CHAPTER II
LITERATURE REVIEW

2.1 Scar

A scar is a permanent patch of skin that grow over the wound.

2.1.1 Scar formation

The scar formation is a natural part of the the wound healing process. A scar is formed after a wound is healed. It is proof and evidence of a healed wound. A scar is a mark that is formed on the skin. There is an inborn ability of the human body to fight against different traumas and ailments. The healing process replaces the wounded or unhealthy/injured tissue with new and healthy tissue. The original tissue is the tissue of the skin and is functional. The replaced tissue is a connective tissue which is also known as a scar in ordinary terms. The scar formation is motivated by the incoming of some specialized cells towards the wounded area. These cells form a scar during the scar formation process (Nauta et al., 2011).

Once an injury has occurred, some reactions take place. These reactions are termed as regeneration, average repair, exaggerated healing and insufficient healing. The first reaction is regeneration in which almost the exact replacement of the tissue takes place. The second reaction is an average repair in which the connective tissue is not an exact replacement of the original tissue but still keeps some of the balance. The third is exaggerated healing. It involves fibrosis and contractures. The last reaction leads to chronic wounds or chronic ulcers in which the wound is not properly healed, either it takes much time or can never be healed.

When a wound occurs, different types of cells that comprise of mediators as well as some enzymes and secretions and many other big and small changes occur at that wounded area. They collectively work and interact with each other performing different functions and roles that result in the wound healing gradually. This is a natural and biological way of body's defense and healing

mechanism. Though the process is natural, a scar is left behind which shows that once there was a wound at this place. These scars are made up of collagen. Collagen is a fibrous protein that is present in the second layer of the skin. It makes up connective tissues.

The replaced skin or the scar gradually improves its strength with time but never attains the strength of the original skin. Scars also have different appearance. There are different types of scars depending upon the nature of the wound, the skin type of the person and the strength of the body's defensive mechanism (Hardy et al., 1989).

2.1.2 Types of scar

2.1.2.1 Normal scar

Normal scar forms after 2–3 weeks, initially appearing pink or red and raised, and then flattening to a linear white line.

2.1.2.2 Widened scar

Widened scar is stretched scar that normally occur in sites of higher tension.

2.1.2.3 Hypertrophic scar

Hypertrophic scar is overgrowths of fibrous scar tissue that remain within the boundaries of the original wound and tend to regress spontaneously.

2.1.2.4 Keloid scar

Keloid scar produces lateral growth of the scar tissue into the surrounding normal skin. They remain over a long period and do not usually spontaneously regress.

2.1.3 The main causes of scar

2.1.3.1 Genetic

Some people are just prone to scarring. This can be partly due to skin type (see below) but can be just realted to the fact that some peoples genetic make up, mean they produce too much collagen when they are healing.

2.1.3.2 Skin Type

Dark skins tend to scar badly and are particularly susceptible to Keloids. People who burn easily in the skin also tend to scar badly.

2.1.3.3 Hormones

Some women develop scars when they are pregnant but not at any other time. Also Keloids, tend to occur during or just after puberty, implying that there is a hormonal link to scar formation.

2.1.3.4 Size of wound

The larger the wound the more likely you are to develop an abnormal scar. This is related to the time to heal.

2.1.3.5 Time to heal

It has been proven that the longer the wound takes to heal, the more likely that you are to scar. The time to heal is dictated by the severity of the wound, wound infections, number of dressing changes and how deep the wound is.

2.1.4 Scar treatment (Niessen et al., 1999).

2.1.4.1 Pressure dressing

It is compressive garments (e.g. Tubigrip™, zinc oxide plaster, custom-made splinting) may be helpful for hypertrophic scars and for contractures. The mechanism of action of pressure dressings is unknown, but may include localized hypoxia causing degradation of fibroblasts and collagen.

Garments should be worn for 24 hours a day and optimal results occur after 6-12 months and patient compliance can be a problem. Adhesive micropore tape may be effective as a preventative measure; its action is thought to be partly through pressure and partly occlusive (by maintaining hydration).

Silicone gel sheets and cream were initially used for burn scars. The silicone gel appears to work independently from pressure, but does not permeate the skin. The sheets are of variable thickness and formed from crosslinked silicone polymers. They are thought to work by occlusion, increasing hydration to the scar. Side effects are local irritation, skin breakdown and poor compliance (they

should be worn 24 hours a day for up to one year). They are effective and are widely used to manage hypertrophic and keloid scars.

