

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

2.1 Wound Dressing

The primary objective in wound care products are the promotion of rapid wound healing with the best functional and cosmetic results. Wound dressings are usually used to encourage the various stages of wound healing and create better healing conditions. They often cover the wound surface to accelerate its healing. An ideal wound dressing should (a) maintain high humidity, (b) protect the wound from secondary infections, (c) absorb exudates, (d) reduce the wound surface necrosis, (e) provide thermal insulation, (f) stimulate the growth factors, (g) anti-bacterial, (h) non-antigenic and non-toxic, and (i) biocompatible (Purna, *et al.*, 2000; Choi, *et al.*, 2001; Shuangyun, *et al.*, 2008). Wound dressings can be prepared into various forms such as hydrogel, film, fiber, sponge, etc.

In this study, Sponge-like wound dressing was prepared because it can meet the requirements such as higher gas permeation and protection of wound from infection and dehydration (Hinrichs, *et al.*, 1992). Among many biomaterials suitable for an ideal wound dressing, we selected cellulose whiskers, chitin whiskers, and silk sericin to prepare sponges composed of each component, then evaluated the potential for wound dressing application in terms of sericin releasing and cytotoxicity.

2.2 Mechanisms of Releasing

The active wound dressings are interested in advance because it can protect wound and heal wound in the same time. The mechanisms of release are divided following the drug delivery system. The development of controlled drug delivery technology is rapidly progressive in the area of medical health care. The mechanism of drug release need to be concerned such as, to know how drug can release from the medium materials to human body, or how long does it take to deriver to termination.

There are three primary mechanisms which active substances can be released from delivery materials explained as follows;

2.2.1 Diffusion Controlled Release

In this case, polymer and drug or other active agents have been blended to form homogeneous system. Diffusion of drug occurs when the drug passes through the polymer matrix to the external environment that is shown in Figure 2.1. While the release continues, the rate of release generally decreased with this system, because drug has longer distance to derive so its need longer diffusion time to release.

For this system, the drug delivery device is basically stable, which does not change in size either swelling or degradation, in the biological environment.

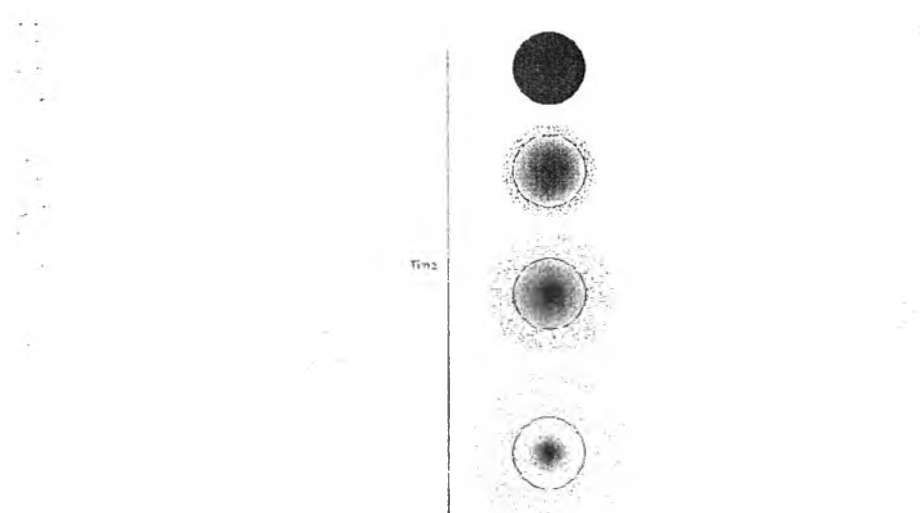


Figure 2.1 Drug delivery from a typical matrix drug delivery system.

2.2.2 Swelling Controlled Release

Controlled drug delivery also possible to be designed so that it is incapable of releasing of its active agents until it is placed in an appropriate biological environment. Generally, swelling controlled release system is firstly dry and when placed in body, it will absorb the water or body fluids and swell. The swelling increases the aqueous solvent content, resulting in the active agents diffuse through the swollen matrices into external environment. Most of the materials that

used in this system are based on hydrogels which are polymers that cannot dissolve when placed in water or biological environment. One of advantages of polymer swelling is it can be triggered by change the surrounding environment, such as pH, temperature, or ionic strength. So that polymer can either shrink or swell upon a change in any of these factors. The Figure 2.2 illustrates the general changing process of swelling controlled release system.

