CHAPTER II
BACKGROUND AND LITERATURE REVIEW

2.1 Wound and Type of Wound

A wound can be described as a defect or a break in the skin, causing from physical or thermal injury or as a result of the presence of an underlying medical or physiological condition. Based on the nature of the repair process, wounds can be classified as acute or chronic wounds. Acute wounds are generally tissue injuries that heal totally, with minimal scarring, within the expected time frame, regularly 8–12 weeks. The main causes of acute wounds include mechanical injuries due to external factors such as abrasions and tears which are caused by frictional interaction between the skin and hard surfaces. Chronic wounds on the other hand arise from tissue injuries that heal slowly, that is have not healed beyond 12 weeks and often reoccur. Chronic wounds include decubitis ulcers (bedsores or pressure sores) and leg ulcers (venous, ischaemic or of traumatic origin) (Joshua S. Boateng *et al.*, 2007).

2.2 Wound Healing Process

Wound healing is a specific biological process correlated to the common phenomenon of growth and tissue regeneration. It is divided into 4 phases as 1) Haemostasis and Inflammation, 2) Migration, 3) Proliferation and 4) Remodeling phases (Joshua S. Boateng *et al.*, 2007).

2.2.1 Haemostasis and Inflammation

Bleeding usually occurs when the skin is injured and serves to flush out bacteria and/or antigens from the wound. Furthermore, bleeding activates haemostasis which is initiated by exudate components such as clotting factors. Blood clot consists of fibrinogen and fibrin network, produces a clot in the wound causing bleeding to stop. The inflammatory phase occurs almost instantaneously with haemostasis, it involves both vascular and cellular responses. The release of exudate into the wound causes vasodilation through release of histamine and serotonin, allows phagocytes to enter the wound and engulf dead cells (necrotic tissue).

Necrotic tissue which is hard is liquefied by enzymatic action to produce a yellowish coloured mass described as sloughy. Platelets liberated from damaged blood vessels become activated as they come into contact with mature collagen and form aggregates as part of the clotting mechanism (Joshua S. Boateng *et al.*, 2007).

2.2.2 Migration

The migration phase involves the movement of epithelial cells and fibroblasts to the injured area to replace injured and lost skin. These cells regenerate from the margins, quickly growing over the wound under the dried scab (clot) accompanied by epithelial thickening (Joshua S. Boateng *et al.*, 2007).

2.2.3 Proliferation

The proliferative phase occurs almost simultaneously or just after the migration phase and basal cell proliferation. Granulation tissue is formed by the in-growth of capillaries and lymphatic vessels into the wound and collagen is synthesized by fibroblasts giving the skin strength and form. By the fifth day, maximum formation of blood vessels and granulation tissue has occurred. Further epithelial thickening takes place until collagen bridges the wound. The fibroblast proliferation and collagen synthesis continues for up to 2 weeks by which time blood vessels decrease and oedema recedes (Joshua S. Boateng *et al.*, 2007).

2.2.4 Remodelling

This phase involves the formation of cellular connective tissue and strengthening of the new epithelium which determines the nature of the final scar. Cellular granular tissue is changed to a cellular mass from several months up to about 2 years (Joshua S. Boateng *et al.*, 2007).

2.3 Bacterial Cellulose

2.3.1 Cellulose

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to over ten thousand $\beta(1\rightarrow4)$ linked D-glucose units, as shown in Figure 2.1. Cellulose is the structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to form biofilms. Cellulose is the most

common organic compound on Earth. About 33% of all plant matter is cellulose (the cellulose content of cotton is 90% and that of wood is 40–50%).

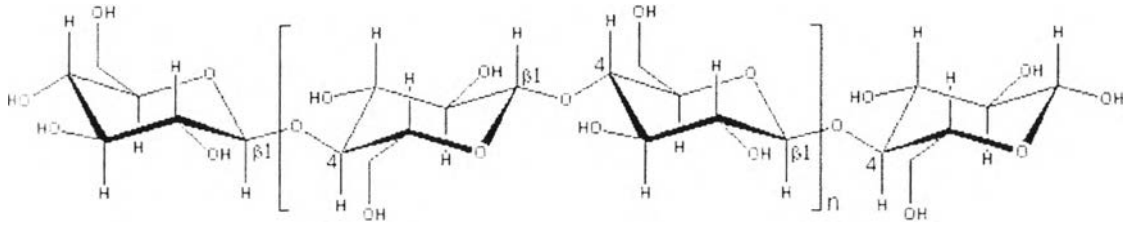


Figure 2.1 The structural unit of cellulose (Chaplin, 2009).

Cellulose is derived from D-glucose units, which condense through β(1→4)-glycosidic bonds. This linkage motif contrasts with that for α(1→4)-glycosidic bonds present in starch, glycogen, and other carbohydrates. Cellulose is a straight chain polymer: unlike starch, no coiling or branching occurs, and the molecule adopts an extended and rather stiff rod-like conformation, aided by the equatorial conformation of the glucose residues. The multiple hydroxyl groups on the glucose from one chain form hydrogen bonds with oxygen atoms on the same or on a neighbor chain, holding the chains firmly together side-by-side and forming microfibrils with high tensile strength. This strength is important in cell walls, where the microfibrils are meshed into a carbohydrate matrix, conferring rigidity to plant cells (Crawford, 1981).

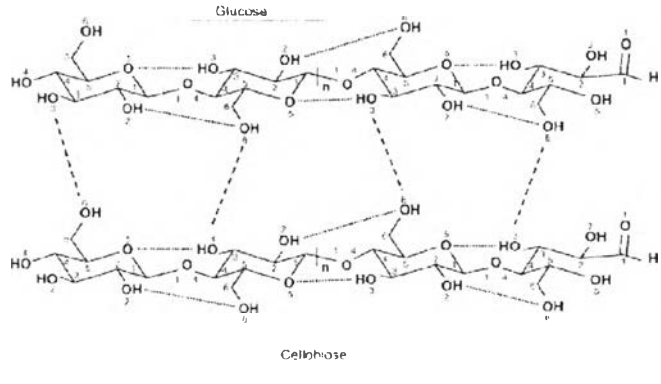


Figure 2.2 Representation of inter- and intra-chain hydrogen bonding network (Sconti, 2010).