2.1.4.2 Steroids

It is intralesional injections of corticosteroids (e.g. 40 mg/ml triamcinolone acetonide at intervals of 4–6 weeks until the scar flattens or discomfort is controlled) can be used to treat hypertrophic and keloid scars. A 5-year response rate of 50–100%, increasing to 85–100% if combined with surgical excision, has been observed. Adverse effects include pain of injection, hypo-pigmentation, telangiectasia, ulceration, fat atrophy and systemic response.

2.1.4.3 Radiation

The use of radiation is controversial due to the potential for malignant transformation, and is not recommended in children or near radiosensitive areas (e.g. breast, thyroid). It is most effective immediately after surgical excision and some studies have shown a prevention of keloid recurrence in about 75% of patients at one-year follow-up. The dose used is usually 1500 Gray, delivered in fractions within the first ten days postoperatively.

Using high-dose-rate iridium-192 brachytherapy after surgical excision of keloid scars is effective in preventing the recurrence of keloid scars, and is associated with few side effects.

2.1.4.4 Laser therapy

Different types of lasers have been studied as treatment options for keloid and hypertrophic scars. They are useful because they are precise and haemostatic, causing minimal trauma to tissue. Three types of laser can be used: pulse-dye, neodymium:yttrium, and erbium:yttrium. The pulse-dye laser is effective in reducing subjective symptoms, colour and height of keloid scars. Combining laser treatment with corticosteroids (intralesional) reduces symptoms of previously resistant keloid scars.

2.1.4.5 Cryotherapy

Liquid nitrogen can be effective if applied to small hypertrophic scars, severe atrophic scars and some keloids. It is applied in 1–3 freeze

cycles (from 10–30 seconds) every 20–30 days. Treatment is delayed to allow postoperative healing to take place. The mechanism of action is cell damage, resultant necrosis, and therefore decreased scar bulk. Adverse effects are pain and permanent hypopigmentation.

2.1.4.6 Other therapies

There is little evidence for the topical use of creams (e.g. vitamin E, allantoin-sulfomucopolysaccharide gel) in scar management. Newer treatments (e.g. skin equivalents incorporating dermis constructs, ciclosporin, verapamil (intralesional), imiquimod cream) are still in development.

Some of the scars diminish and eventually vanish with the passage of time, while others need some kind of treatment. Pressure dressing is a gel that reduces the chances of scar formation process or minimizes it. Pressure dressing not only speeds up a wound healing process but also prevents scar formation.

2.2 Natural rubber

Natural rubber (NR), *cis*-1,4-polyisoprene (Figure 2.1), is an important raw materials used in many thousands of products and hundreds of medical applications. NR is obtained from latex and harvested by tapping the rubber tree. Although more than 2,500 plant species are produced natural rubber, currently threr is only one important commercial source, *Hevea brasiliensis* trees (Puskas et al., 2006). Natural rubber latex is composed of about 36% of rubber, 5% of non-rubbers components (proteins, lipids, sugars, and ash) and water accounting for the remaining 59% (Sansatsadeekul, et al., 2011).

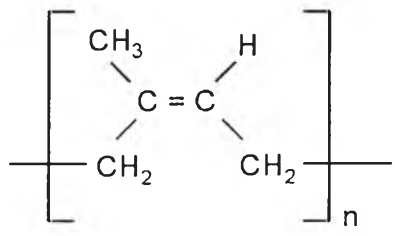

Figure 2.1 Chemical structure of *cis*-1,4-polyisoprene (Puskas et al., 2006).

Thailand has most of natural rubber as one of its economically agricultural goods because of low cost, available and renewable natural resource (Chuayjuljit et al, 2009). The excellent properties of natural rubber are flexibility, high strength, good crack growth resistance and good processability. (Ripel *et al.*, 2009). NR is mainly used in tires and tires for trucks. The unique mechanical properties of NR result from both its highly stereoregular microstructure and the rotational freedom of the R-methylenic C-C bonds and from the entanglements resulting from the high molecular weight which contributes to its high elasticity. The properties of NR can be tailored by the addition of fillers of varying surface chemistry and aggregate size/aspect ratio to suit the application concerned (Nair et al., 2003).

The protein of natural rubber latex (NRL) constitutes about 1-2% by weight. These naturally occurring proteins can relate to hypersensitivity reactions (type I) in some sensitive human who contact with them, but same of associated proteins on rubber particles also help to maintain the latex stability (Sakdapipanich *et al.*, 2007). For this reason, when these proteins are removed or degraded, coagulation properties and destabilization of the latex can be occurred. There are several methods of reducing the amount of these protein antigens in NRL. There are two effective methods which were used to remove protein in NR latex including double centrifugation and creaming of NRL. In addition, leaching, chlorination and enzymatic treatment were used to remove these antigenic proteins in wet-gel and dry-films of NR (Perrella *et al.*, 2002). Comparing with post-washing NRL products, enzymatic treatment is very effective in reducing

antigenic protein in NRL and it is still quite cost-effective because the enzyme treatment can remove protein from natural rubber latex more than 99%. This is the important necessity for especially manufacture products in health care products such as medical gloves and condoms (Sansatsadeekul *et al.*, 2011).