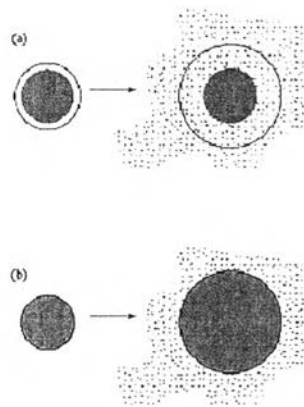


Figure 2.2 Drug delivery from environmentally sensitive release system.

2.2.3 Erosion Controlled Release

All of previously mentioned systems are based on polymers that do not change their chemical structure during swelling. However, a great deal of drug delivery research is being concerned with biodegradable polymers. So, the materials that can degrade within body resulting in eliminating to remove them after the active agents have completely been released. Most of biodegradable polymers are created to degrade as a result of hydrolysis to smaller chains into biologically acceptable compounds. Degradation may occur as shown schematically in Figure 2.3. Some degradable polymer matrices, degrade through bulk hydrolysis which throughout the matrices. But some polymers, the degradation occurs only at the surface of the polymers, resulting in the release rate is proportional to the surface area of the polymer matrix.

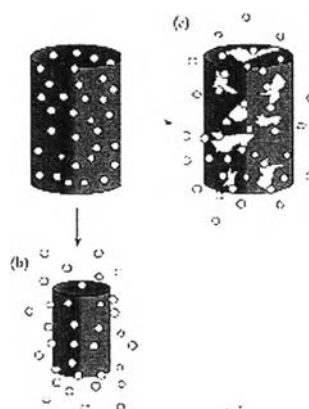


Figure 2.3 Drug delivery from (a) bulk erosion and (b) surface erosion systems.

2.3 Silk

Silk is a natural protein fiber that silk filaments are obtained from the mulberry silkworm (*Bombyx mori*.) The *Bombyx mori* cocoon is spun into a single unbroken strand, which usually has length between 600-1500 meters. The strand is called a bave which is made of two tiny sub-strands called brins. The brins are made of a protein called fibroin, which are held together by another protein called sericin — or silk gum (Figure 2.1). When sericin still coats on the fibroin, makes the strand tough and hard like paper.

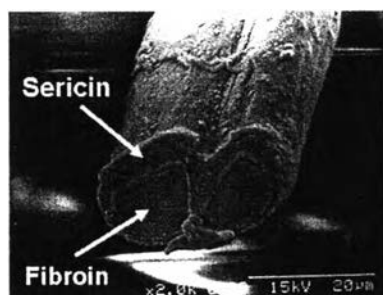


Figure 2.4 Structure of silk fiber.

Table 2.1 Composition of silk in *Bombyx mori* (Gulrajani, 1988)

Component	Percentage (%)
Fibroin	70-80
Sericin	20-30
Wax matter	0.4-0.8
Carbohydrates	1.2-1.6
Inorganic matter	0.7
Pigment	0.2
Total	100

During raw silk production, most of sericin must be removed by boiling the silk in a mild alkali surfactant bath, leaves the silk soft and lustrous. This process is called degumming.

2.3.1 Sericin

Sericin is a macromolecular protein and exists about 30% of the total cocoon weight. Molecular weight of sericin ranges between 24-400 kDa (kilodalton) and is composed of 18 amino acids — which similar to proteins in a human body. (Kundu, *et al.*, 2008) The main contents of amino acids in sericin are serine and aspartic acid, which contain many polar side groups such as hydroxyl, carboxyl and amino groups, as shown in Table 2.2.



However sericin is insoluble in cold water, however it is easily hydrolyzed, which a long protein chain breaks down to smaller fractions, leads to easily disperse, or soluble in hot water (Gulrajani, 1988). Sericin occurs mainly in an amorphous random coil and can change into a β -sheet conformation, which the random coil is easier to soluble than the β -sheet structure.