Several different crystalline structures of cellulose are known, corresponding to the location of hydrogen bonds between and within strands. Natural cellulose is cellulose I, with structures I_α and I_β. Cellulose produced by bacteria and algae is enriched in I_α while cellulose of higher plants consists mainly of I_β. Cellulose in regenerated cellulose fibers is cellulose II. The conversion of cellulose I to cellulose II is irreversible, suggesting that cellulose I is metastable and cellulose II is stable. With various chemical treatments it is possible to produce the structures cellulose III and cellulose IV (Serge Pérez and William Mackie, 2001).

2.3.2 Principal Pathways to Cellulose

There are four different pathways to synthesize the biopolymer cellulose that are described schematically in Figure 3. The first one is the most popular and industrial important isolation of cellulose of plants including separation processes to remove lignin and hemicelluloses. The second way is biosynthesize of cellulose by different types of microorganisms such as algae (Vallonia), fungi (Saprolegnia, Dictysteliumdiscoideum) and bacteria (Acetobacrter, Achromobacter). The third way is enzymatic in vitro synthesis starting from cellobiosyl fluoride. The last way is the first chemosynthesis from glucose by ring-opening polymerization of benzylated and pivaloylated derivatives (Klemm *et al.*, 2001).As mentioned above, Cellulose can be synthesized by bacteria. But not all of these bacterial species are able tosecrete the synthesized cellulose as fibers extracellular (Jonas & Farah, 1998) as shown in Table 1.

2.3.3 Cellulose Synthesis Using *Acetobacter xylinum*

Acetobacter xylinum is a gram negative, rod-shaped, non-pathogenic and aerobic bacterium which is interested for many studies due to the large quantity of cellulose product (Ross *et al.*, 1991). For the first time, the bacterium *A. xylinum* was decribed in 1886 by Brown. He identified a gelatinous mat formed in the course of vinegar fermentation on the surface of the broth as chemically equivalent to cell-wall cellulose. Microorganism of the genus Acetobacter are obligate aerobes and usually found on fruit, vegetables, in vinegar, fruit juices, and alcoholic beverages. The synthesis mechanism helps the aerobic bacterial cells to arrive the oxygen-rich surface. Like-wise, the pellicle protects the cell from the lethal effect of UV-light,

enhances colonization on fruits, retains moisture to prevent drying, and hold the bacteria in the aerobic environment (Klemm *et al.*, 2001). This family is able to oxidize alcohols to acids and ketones, especially for the production of vinegars using ethanol, wine or cider as carbon sources. The optimal pH range for cellulose production is 4-7 and the optimal growth temperature is 25-30°C (Jonas & Farah, 1998). Surma-Slusarska *et al.* (2008) found that the optimum condition of cellulose production was 7 days at 30°C. Glucose and mannitol were the good carbon source that resulted in high yielding of bacterial cellulose. Furthermore, bacterial cellulose exhibited a high thermal stability which degraded around 300°C.

The molecular structure of cellulose synthesized by *A. xylinum* is same as plant cellulose. Unlike plant cellulose, bacterial cellulose has no lignin, pectin, and hemicellulose. Moreover, the characteristics of bacterial cellulose differ from plant cellulose due to its high crystallinity, high water absorption, and mechanical strength in the wet state, ultra-fine network structure (nanoscale fiber diameter) and mould ability (Klemm *et al.*, 2001).

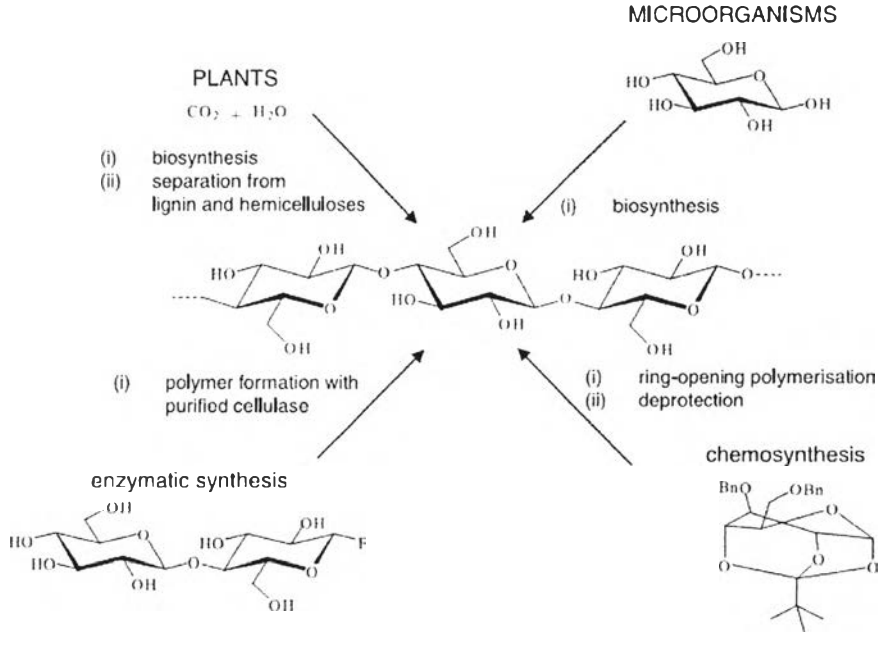


Figure 2.3 Pathway to synthesize cellulose (Klemm *et al.*, 2001).

Table 2.1 Bacterial cellulose producers. (Jonas & 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

The cellulose formation includes five fundamental enzyme mediated steps: the transformation of glucose to UDP-glucose-6-phosphate and glucose-1-phosphate and finally the addition of UPD-glucose to the end of growing polymer chain by cellulose synthase as shown in Figure 2.4. Cellulose synthase (UPD-glucose: 1,4-β-D-glycosyltransferase) is regarded as the essential enzyme in the synthesis process. It is subjected to a complicated regulation mechanism, which controls activation and inactivation of the enzyme (Klemm *et al.*, 2001).

