was brittle.

Senakoon *et al.* (2009) studied on the antibacterial properties of sericin against *Escherichia coli* and *Staphylococcus Aureus* in cellular level. The critical concentration of sericin to inhibit the growths of *E. coli* and *S. aureus* were 0.2 μg/mL and 30 μg/mL, respectively. From scaning eletron micrograph (SEM), after treated both *E. coli* and *S. aureus* with sericin, the bacterial cell membranes were dysfunctioned. Moreover, in the case of sericin-treated *S. aureus* , the failure process on cell division was observed.

Padamwar *et al.* (2005) studied the moisturizing efficiency of sericin. The results proved an increase in the intrinsic moisturization of skin, such as a decrease in skin impedance, an increase in hydroxyproline level, a reduction of trans epidermal water loss (TEWL), and enhancement of smoothness on the upper layer of skin. Moreover, it was described that amino acid of sericin contributed 40 % of total natural moisturizing factor because sericin is mainly composed of serine, glycine, threonine, and proline. Restoration of proline might convert to hydroxyl proline, if moisture was present in the skin, hence increasing hydroxyproline level as mentioned above.

Kato *et al.* (1998) investigated the use of sericin to inhibit lipid peroxidation and tyrosinase activity. In the presence of sericin, a decrease in the value of lipid peroxidation (TBARS) and tyrosinase activity was observed. The inhibition of TBARS and tyrosinase activity increased with increasing the sericin concentration. Furthermore, the mechanism of sericin in antioxidant activity was described to relate to a high amount of hydroxy amino acids of sericin and threonine (serine and threonine contents of about 40%). These hydroxyl groups were responsible for the chelating with elements, such as copper and iron.

Dash *et al.* (2007) reported the antioxidant efficiency of sericin against hydrogen peroxide–induced oxidative stress in skin fibroblasts. Treating cells with sericin provided more viable cells than hydrogen peroxide treated cells. Hydrogen peroxide caused cell damage, resulting in a shrunk cell shape. In addition,

sericin reduced the amount of catalase, lactase dehydrogenase, and malondialdehyde activities. Thus, it was implied that the antioxidant efficiency of sericin promoted wound healing process.

Ahn *et al.* (2000) developed a novel muscoadhesive polymer by template polymerization of acrylic acid in the presence of silk sericin. The FT-IR results showed that poly(acrylic acid) (PAA) formed hydrogen bonding with sericin. The glass transition temperature (Tg) of PAA and sericin in the PAA/sericin complex were inner shifted towards the T_g of sericin and PVA individually, indicating the miscibility of PAA-sericin due to hydrogen bondings. Moreover, the mucoadhesive force of PAA/sericin complex had potential similar to that of the commercial product.

Teramoto *et al.* (2005) prepared sericin hydrogel by adding ethanol in a sericin solution. Freeze drying process contributed the porous hydrogel. The FT-IR results indicated that the sericin solution possessed random coil conformation. After the addition of ethanol, the sericin hydrogel showed β-rich structure because ethanol induced strong intermolecular hydrogen bond. Therefore, the structure of sericin changed from random coil to β-sheet structure and formed network structure of hydrogel after exposure to ethanol. Moreover, it was suggested that the study was useful for the development of biomedical material because it was prepared without any chemical cross-linking agent.

Tao *et al.* (2005) prepared the porous sericin materials by freeze drying method. During freeze drying, the sublimation of ice in porous sericin material occurred. Smaller pore size and bigger pore density were observed at a lower freezing temperature and at a higher sericin concentration due to the restriction of the movement of water molecules. Moreover, the XRD results also showed an interior condensed structure of sericin which possessed mostly amorphous structure and a few of crystal structure. The addition of poly(ethylene glycol) diglycidyl ether as a cross-linking agent increased the crystal structure content.

Namviriyachote *et al.* (2009) studied the physical properties of sericin/polyvinyl alcohol (PVA) films for wound dressing application. The surface density and tensile modulus of the sericin/PVA film increased with increasing a

sericin concentration but increasing sericin concentration decreased light transmission. From the dissolution testing, protein released at the maximum concentration was achieved at about 9 h. Moreover, it was described that pure sericin was fragile. On the other hand, sericin/PVA blended film had a higher mechanical property. However, all of these compositions were still brittle and fragile which still needed further improvement.

Mandal *et al,* (2009) prepared sericin/gelatin scaffold and film for tissue engineering applications. Blends of sericin/gelatin contributed high porosity, greater mechanical properties, and high swellability — these properties are crucial for tissue engineering and biomedical applications, while pure sericin was fragile. Furthermore, the blended film also promoted cell attachment and cell viability as well as exhibited low immunogenicity.

Aramwit *et al.* (2010) developed silk sericin/PVA porous three dimensional scaffolds with and without glycerin and genipin as a plasticizer and a cross-linking agent, respectively. An increase in genipin concentration resulted in a smaller pore size, a better homogeneous mixing, a higher degree of cross-linking, higher moisture absorption, a higher swelling ability, and a greater mechanical strength; but exhibited a lower level of protein released, as compare to sericin/PVA/glycerin and sericin/PVA. The findings implied the tendency for biomedical utilizations, such as wound dressing and tissue engineering applications.

Mandal *et al.* (2011) successfully prepared silk sericin/PVA hydrogel. The existence of sericin enhanced swelling property as well as the adhering and spreading of animal cells. Therefore, the sericin/PVA hydrogel had high potential for being used as biocompatible and biopolymeric material in tissue engineering and biotechnological applications.

Akturk *et al.* (2011) prepared the sericin/collagen membrane for a possible use as a wound dressing biomaterial. The water swelling property of the obtained membranes was derived from the hydrophilic property of sericin. A high content of sericin decreased tensile strength and elongation at break, but increased modulus of the membrane. However, these values were still acceptable for wound dressing application. In addition, the membrane also prevented the penetration of

microorganisms. The sericin/collagen membrane was biocompatible and supported cell attachment and proliferation of fibroblasts and keratinocytes.

Ang-atikarnkul *et al.* (2011) developed the cellulose whisker/chitin whisker/silk sericin bionanocomposite sponges for potential use in wound dressing application. Adding glutaraldehyde as a cross linking agent improved dimensional stability of the bionanocomposite sponge when immersed in water. In addition, the presence of sodium chloride (NaCl) in releasing media increased an amount of sericin releasing from bionanocomposite sponges because NaCl destroyed the hydrogen bonding between sericin and chitin whiskers. Whereas, lysozyme, an enzyme in human body that can degrade chitin, was insignificantly affected sericin releasing behavior due to the adhesion of sericin on the surface area of the whiskers, leading to a lower interaction between chitin and lysozyme. However, the remaining sericin in bionanocomposite sponges had a good advantage in providing moisturizing at the wound area.