Table 2.2 Amino acid composition of sericin obtained from cocoon mulberry wild silkworm (Yamada, 1978)

Amino acid	R	Percentage (%)
Serine	-CH ₂ OH	28.58
Aspartic acid	-CH ₂ COOH	18.46
Glycine	-H	16.80
Threonine	-CH(OH)CH ₃	9.92
Residual amino acids	-	26.24

Sericin is very useful because of its properties such as antioxidant, antibacterial, UV resistant, and absorbs and releases moisture easily. (Zhang, 2002) Applications of sericin range from cosmetics to biomedical products, which include its use in anticancer drugs, anticoagulants, and cell culture additives. (Kundu, *et al.*, 2008) Also, many researches of sericin composite have been reported such as biodegradable materials, biomedical materials, functional membranes, fibers and fabrics. (Zhang, 2002)

For example, blend sericin with polyurethane foam. (Hatakeyama, 1996) The resulting polyurethane could be made into film, fiber and molded material with excellent mechanical and thermal properties. Moreover, these blended materials are biodegradable because they contain biodegradable sericin segment.

Akiyama, *et al.*, (1993) investigated sericin copolymerized with a mixture of poly(acrylic acid) (PAA) and acrylamide. This polymer could absorb water up to 180 times of its weight by using molecular weight of sericin over 60 kDa.

In 2001, Nagura *et al.*, prepared a sericin hydrogel with DMU as cross-linking agent, the 2% (w/v) of sericin solution was mixed with DMU at 80°C, casted and dried at 30°C for 24h to prepare sericin hydrogel. The hydrogel revealed that crosslinked sericin is tough at all portions of DMU, while non-crosslinked sericin is brittle.

Due to the antioxidant and the antibacterial properties of sericin, it was used to coat on nylon and polyester for use as an air filter (Saravart, *et al.*,

2003). They found the coated fibers had smooth surface, brittle and had strong potential to use for the indoor air filter to reduce the amount of toxic free radicals, fungi, and bacteria.

Zhang, *et al.*, (2006) investigated sericin-insulin bioconjugated by using glutaraldehyde as cross-linking agent due to largely amount of hydroxyl and amino side groups of sericin. They found sericin can be conjugated covalently with insulin and sericin can improve both physicochemical and biological stability.

Applications of sericin in medical fields have been studied. For instant, sericin/gelatin scaffold and film were prepared for tissue engineering applications (Mandal, *et al.*, 2009). Sericin/gelatin blends enhanced mechanical strength, high porosity, high swelling — all of these are important for tissue engineering and biomedical applications. In addition, the blends also enhanced cell attachment, viability and low immunogenicity.

Moreover, sericin is suitable for used as drug-release biomaterials of charged protein drugs — fluorescein isothiocyanate–albumin (FA) (Nishida, *et al.*, 2011). It found drug release from these preparations sustained longer when the concentration of sericin greater.

In 2005, Sericin was found enhancing the attachment of cultured human skin fibroblasts (Tsubouchi, *et al.*, 2005). The percentages of living cell after 3days was enhanced to 250% compared to no-sericin control, which this ability is important for wound care. Moreover, sericin was used as mitogenic factor for mammalian cell culture (Terada, *et al.*, 2005). They found sericin accelerate cell proliferation which low-molecular weight sericin showed better cell viability than high-molecular weight sericin.

Antioxidant of sericin has beneficial properties especially in wound healing fields because it against oxidation which is the wound damage process. In previous research found that sericin against the oxidative stress induced by hydrogen peroxide of wound skin fibroblast resulting in promote cell viability of AH927 cells (Dash, *et al.*, 2007). In skin wound healing in rats, it found sericin acted as free radical scavengers during wound healing process (Gupta, *et al.*, 2002).

2.4 Whiskers

In the previous decade nanocomposites, are very interested, which that the composites with at least one component having a dimension in 1-1000 nm. The most popular methods are adding nanofillers to reinforcing the matrix, which enhances properties of the matrix.