A. xylinum forms the cellulose between the outer and the cytoplasma membrane. The cellulose-synthesizing complexes or terminal complexes (TC) are linearly arranged, and in association with pores at the surface of the bacterium. In the first step of cellulose formation glucan chain aggregates consisting of approximately 6-8 glucan chains are elongated from the complex. These subelementary fibrils are assembled in the second step to form microfibrils followed by their tight assembly to form a ribbon as the third step that presented in Figure 5. The Matrix of the interwoven ribbons constitutes the bacterial cellulose membrane or pellicle. Bacteria cellulose ribbon produced by one bacterial cell as shown in Figure 6 and Figure 7 demonstrate that A. xylinum cells are distributed throughout the network of the cellulose ribbons.

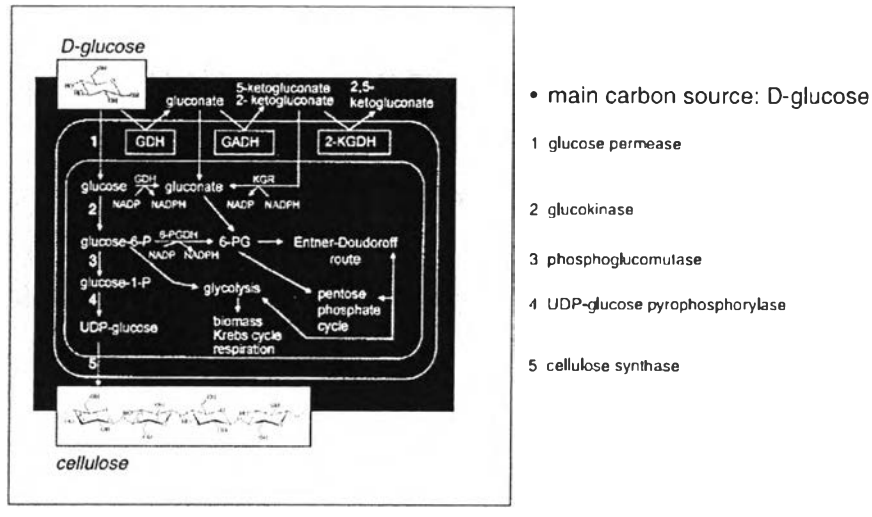


Figure 2.4 Pathways of carbon metabolism in *Acetobacter xylinum* (Klemm *et al.*, 2001).

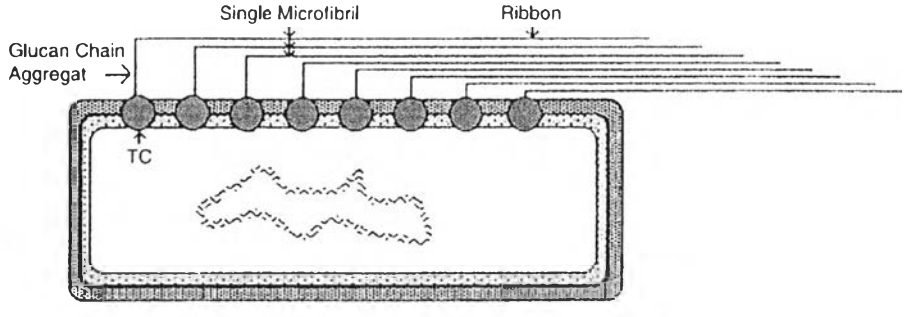


Figure 2.5 Formation of bacterial cellulose (Klemm *et al.*, 2001).

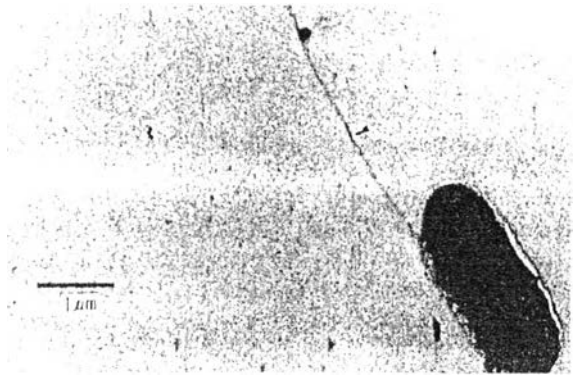


Figure 2.6 TEM image of bacterial cellulose ribbon produced by a bacterial cell (Klemm *et al.*, 2001).

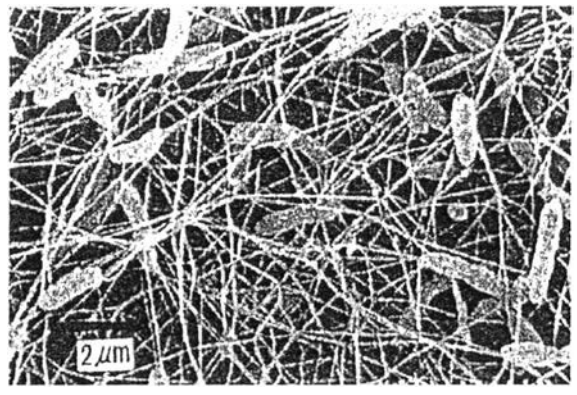


Figure 2.7 SEM image of a bacterial cellulose network including the bacterial cells (Klemm *et al.*, 2001).

2.3.4 Structure of Bacterial Cellulose

Numerous researches on bacterial cellulose revealed that it is chemically identical to plant cellulose, but it's different in macromolecular structure and properties as shown in Figure 8. In addition, dimensions of bacterial cellulose fibrils are 20-50 nm width, at least 10µm long. The ultrafine ribbon network structure was stabilized by extensive hydrogen bonding (Yamanaka *et al.*, 1989). The degree of polymerization is 13000-14000 for plant and 2000-6000 for bacterial cellulose (Jonas & Farah, 1978).

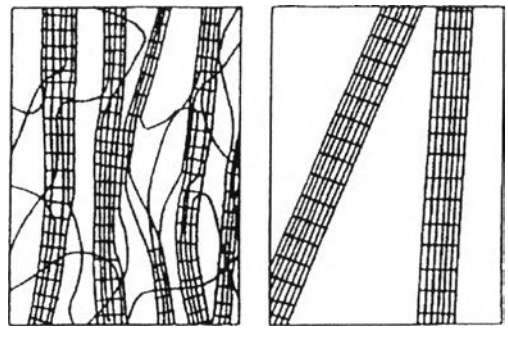


Figure 2.8 Schematic model of BC microfibrils (right) drawn in comparison with the 'fringed micelles'; of PC fibrils(left) (Iguchi *et al.*, 2000).