Natural rubber is widely used in many industries and also interested in environment. Thus, it has been an increased biodegradability. Biodegradable materials can be degraded by enzymatic reactions of living organisms, such as bacteria, fungi and algae. These biodegradable materials are designed to be easily degraded and finally mineralized in natural environments such as soils, sediments, and landfill sites (Chuayjuljit et al, 2009).

Nanocomposite materials were prepared from natural rubber latex as a matrix and a crab shell chitin whisker as a reinforcing phase (Nair et al., 2003). The chitin whisker was prepared by acid hydrolysis of chitin from crab shell, aspect ratio close to 16. The reinforcing effect of chitin whiskers strongly depended on their ability to form a rigid three-dimensional network, resulting from strong interactions such as hydrogen bonds between the whiskers. Dynamic mechanical analysis showed that the rubbery modulus of unvulcanized evaporated natural rubber is improved by the incorporation of chitin whiskers. For filler contents higher than 5 wt%, an improvement in thermal stability of the composite is also noticed up to 220-230 °C. Tensile and conventional modulus values are higher for unvulcanized evaporated samples than for vulcanized evaporated ones since the existence of a three-dimensional chitin network formed as a result of hydrogen bonding within the evaporated samples.

Polymer composite was obtained from the wastes of natural rubber glove (NRG) and polystyrene foam blended with cellulose from sugar cane leaves via the laminate method (Riyajan et al., 2012). The toluene resistance of the polymer blend was improved after adding MA and cellulose. The highest toluene resistance was achieved when using 12% cellulose. The hardness of the polymer blend and composite increased as a function of PSF. In addition, their impact strength increased with increasing NRG and cellulose contents.

Nanocomposite materials were prepared by using a latex of natural rubber as the matrix and an aqueous suspension of waxy maize starch nanocrystals as the reinforcing phase (Angellier et al., 2005). Starch nanocrystals were obtained after sulfuric acid hydrolysis of waxy maize starch granules. After mixing the latex and the starch nanocrystals, the resulting aqueous suspension was cast and evaporated. Scanning electron microscopy confirmed that the filler was evenly distributed within the NR matrix, showed that good mechanical properties. Wide-angle X-ray diffraction analysis showed that the processing did not affect the crystallinity of the starch nanocrystals. By adding starch nanocrystals in NR, the swelling by toluene decreases and the swelling by water increases. It was assumed that these phenomena were due to the formation of a starch nanocrystals network through hydrogen linkages between starch nanoparticles clusters and also to favorable interactions between the matrix and the filler. The platelet-like morphology of starch nanocrystals seems to be responsible for the decrease of both the permeability to water vapor and oxygen of natural rubber filled films. The surface chemical modification of starch nanocrystals results in a favored swelling behavior with toluene and a diminution of the water uptake.

2.3 Pluronic

Pluronic or poloxamer is nonionic triblock copolymers consist of hydrophilic poly(ethylene oxide) (PEO) and hydrophobic poly (propylene oxide) (PPO) blocks arranged in A-B-A triblock structure: PEO-PPO-PEO (Batrakova et al., 2008). The general chemical structure of a pluronic triblock copolymer is shown in figure 2.3.

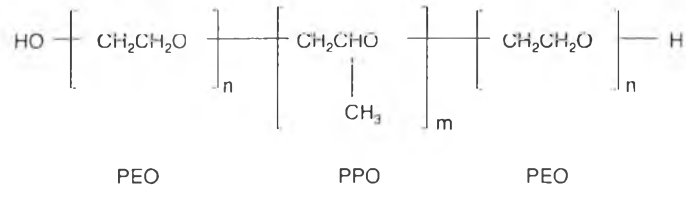

Figure 2.2 The chemical structure of pluronic triblock copolymers (Fusco et al., 2006).

This arrangement is an amphiphilic copolymer in which the number of hydrophilic (PEO) and hydrophobic (PPO) repeating units can be varied for change the size, hydrophilicity, and hydrophobicity (lipophilicity). Thereby, the amphiphilic behaviors of pluronic depend on the molecular architecture, such as total molecular weight, relative block size and block sequence as well as thermodynamic parameters, such as temperature and pressure.