Nanofibrils, or called whiskers, have been interested to use as reinforcing fillers due to their high aspect ratio and highly crystalline structure (Wattanapanit, *et al.*, 2008). Many researches use inorganic whiskers, such as layered silicate, silica, cadmium sulfide nanoparticles and carbon nanotubes (Roohani, *et al.*, 2008). These inorganic whiskers are limited in processability, biocompatibility and biodegradability (Nair and Dufresne, 2003a). Whereas natural whiskers have low cost, renewability, availability, good biocompatibility, low density, and ease to modified by chemical and mechanical methods (Lu, *et al.*, 2004; Nair and Dufresne, 2003a).

The use of natural whiskers as reinforcing materials have also been investigated which can be prepared from various polysaccharides, such as chitin, cellulose and starch. In this study mention only chitin and cellulose whiskers.

2.4.1 Cellulose Whisker

Cellulose is the most abundant natural polysaccharides, which is found in plant cell walls and can also be produced by bacteria (Azizi Samir, *et al.*, 2005). Because cellulose is made of the repeat units of the glucose monomer, it is called polysaccharide which it is a linear polymer of poly- $\beta(1\rightarrow4)$ -D-glucose (Zuluaga *et al.*, 2007). Similar to chitin, cellulose has strong inter- and intra-molecular hydrogen bonding due to hydroxyl group at C2 position.

Cellulose is odorless, hydrophilic, biodegradability and has no taste. It is insoluble in water and common organic solvents. However, it can be broken down into its glucose units by treating with concentrated acids at high temperature (Visakh and Thomas, 2010). Cellulose has six different polymorphs; cellulose I, II, III_I, III_{II}, IV_I, and IV_{II} with possibility of conversion from one structure to another (Azizi Samir, *et al.*, 2005).

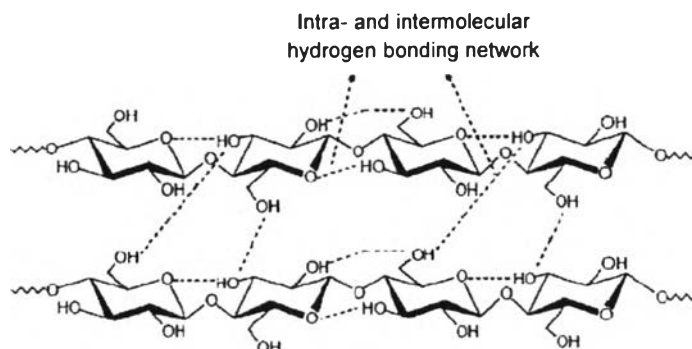


Figure 2.5 Representation of the extensive intra- and intermolecular hydrogen bonding network occurring in cellulose.

Cellulose whiskers are also extracted by an acid hydrolysis which is removed the amorphous regions. The use of hydrochloric acid (HCl), the prepared cellulose whiskers have no charges on the whisker surfaces. Whereas, the sulfuric acid (H₂SO₄)-prepared cellulose whiskers gave negatively charged surface and more stable than the HCl hydrolysis (Azizi Samir, *et al.*, 2005; Elanthikkal, 2010).

The obtained cellulose whiskers have the diameter about 5-10 nm and the length ranges from 100 nm to several micrometers depending on the sources of cellulose. Properties of cellulose whiskers from earlier researches are shown in the Table 2.3 (Oksman, *et al.*, 2006; Mathew and Dufresne, 2002).

Table 2.3 Properties of cellulose crystallites

Property	Cellulose crystallites
Length (nm)	300–2000
Diameter (nm)	5–20
Aspect ratio (L/d)	20–60
Tensile strength (Mpa)	10,000
E-modulus (GPa)	150

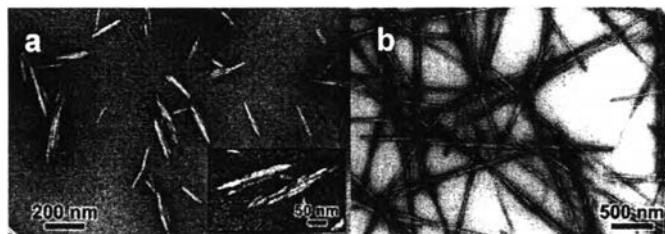


Figure 2.6 TEM micrographs of negatively stained cellulose whiskers obtained by sulfuric acid hydrolysis of (a) cotton and (b) tunicate (Elazzouzi-Hafraoui *et al.*, 2008).