The production of bacterial cellulose has quite successfully in static culture that resulted in pellicle formed on the surface of static culture as shown in Figure 9. But there are low productivity and labor intensive (Yang *et al.*, 1997). In

case of, bacterial cellulose in agitated culture produced in well-dispersed slurry as irregular mass as shown in Figure 10. (Hestrin & Schram, 1954). The agitated culture has not been successful in bacterial cellulose production due to its low yield (Byrom, 1991). Another problem for agitated culture is associated with the culture instability that resulted in loss of cellulose producing cells because of non-producing mutants (Valla & Kjosbakken, 1982). However, some researchers suggested that the agitated culture might be suitable for economical scale production (Yoshinaga *et al.* 1997).

Figure 2.9 BC pellicle formed in static culture (Bielecki *et al.*, 2002)

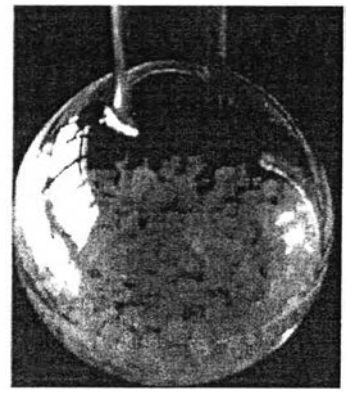


Figure 2.10 BC pellets formed in agitated culture (Bielecki *et al.*, 2002)

The I_α , I_β content and percent crystallinity from FTIR and XRD measurement showed higher percent crystallinity and I_α-rich cellulose in stationary condition, as shown in table 2.(Czaja, 2004). In case of agitated culture, the interference during the crystallization process of bacterial cellulose may lead to the formation of crystallites in a smaller size. Therefore, the formation process of cellulose I_β may be preferentially induced (Watanabe *et al.*, 1998).

Table 2.2 Cellulose I_α and I_β content (%) and Percent crystallinity. (Czaja, 2004)

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

2.3.5 The Physical and Mechanical Properties of Bacterial Cellulose

The physical and mechanical properties of bacterial cellulose arise from the unique 3-D ultrafine network structure. Preliminary study has measured the Young's modulus of bacterial cellulose as high as (>15 GPa) , in any direction across the plane of sheet. It is considered that the high mechanical strength arise from the high density of interfibrillar hydrogen-bonds, due to the very fine fibrils and large contact area. In addition, there is no significant effect of varying cultivation time and amount of cellulose content on mechanical properties. Furthermore, the pulp derived from bacterial cellulose can enhance reinforcement to the ordinary cotton lint pulp (Yamanaka *et al.*, 1989).

This unique structure can also enhance to absorb a large amount of water (up to 200 times of its dry mass) because of the large surface area. Moreover, bacterial cellulose in wet state show great elasticity, high wet strength, high conformability and transparency (Klemm *et al.*, 2001; Czaja *et al.*,2006).

2.3.6 Bacterial Cellulose in Wound Dressing Application

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 has been divided into four phases: homeostasis, inflammation, granulation tissue and remodeling (Eming *et al.*, 2002). Wound dressings can be classified into traditional and modern wound dressings (moist wound dressings such as hydrocolloid, alginate and hydrogel). Modern wound dressings have been developed because it provide moist environment which facilitate for wound healing process (Boateng *et al.*, 2008). In 1962, George

Winter found that the re-epithelization was accelerated if the wound was kept moist. In addition, the moist wound environment can enhance eshar and clot removal, reepithelialization and collagen synthesis which promote proteolytic environment and the growth factor over the dry wound (Chen *et al.*, 1992). Thus, moist wound dressingshave been developed as an improvement on the traditional wound dressings. Moreover, for highly water vapor permeable wound dressing (PEU) can promote a high amount of fibrinogen and fibronectin which associated with accelerated epithilization during wound healing process (Jonkman *et al.*, 1990).

Due to its unique properties, bacterial cellulose has shown great potential for using as wound dressing material as shown in table 3. Bacterial cellulose actually performed better than conventional wound dressings in 1.conforming to the wound surface (excellent molding toall facial contours and a high degree of adherence even to thecontoured parts such as nose, mouth, etc. were observed), 2. maintaining a moist environment within the wound, 3. significantly reducing pain, 4.acceleratingre-epithelialization andthe formation of granulation tissue, and 5.reducing scarformation (Czaja *et al.*, 2007)

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

Properties of ideal wound care dressing	Properties of bacterial cellulose
Maintain a moist environment at the wound/dressing surface	High water holding capacity (typical membrane can hold up to 200g of its dry massin 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

Properties of ideal wound care dressing	Properties of bacterial cellulose
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^2 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 BC 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)

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 can help to promote healing of many skin injuries 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 graft (Fontana *et al.*, 1990). Farah *et al.* (1990) described many advantages of Biofill product on the lesion region such as close adhesion to anybody location, enhancing the absorption of exudates, reduced pain (isolated nerve ending), reducing scar formation, no allergic reaction and easily stored.

Another bacterial cellulose product is Xcell. Unlike other wound dressing products in the market, Xcell product has ability to manage the moisture balance by absorbing excess exudates and donating moisture in wound area. Alvarez *et al.* (2004) reported that Xcell success with the chronic venous ulceration treatment. The combination of bacterial cellulose wound dressing and compression bandage resulted in less wound pain, improved autolytic debridement and developed of granulation tissue as compared with standard wound care. Moreover, Heasley *et al.* (2003) proved that Xcell can be effectively used to treat on the diabetic foot ulcers.

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

2.4 Chitosan

2.4.1 Background of Chitosan

Chitosan is the N-deacetylated derivative of chitin, as shown in Figure 15, although this N-deacetylation is almost never complete. A sharp nomenclature with respect to the degree of N-deacetylation has not been defined between chitin and chitosan. The structures of chitin and chitosan are shown in Figure 13 and 14. Chitin and chitosan are of commercial interest due to their high percentage of nitrogen (6.89%) compared to synthetically substituted cellulose (1.25%). These

naturally abundant materials also exhibit a limitation in their reactivity and process ability. In this respect, chitosan is recommended as suitable functional materials, because these natural polymers have excellent properties such as biocompatibility, biodegradability, non-toxicity, adsorption properties, etc.