Pluronic triblock copolymers have amphiphilic structures and self-assemble into micelles to form a variety of close packed structures including spherical rods, pancake-shaped micelles, as well as complex-structured fluids like bicontinuous microemulsions (Fusco et al., 2006).

Hydrophobicity have in the micellization process. The hydrophilic-lipophilic balance (HLB) is presented by the following equation:

where n and m are the number of repeating units in the PEO segment and PPO segment of the polymer, respectively.

The higher HLB show that the higher trend to form micelles. The cloud point is the temperature which the copolymer solution starts to separate depend on PEO percentage, ranges from about 10°C to above 100°C. The higher PEO content show that the higher cloud point.

At room temperature, water is a high solubility for pluronic due to the high PEO percentage. When the temperature increases, water is a lower solubility for the PPO segment that causes to form micelles.

Some of these systems show reverse thermal gelation (RTG) or thermothickening behavior. This phenomenon, which occurs in the self-assembling of the macromolecules above certain temperature conditions, is reverse to the gelation processes usually observed upon cooling (Fusco et al., 2006).

The self-assembling process occurs through a micellization characterized by two key parameters which are the critical micellization concentration (CMC) and critical micellization temperature (CMT) (Kurumada et al., 2004).

These parameters depend on physical–chemical properties of PEO-PPO-PEO systems such as total molecular weight and block composition (PEO/PPO ratio) as well as the respective PEO and PPO block length. Therefore, CMC and CMT can be adjusted to obtain widely applications (Fusco et al., 2006).

By fixing the temperature and molecular weight of the PEO blocks, the CMC decreased with increasing the PPO segment. Similarly, by fixing the concentration, the CMT decreased with increasing the PPO segment. Moreover, by fixing the PPO content, both CMC and CMT small increase with increasing the PEO segment (Fusco et al., 2006). Conclusion, the dynamics for micelle formation, in terms of CMC and CMT, are influenced mainly by the hydrophobic PPO segment.

Interactions of Pluronic are a very complicate to assess since the PEO and the PPO behave differently in a solvent, such as water, which soluble in one segment and insoluble in the other. This phenominon leads to amphiphilic behavior and, therefore, to a tendency of the copolymers to self-assemble into structures, such as micelles (Fusco et al., 2006).

Micellization occurs in dilute solutions of block copolymers in selected solvents above the critical micellar concentration, at a given temperature. At higher concentrations, above a critical gel concentration, the micelles can order into a lattice (Escobar-Chavez et al., 2006).

At low temperature both PPO and PEO are water soluble and the copolymer chains are fully soluble in aqueous solution. As the temperature increase, the PPO block becomes hydrophobic and when the concentration is larger than the critical micelle concentration, individual micelles begin to form with a PPO core and a PEO corona (Boucenna et al., 2009).

Pluronics show thermoreversible gelation around body temperature and no organic solvents or toxic crosslinkers are involved during gelation so they are suitable for biomedical applications such as drug delivery, gene therapy and tissue engineering (Fusco et al., 2006).

In addition, these polymers are commonly used in industrial applications, cosmetics, and pharmaceuticals and were shown to sensitize drug resistant cancers to chemotherapy (Boucenna et al., 2009 and Fusco et al., 2006). Moreover, in bioprocess applications, pluronic is also used in cell culture media for its cell cushioning effects because its addition leads to less stressful shear conditions for cells in reactors. Among other things, they can be used to increase the water solubility of hydrophobic, oily substances or otherwise increase the miscibility of two substances with different hydrophobicities (Du et al., 2011 and Fusco et al., 2006).

Pluronic have a lot of grades so different properties. For the Pluronic tradename, coding of these copolymers starts with a letter to define its physical form at room temperature (L = liquid, P = paste, F = flake (solid)) followed by two or three digits, The first digit (two digits in a three-digit number) in the numerical designation, multiplied by 300, indicates the approximate molecular weight of the hydrophobe; and the last digit x 10 gives the percentage polyoxyethylene content (e.g., L61 = Pluronic with a polyoxypropylene molecular mass of 1,800 g/mol and a 10% polyoxyethylene content) (Alexandridis et al., 1995).

Aqueous solutions of pluronics are stable in the presence of acids, alkalis, and metal ions. Commonly used pluronic include the 188 (F-68 grade), 237 (F-87 grade), 338 (F-108 grade) and 407 (F-127 grade) types, which are freely soluble in water (Escobar-Chavez et al., 2006).

The transition from solution to micelle gel system has been investigated by using rheological and diffusion techniques.