Cellulose whiskers have good mechanical properties, maybe due to the binding bonds if neighboring atom (Azizi Samir, *et al.*, 2005). Thus, they are common used as the reinforcing fillers in many applications. The isolated cellulose whiskers can be extracted from various sources, such as tunicate, commercial microcrystalline cellulose (MCC), cotton, grass, bacterial cellulose etc.

First of all, the cellulose whiskers were successful extracted from tunicate — a sea animal — which the whiskers are called tunicin (Faveir, *et al.*, 1995). After passed through the sulfuric acid treatment, the tunicin crystals were parallel-piped rods with the widths ranged 10-20 nm and the lengths ranged from 100 nm to several micrometers. Then, the suspension of tunicin whiskers was mixed with the copolymer of styrene (35% w/w), buthyl acrylate (65% w/w) and a small amount of acrylic acid to form nanocomposite films. The films showed the mechanical properties are improved with a small percentage of tunicin whiskers.

Since then, the cellulose whiskers were isolated from commercial microcrystalline cellulose (MCC) by acid hydrolysis. From previous studies showed the average aspect ratio of cellulose whiskers which derived from MCC around 11, that lower than tunicin whiskers (Capadona, *et al.*, 2009). The group of Oksman prepared cellulose whiskers/poly(lactic acid) nanocomposites. Cellulose whiskers usually aggregate due to their strong hydrogen bonds between the individual cellulose whiskers and difficult to disperse uniformly in a non-polar solvent because of their polar surface. Oksman, *et al.*, (2006) found that DMAc/LiCl can be used as swelling/separating agent for MCC, resulting in improving the mechanical

properties. After that, they treated cellulose whiskers with either *tert*-butanol or a surfactant (Petersson, *et al.*, 2007). Both treatments improved the storage modulus of PLA at higher temperature and the thermal stability still stabled.

Other sources of cellulose whiskers have been observed. For example, sisal whiskers with the aspect ratio about 60 were extracted from an acid hydrolysis of sisal fibers, to reinforcing the PVAc matrix (Garcia de Rodriguez, *et al.*, 2006). Cellulose whisker derived from cotton linter which obtained the aspect ratio around 11-12 (Roohani, *et al.*, 2008). The cotton cellulose whiskers were mixed with the copolymer of PVA and PVAc. Otherwise, grass of Korea was investigated to use as starting cellulose sources (Pendey, *et al.*, 2008 and 2009). Then, the cellulose whiskers were modified with maleic anhydride (MA) and mixed with PLA. Cassava bagasse nanofibrils were also studied (de Moris Teixeira, *et al.*, 2009) and used to reinforcing the thermoplastic cassava starch. All results revealed the same tendency that the presence of cellulose whiskers enhanced the thermal and the mechanical behaviors.

Recently, the cellulose whiskers were prepared from new sources to investigate their structure, morphology and properties. Cellulose whiskers could be extracted from the branch-barks of mulberry by alkaline treatment and subsequently an acid hydrolysis (Li, *et al.*, 2009). The cellulose whiskers had high crystallinity with the diameters ranged 20-40 nm and had 400-500 nm in lengths.

Similar to banana plants which have been investigated to use as starting materials for cellulose whiskers. Zuluaga, *et al.* (2007) isolated cellulose microfibrils from banana farming residues. The banana rachis was passed through mechanical and chemical treatments. Two different chemical treatments; peroxide/organosolv (PO) and peroxide/homogenization (PH), were evaluated. The width of individual cellulose microfibrils closed to 5 nm in both treatments. The PH treatment showed longer microfibrils, and individualized or aggregation into bundles could be seen. In contrast, the PO-microfibrils generally aggregated and had higher crystallinity that maybe due to more efficient removal of non-cellulosic substances, such as hemicelluloses and lignin.

After that, they continued studied the effect of alkaline treatments on structure and morphology of cellulose microfibrils from banana rachis (Zuluaga, *et al.*,

2009). The cellulose microfibrils were treated with four different alkaline treatments; peroxide alkaline, peroxide alkali-hydrochloric acid, 5 wt% and 18 wt% potassium hydroxide. The first three treatments estimated the fibrils with 3-5 nm. in the diameters, several micrometers in the lengths and they consisted of cellulose IV₁ form. While the fibrils from 18wt% KOH were still microfibrils, but consisted of cellulose II structures.