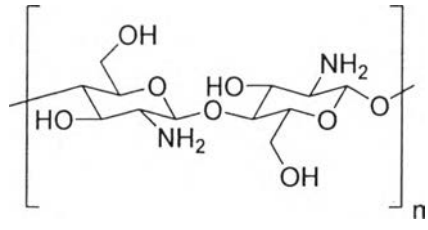


Figure 2.11 The Structural of chitosan.

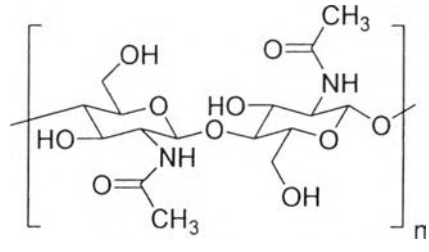


Figure 2.12 The Structural of chitin.

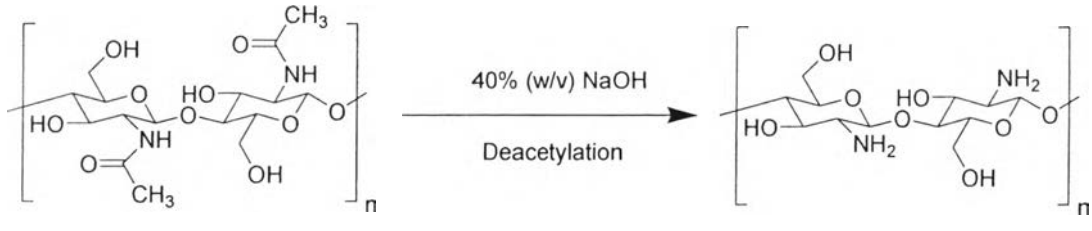


Figure 2.13 Deacetylation of chitin.

2.4.2 Characteristics of Chitosan

2.4.2.1 Antioxidant Activity

Park *et al.* (2004a) prepared three kinds of partially deacetylated hetero-chitosan such as 90% deacetylated, 75%deacetylated, and 50% deacetylated chitosan from crab chitin, and their antioxidant properties were measured using electron spin resonance spectrometry. Park and coworkers found that

their antioxidant activities were dependent on the DD, and the 90% deacetylated chitosan showed the highest free radical scavenging activities.

2.4.2.2 Antimicrobial Activity

Many studies have been conducted on the antimicrobial activity of chitosan since Allan and Hadwiger (1979) first reported that chitosan and its derivatives had broad-spectrum antimicrobial effects. The group of researches proved that chitosan is capable of inhibiting the growth of some microorganisms including bacteria, yeasts, and fungi.

2.4.2.3 Anti-Inflammatory Activity

Inflammation involving a wide variety of physiological and pathological processes is a kind of defense mechanism in an organism and is initiated by the invasion of pathogens or by tissue injury caused by biological, chemical, or physical damage. Inflammation is largely divided into acute and chronic inflammation. Acute inflammation is a short-term normal response, whereas chronic inflammation may progress from acute inflammation if the stimuli persist, causing individual tissue damage (Drayton *et al.*, 2006).

2.4.3 Biomedical Application of Chitosan

Chitosan's properties allow it to rapidly clot blood, and have recently gained approval in the United States and Europe for use in bandages and other hemostatic agents. The U.S. Marine Corps has been shown in testing that the chitosan hemostatic products are quickly stop bleeding and reduce blood loss in comparison to gauze dressings, and result in 100% survival of otherwise lethal arterial wounds in swine. Moreover, it can increase patient survival. Chitosan has natural antibacterial properties, and it is hypoallergenic, which further support its use in field wound dressing.

R.A.A. Muzzarelli (1997) studied on the human enzymatic activities related to the therapeutically administration of chitin derivatives. The results revealed that chitosan has important applications in photography due to its resistance to abrasion, its optical characteristics, and film forming ability. Silver complexes are not appreciably retained by chitosan and therefore can easily be penetrated from one layer to another of a film by diffusion.

H.F. Mark *et al.* (1985) reported that the applications of chitosan in cosmetic field, organic acids are usually good solvents; chitosan has fungicidal and fungi static properties. Chitosan is the only natural cationic gum that becomes viscous on being neutralized with acid. These materials are used in creams, lotions and permanent waving lotions and several derivatives have also been reported as nail lacquers.

Malette *et al.* (1986) studied the effect of treatment with chitosan and saline solution on healing and fibroplasia of wounds made by scalpel insertions in skin and subcutaneous tissue in the abdominal surface of dogs.

Yannas *et al.* (1982) proposed a design for artificial skin, applicable to long-term chronic use, focusing on a nonantigenic membrane, which performs as a biodegradable template for synthesis of neodermal tissue. It appears that chitosan, having structural characteristics similar to glycosamino glycans, could be considered for developing such substratum for skin replacement.

Sparkes and Murray (1986) developed a surgical dressing made of a chitosan–gelatin complex. The procedure involves dissolving the chitosan in water in the presence of a suitable acid, maintaining the pH of the solution at about 2–3, followed by adding the gelatin dissolved in water. The ratio of chitosan and gelatin is 3:1 to 1:3. To reduce the stiffness of the resulting dressing a certain amount of plasticizers such as glycerol and sorbitol could be added to the mixture. Dressing film was cast from this solution on a flat plate and dried at room temperature. It was claimed that, in contrast to conventional biological dressings, this experimental dressing displayed excellent adhesion to subcutaneous fat.

Biagini *et al.* (1991) developed an N-carboxy-butyl chitosan dressing for treating plastic surgery donor sites. A solution of N-carboxy-butyl chitosan was dialyzed and freeze-dried to produce a $10\times20\times0.5$ cm^3 soft and flexible pad, which was sterilized and applied to the wound. This dressing could promote ordered tissue regeneration compared to control donor sites, better vascularization and the absence of inflammatory cells were observed at the dermal level, while fewer aspects of proliferation of the malpighian layer were reported at the epidermal level.

Muzzarelli (1995) introduced another chitosan derivative, 5-methyl-pyrrolidinone chitosan, which is believed to be very promising in medical

applications. This polymer is claimed to be compatible with other polymer solutions, including gelatin, poly(vinyl alcohol), poly(vinyl pyrrolidone) and hyaluronic acid. The advantages include healing of wounded mensical tissues, and of decubitus ulcers, depression of capsule formation around prostheses, limitation of scar formation and retraction during healing.