Thus, cellulose is the best material since it is the most abundant renewable polysaccharide found on earth, biopolymer, biocompatibility, hydrophilicity. Moreover, it has high aspect ratio, lead to high mechanical properties and easy to fabricate.

2.4.2 Chitin Whisker

Chitin is the second most abundant polysaccharides in nature (after cellulose). It mainly consists of the aminosugar N-acetylglucosamine — β -(1,4)-2-deoxy-D-glucopyranose — which is partially deacetylated. The mostly deacetylated form of chitin is called chitosan, which degree of deacetylation is higher than 50% (Khor and Lim, 2003). Chitin and chitosan are slightly different in structures. Chitin contains an acetyl amide group, whereas chitosan contains an amine group at the C2 position. In addition, chitin is often considered with cellulose because both have closely structures, like chitosan. At the C2 position, cellulose contains a hydroxyl group.

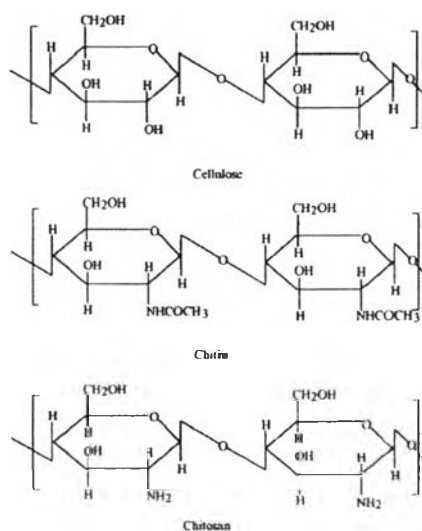


Figure 2.7 Structure of cellulose, chitin and chitosan.

However both chitin and cellulose are natural polysaccharides, they are found in different sources. Chitin is nitrogenous polysaccharides that are found in the outer of skeleton of insects, crabs, shrimps, lobsters and in the inner structures of other invertebrates, which are shown in the Table 2.4.

Table 2.4 Sources of chitin and chitosan (Mathur and Narang, 1990)

Sea animals	Insects	Microorganisms
Annelida	Scorpions	Green algae
Mollusca	Spiders	Yeast (β -type)
Coelenterata	Brachiopods	Fungi (cell walls)
Crustaceans:	Ants	Mycelia Penicillium
Lobster	Cockroaches	Brown algae
Crab	Beetles	Spores
Shrimp		Chytridiaceae
Prawn		Ascomydes
Krill		Blastocladiaceae

Chitin is a tough semitransparent substance and has a different polymorphic structure, depends on its function in animal body. The α -chitin has much tighter crystalline, due to an anti-parallel arrangement of the chains, while a β -chitin has a parallel arrangement with weaker intermolecular hydrogen bonding. In addition, the β -chitin is more reactive than the α -chitin (Rinaudo, 2006).

Chitin whisker is ordered nanocrystallites embedded into low-ordered nano-domains, as shown in Figure 2.9a, which mostly found in the exoskeleton of crustacean. Chitin whisker is prepared by acid hydrolysis to remove low-ordered region, then high crystalline chitin is obtained as shown in Figure 2.9b. The vigorous mechanical shearing will generate individual chitin fibril or called chitin whisker as illustrate in Figure 2.9c (Nair and Dufresne, 2003a). The nanocrystallites of chitin are obtained which having aspect ratio ranges between 10-120, depending on the sources (Wongpanit, *et al.*, 2007).

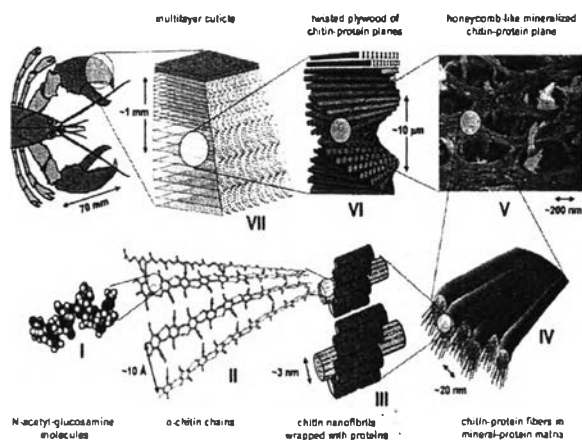


Figure 2.8 Multi-level supermolecular architecture of chitin.