Shin-YeuOng *et al.* (2008) studied on the development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. A new chitosan-polyphosphate dressing has been developed that accelerated blood clotting, platelet adhesion, thrombin generation, and absorbed significantly more blood than chitosan. These results provide the first evaluation and optimization of a chitosan-polyphosphate complex for hemostatic applications. Further investigation will more precisely delineate the mechanisms behind the improved hemostatic activity of chitosan-polyphosphate, and validate the findings in vivo. The chitosan-polyphosphate with incorporated silver was also effective in reducing mortality compared to standard gauze treatment in a full-thickness wound model contaminated with high levels of *P. aeruginosa*.

Jaehwan Kim *et al.* (2011) prepared and characterized a bacterial cellulose/chitosan composite for potential biomedical application. Bacterial cellulose/chitosan composite has been prepared by immersing wet bacterial cellulosepellicle in chitosan solution. SEM images show that chitosan molecules can penetrate into BC multilayered structure. By incorporation of chitosan in bacterial cellulose, crystallinity tends to decrease while the thermal stabilityhas certain improvement. At the same time, the mechanical properties of bacterial cellulose/chitosan composite maintain some levels. Cell culture test results indicate that bacterial cellulose/chitosan composite has much better biocompatibility than pure bacterial cellulose.

Jyh-Ping Chen *et al.* (2008) developed electrospun collagen/chitosan nanofibrous membrane as wound dressing. The membrane was found to promote wound healing and induce cell migration and proliferation. The Young's modulus increased after crosslinking, however, the ultimate tensile strength, tensile strain, and water sorption capability decreased after crosslinking. The nanofibrous membrane showed no cytotoxicity toward growth of 3T3 fibroblasts and had good in vitro

biocompatibility. From animal studies, the composite nanofibrous membranes were better than gauzeand commercial collagen sponge in wound healing rate.

Muenduen Phisalaphong *et al.* (2008) studied on the biosynthesis and characterization of bacteria cellulose–chitosan film. Modifying bacterial cellulose by means of adding 0.25–0.75 (%w/v) of chitosan in the culture medium during biosynthesis by *A. xylinum* caused a number ofvaluable features including high mechanical properties in a wet and a dry state, a high water absorption capacity (WAC) and high average surface area. Moreover, the FTIR spectra of the BCC films indicated intermolecular interaction between the hydroxyl groups of cellulose fiber and the aminogroups of chitosan.

Tao Wang *et al.* (2012) prepared hydrogel sheets of chitosan, honey and gelatin as burn wound dressings. A hydrogel sheet composed of chitosan, honey and gelatin (HS; 0.5:20:20, w/w) was developed as a burn wound dressing. HS showed powerful antibacterial efficacy up to 100% to *Staphylococcus aureus* and *Escherichia coli*, significantly superior to chitosan and honey used separately. A series of toxicological evaluations demonstrated that HS is not toxic and not irritant to skin and body. Therefore, HS demonstrated its potential as a treatment.

Shuichi Aoyagi *et al.* (2007) developed a novel chitosan wound dressing loaded with minocycline for the treatment of severe burn wounds. Novel wound dressings composed of chitosan (CH) film and minocycline hydrochloride (MH) were prepared using commercial polyurethane film (Tegaderm) as a backing. These dressing showed the sustained release of minocycline in vitro. Various formulations were applied to severe burn wounds in rats in the early stage, and the wound status and change in the wound surface area were examined. The obtained dressing is suggested as a useful formulation for the treatment of severe burn wounds.

Jen Ming Yang *et al.* (2004) studied on the properties of chitosan containing PP-g-AA-g-NIPAAm bigraft nonwoven fabric for wound dressing. PP-g-AA-g-NIPAAm-Chitosan with high water content was prepared in this study. By using the thermosensitive behavior of poly(NIPAAm), the modified nonwoven fabric can be easily stripped off from the skin. As the values of WVTR, permeance and permeability of the PP-g-AA-g-NIPAAm-Chitosan are comparable to the

commercial products and no bacterial transport results, the modified nonwoven fabric may be considered for wound dressing.

T. Takei *et al.* (2012) synthesized a chitosan derivative soluble at neutral pH and gellable by freeze–thawing, and its application in wound care. A chitosan derivative soluble in an aqueous solution at neutral pH and gellable by freeze–thawing was developed. The derivative had extremely low cytotoxicity. The cryogels showed low cellular adhesiveness. The time necessary for complete degradation of the gels by lysozyme was tunable by the derivative concentrationin the gels. The gels applied to full-thickness skin wounds promoted accumulation of inflammatory cells, especially PMN, andaccelerated wound healing. It is concluded that the chitosan gels are effective for wound care.

Moustafa M.G. *et al.* (2009) used the chitosan/polyamine biopolymers based cotton as a model system to prepare antimicrobial wound dressing. The antimicrobial activity of chitosan/polyvinyl amine system showed promising results. Further studies using different anchors will be done, as well as different microorganisms will be tested also. The resulting antimicrobial dressing based cotton can be used successfully in healing wounds, burns and ulcers as well as diabetic foot ulcers.

2.5 Sericin

2.5.1 Background of Sericin

Silk derived from silkworm *Bombyx mori* is a natural protein that is mainly made of sericin and fibroin proteins as shown in Figure 11. Sericin constitutes 25–30% of silk protein and it envelops the fibroin fiber with successive sticky layers that help in the formation of a cocoon. Sericin ensures the cohesion of the cocoon by gluing silk threads together. Most of the sericin must be removed during raw silk production at the reeling mill and the other stages of silk processing. At present, sericin is mostly discarded in silk processing wastewater. The cocoon production is about 1 million tons (fresh weight) worldwide and this is equivalent to 400,000 tons of dry cocoon. Processing of this raw silk produces about 50,000 tons

of sericin. If this sericin protein is recovered and recycled, it can represent a significant economic and social benefit (Zhang, 2002).

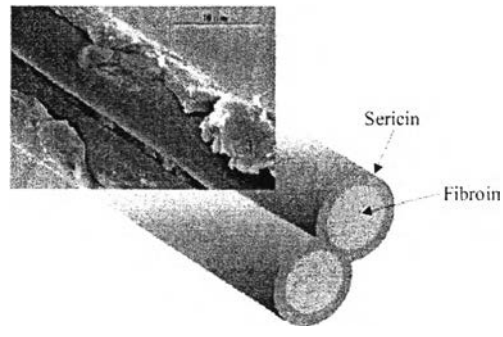


Figure 2.14 Structure of silk (Rigueiro *et al.*, 2001).

Sericin is a macromolecular protein (Figure 12). Molecular weight of sericin ranges widely from about 10 to over 300 kDa. The sericin protein is composed of 18 amino acids. Most of these amino acids have strongly polar side groups such as hydroxyl, carboxyl, and amino groups (Zhang, 2002), as shown in Table 4 and 5.

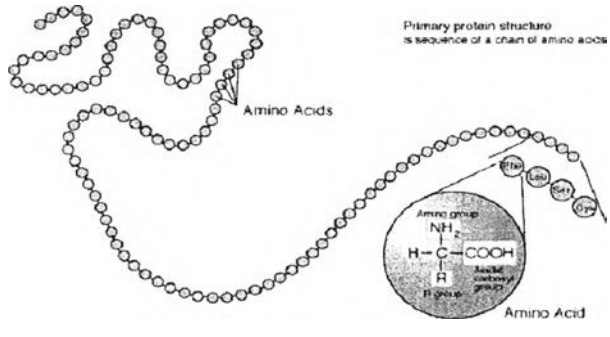


Figure 2.15 Structure of primary protein.

Table 2.4 Classification for amino acid of silk sericin. (Lehninger, 1971)

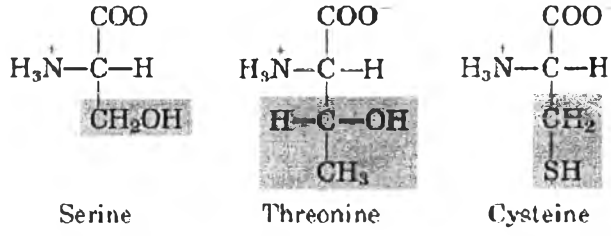
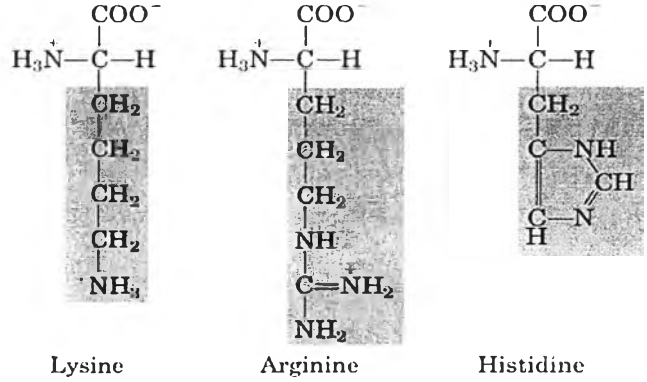
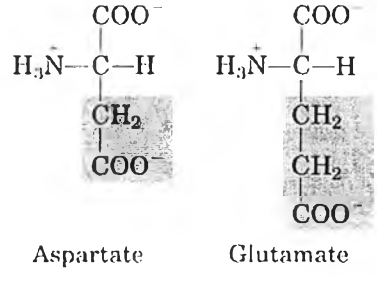
Classification	Amino acid
Polar uncharged amino acid	 Serine Threonine Cysteine
Polar amino acids with positively charged side chains	 Lysine Arginine Histidine
Polar amino acids with negatively charged side chains	 Aspartate Glutamate

Table 2.5 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%

Sericin is a water-soluble protein. When sericin is dissolved in a polar solvent, hydrolyzedin acid or alkaline solutions, or degraded by a protease, the size of the resulting sericin molecules depends on factors such as temperature, pH, and the processing time. Lower molecular weight sericin peptides (≤20 kDa) or sericin hydrolysates are used in cosmeticsincluding skincare and haircare products, health products, and medications. High-molecular weight sericin peptides (≥20 kDa) are mostly used as medical biomaterials, degradablebiomaterials, compound polymers, functional biomembranes, hydrogels, and functional fibers and fabrics (Zhang, 2002).

2.5.2 Characteristics of Silk Sericin

2.5.2.1 Gelling Property

Sericin exists in random coil and β-sheet structure. Sericin has random coil structure when dissolve in hot water. The structure of sericin convert from random coil to β sheet structure when decrease temperature that resulted in gel

formation (Zhu *et al.*, 1998).

2.5.2.2 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).

2.5.2.3 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).

2.5.3 Biomedical Applications of Silk 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 & Pawar, 2004). Moreover, as the mentioned above, the amino acids of sericin contain polar side groups (-OH,-COOH,-NH_2) which can be formed covalent bonding, crosslinked, copolymerized or blend with other material to the formation of new biopolymer with improve properties (Kundu *et al.*, 2008).

Tsobouchi *et al.* (2005) found that sericin could enhance the attachment of human skin fibroblasts for healing skin lesions. Sericin coating, the living cell number after cultivated for 72 h attained 250% of the no sericin coating sample. The result of sericin coating was similar to collagen coating. Phase-contrast microscopic illustrated that cells growing on sericin coating exhibit a well-extend fibrous shape, whereas no sericin coating showed a round cell shape. Thus, sericin coating is suitable for using as substratum of cultured human skin fibroblast.

Aramwit *et al.* (2007) studied the effect of sericin on wound healing in rats. The results revealed that 8% sericin cream promoted less inflammation, faster wound size reduction, less healing time, denser collagen, and full recovery of epidermis growthwhen compared with untreated ones. While, betadine and cream base (control) showed less potential for wound healing when compare to sericin cream as mentioned above. Hence, sericin cream has a high potential for wound healing.

Teramoto *et al.* (2008) prepared sericin gel film from mixing sericin with ethanol by molding process. FTIR results indicated that sericin form water stable networks of intermolecular β sheet during gelation step which provide good

handling property (flexible) in wet state. In addition, the stretched sericin gel film can generate molecular orientation with increasing maximum stress. Due to most of sericin contain hydrophilic amino acids, it can swell rapidly in water and equilibrate at a water content of about 80% within 3 mins. Moreover, cell proliferation testing resulted in no toxicity. They also described that low cell adhesion on sericin may provide less damaging of new regenerated tissues while gel film was peeled off. All of these reasons, sericin gel film appropriate for wound dressing material.