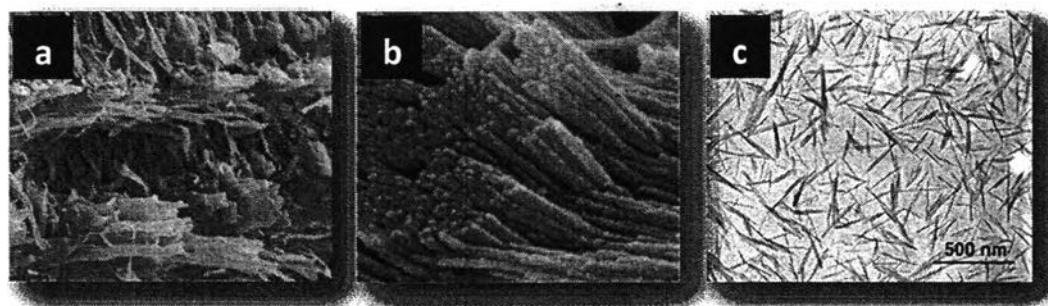


Figure 2.9 Illustration of (a) chitin, (b) crystallite chitin, and (c) chitin whisker.

Chitin whiskers have been successfully prepared from crab shells (Nair and Dufresne, 2003a) via an acid hydrolysis, based on the method for squid pen (Paillet and Dufresne, 2001) and *Riftia* tubes (Morin and Dufresne, 2002). The results indicated that the chitin whiskers had slender parallel-piped rods with an aspect ratio about 16. Moreover, chitin whisker extracted from squid pen with the average aspect ratio closed to 15, to reinforcing the copolymer of styrene and butyl acrylate (Paillet and Dufresne, 2001). High aspect ratio of chitin whisker could be obtained from *Riftia* tubes by an acid hydrolysis (Morin and Dufresne, 2002). The obtained chitin whiskers with the aspect ratio around 120 used to reinforcing phase in the poly(caprolactone) matrix.

That all of these previously researches revealed chitin whisker in reinforcing field. However chitin is very useful in many applications such as water

treatment, textile, paper, cosmetic, wound dressing and biotechnology (Goosen, 1997). Especially, chitin has an advantage in biocompatibility, which better than chitosan, because an acetyl amide group of chitin is similar to an amide group of protein in living body (Muzzareli, 1985). Moreover, chitin is very interesting in biomedical fields because when applied chitin to human wounds and surgical cloths, it accelerates the skin healing process (Usami, *et al.*, 1998).

For instant, chitin could induce the collagen production (Minami, *et al.*, 1996). Collagen deposition is important for the proliferation phase of wound healing process which can reduce scar tissue. Moreover, chitin was found that less extensive inflammatory reaction compared with chitosan, which coated polyester nonwoven fabric (Kojima, *et al.*, 1998).

Wongpanit, *et al.*, (2007) mixed the chitin whiskers with silk fibroin to improving a dimensional stability of silk fibroin sponges by using freeze-drying method. The chitin whiskers were isolated from shrimp shells with the average aspect ratio around 10. The results revealed the chitin whiskers not only improved the dimensional stability, but also promoted a cell spreading. Moreover, the existence of chitin whiskers indicated that the sponges still cytocompatible, leading to suitable for tissue engineering applications.

Then, the chitin whiskers reinforced alginate nanocomposite fibers were investigated in the objective of biodegradability (Wattanaphanit, *et al.*, 2008). The chitin whiskers were prepared from shrimp shells which gave the average aspect ratio about 7.5. The mechanical and the thermal properties of the nanocomposite fibers significantly increased at the low content of chitin whiskers. The investigation of biodegradability using lysozyme/Tris-HCl solution found that the chitin whiskers in the nanocomposite fibers accelerate the biodegradation process in the presence of lysozyme.