Aramwit *et al.* (2009) investigated the inflammatory mediators activated by sericin, using a rat wound healing model. MTT assay showed that sericin is non-toxic to cells as shown in the cell proliferation results. Fifteen days after dermatotomy, sericin-treated wound promoted better epithelization, higher wound size reduction, faster in wound healing process as compared with the control wounds (NS, base cream). The inflammatory mediators, TNFα and IL-1β ofsericin-treated wound showed lower level than NS and base cream. Thus, sericin has a high potential in wound healing process.

Sarovart *et al.* (2003) could successfully enhance the antioxidant and antimicrobial properties to air filter by coating sericin on nylon and PET fibers. They found that sericin has good antioxidant property because of the reduction in hydroxyl radicals level. The antifungal and antimicrobial efficiency are increased with sericin concentration. Furthermore, the morphology sericin coating revealed that it was smooth surface and brittle.

Senakoon *et al.* (2009) studied on the antibacterial properties of sericin against E.coli and S.aureus in cellular level. The critical concentration of sericin for anti E-coli and S.aureus was 0.2 μg/ml and 30 μg/ml respectively. From SEM micrograph, after treated both E.coli and S.aureus with sericin appeared in membrane dysfunction. Moreover, in case of sericin-treated on S.aureus illustrated in the failure process on cell division.

Padamwar *et al.* (2005) studied the moisturizing efficiency of sericin. The results proved an increase in the intrinsic moisturization of skin such as decrease in skin impedance, increase in hydroxyproline level, reduce trans epidermal water loss (TEWL) and enhance smoothness on the upper layer of skin. Moreover, they

described that amino acid contributes 40% of total natural moisturizing factor. Sericin is mainly composed of serine, glycine, threonine and proline. Restoration of proline may convert to hydroxyl proline because of moisture present in the skin, and thus increases its content as the mentioned above.

Kato *et al.* (1998) investigated the useful of sericin in the inhibition of lipid peroxidation and tyrosinase activity. In the presence of sericin, resulted in decrease the value of lipid peroxidation (TBARS) and tyrosinase activity. The inhibition of lipid peroxidation and tyrosinase activity are increase with sericin concentration. Furthermore, the mechanism of sericin in antioxidant activity was described because of the high amount of hydroxy amino acids (serine and threonine ~ 40%) in sericin. These hydroxyl groups can be formed with chelating elements such as copper and iron.

Dash *et al.* (2007) reported the antioxidant efficiency of sericin to protect hydrogen peroxide –induced oxidative stress in skin fibroblasts. Treating cells with sericin provided more viable cells than H_2O_2 treated. H_2O_2 caused cell damage that illustrated in shrunk cell shape. In addition, sericin reduced the amount of catalase, lactase dehydrogenase and malondialdehyde activity. Thus, they implied that the antioxidant efficientcy of sericin can promote in wound healing process.

Ahn *et al.* (2000) developed a novel muscoadhesive polymer by template polymerization of acrylic acid in the presence of silk sericin. FTIR results reported that poly(acrylic acid)(PAA) formed hydrogen bonding with sericin. The T_g of PAA and sericin in the PAA/sericin complex were inner shifted between the T_g of sericin and PAA individually which indicated the miscibility of PAA-sericin due to hydrogen bonding. The dissolution rates of PAA/sericin complex depend on pH. Moreover, the mucoadhesive force of PAA/sericin complex had a potential similar to the commercial product.

Teramoto *et al.* (2005) prepared sericin hydrogel by adding ethanol in sericin solution. Freeze drying process contributed the porous hydrogel. From FTIR results indicated that sericin solution was random coil conformation. In case of, the sericin hydrogel was β-rich structure because ethanol can induce strong intermolecular H-bond. Therefore, the structure of sericin changed from random coil

into β-rich structures which form network structure of hydrogel. Moreover, they suggested that this study was useful for biomedical material because it was prepared without any chemical crosslinking 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 which remained many pores in them. The smaller pore size and bigger pore density were observed when used at lower freezing temperature and higher sericin concentration due to the restriction on the movement of water molecules. Moreover, XRD results showed the interior condensed structure of sericin which contained mostly amorphous structure and a few of crystal structure. The addition of PEG-DE as crosslinking agent resulted in the increasing of β sheet structure.

Wu *et al.* (2006) investigated the interpenetrating polymer network hydrogel based on sericin and poly (N-isopropylacrlamide) by using GA and MBAAM as crosslinking agent. From DSC measurement showed a single T_g value which indicated PNIPAAM- sericin formed miscible pair. Morphology of swollen samples illustrated that the porous distributed homogeneusly. The swelling ratios depend on both temperature and pH value. The swelling ratio decreased at temperature above LCST of IPN hydrogel. At pH 4.4 and 7.5 exhibited low swelling ratios because of polyisoelectric points of sericin and salting out effect.

Namviriyachote *et al.* (2009) studied the physical properties of sericin-PVA films for wound dressing application. The surface density, tensile modulus increased with sericin concentration but decreased in light transmission. From dissolution testing, showed the release of protein with maximum concentration about 9 hours. Moreover, they described that pure sericin was fragile. Sericin/PVA blend film can enhance mechanical property. However, all of these compositions were still brittle and fragile that needs to further improve.

Mandal *et al.* (2009) prepared sericin/gelatin scaffold and film for tissue engineering applications. Blends of sericin/gelatin contribute high porosity, improved mechanical properties and high swellability – with these properties are crucial for tissue engineering and biomedical applications, while pure sericin resulted

in fragile. Furthermore, it can be support cell attachment, cell viability and low immunogenicity.

Aramwit *et al.* (2010) developed silk sericin-PVA porous 3-D scaffold with and without glycerin and genipin as plasticizer and crosslinking agent respectively. The increasing of genipin concentration resulted in the smaller pore sizes, better uniformity, higher degree of crosslinking, higher moisture absorption, higher swelling ability and enhanced mechanical strength but lower level of protein released as compare with sericin/PVA/glycerin and sericin/PVA. All of these results revealed to the tendency for biomedical products such as wound dressing, tissue engineering applications.