Poloxamer 188 (P188) or pluronic F-68 may improve capillary blood flow and reduce the zone of stasis (Harting et al., 2008) and also decrease the oxidative outbreak response. P188 can relieve the progressive injury in the wound.

Poloxamer 188 may interacted with wound or broken sites on the membrane, repaired the cell membrane, sealing up the cell membrane, and protected cell activity (Hannig et al., 2000)

The effect of poloxamer 188 on deepening of deep second-degree burn wounds in early stage after burn were studied (Shi et al., 2012). Systemic application of P188 on deep second-degree burn wounds at the early stage may alleviate wound deepening, whose mechanism may be related to timely sealing up the damaged cell membrane and inhibiting the inflammatory reaction.

The rheological properties of polyvinyl butyric (PVB)/Pluronic F127/ polyethylene glycol (PEG) 200 blend were studied (Wei et al., 2011). Their were investigated by a rotational rheometer with parallel plates. The blend show an upper critical solution temperature (UCST) behavior.The blend approach homogeneous state at 140, 150 and 160 °C while the rheological properties of the blend differ from the homogeneous systems at 120 °C. Shear thinning behavior increase with the increase of Pluronic F127 content. The viscous and elastic response depend on the composition of the blend. The complex viscosity, storage modulus, loss modulus, zero-shear activation energy and flow recovery of the blend enhanced by increasing the Pluronic F127 loading.

The polymorphism and thermoresponsive properties of poly(vinylidene fluoride) (PVDF) membranes with amphiphilic copolymer Pluronic F127 were investigated (Du et al., 2011). FTIR revealed that Pluronic existed stably in the blend membranes and some of them had migrated onto the surface of the membranes. SEM indicated that the pore structure of the membrane changed from irregular finger-like structure to regular finger-like structure when increased the Pluronic in membranes and also a more sponge-like structure appears at the bottom surface of the

membranes. PVDF/F127 blend membranes exhibited good thermoresponsive properties.The water flux of the membranes increased with the increase of the temperature, decreased with the decrease of the temperature, and the increasing and decreasing curves were almost overlapped. Contact angles and permeation of membranes indicated that the Pluronic could improve the hydrophilicity and fouling resistance.

The synthesis and characterization of the Pluronic on the poly(butyl methacrylate) (PBMA) latex film were studied (Ye et al., 2003). The influence of two different Pluronics block copolymers, PEO-PPO-PEO and PPO-PEO-PPO, on the diffusion rate in PBMA latex films was also studied. The small amount of PPO-PEO-PPO increase the PBMA diffusion rate. This indicates that the PPO-PEO-PPO plasticized the PBMA polymer and enhanced its diffusivity. The small amount of PEO-PPO-PEO also increases the PBMA diffusion rate, but the effect appears to saturate and PEO-PPO-PEO has limited miscibility in PBMA films at 70 °C. DSC revealed that PEO-PPO-PEO appears a smaller influence on the glass transition temperature of PBMA to its lower solubility in PBMA. This result is consistent with films formed from nonpolar latex like SBR (styrene-butadiene rubber), where nonionic surfactants with lower HLB values are more miscible than those with higher HLB values. The HLB value for PEO-PPO-PEO is higher than PPO-PEO-PPO.

The thermal and rheological properties of laponite clay on Pluronic triblock copolymer were studied (Boucenna et al., 2009). The high concentration 17 wt% Pluronic F127 aqueous solutions with the addition of laponite as a novel temperature-sensitive hydrogel system was investigated. The critical micelle temperature (cmt) and viscoelastic properties of mixtures was characterized by oscillatory experiments and differentail scanning calorimetry. The critical micelle temperature (cmt) was found around 18 °C for all mixtures, showing that laponite particles has no significant influence on the cmt. Laponite particles can be used to increase the temperature of gelation for the Pluronic F127 copolymer. Moreover, the sol-to-gel transition temperature increases when mixing 2 and 3 wt% of laponite

particles so laponite can be used to adjust the gelation temperature in medical applications.

Degradable reverse thermo-sensitive materials were prepared by incorporating (oligo)ester blocks along the backbone of PEO–PPO–PEO-based chain extended polymer (Cohn et al., 2006). There are two-step synthesis. First, the PEO-PPO-PEO triblocks were end-capped with LA or CL oligo(ester)s whereby pentablocks were produced. Then, the different precursors were chain extended using hexamethylene diisocyanate to create the respective polymers. The end-capping of the PEO–PPO–PEO triblock affected the size of the micelles formed which demonstrate that the size of the aggregates increases with the molecular weight of the LA blocks.The increasing of the gelation temperature indicates that materials containing longer LA units failed to show any reverse thermo-responsiveness.The presence of the oligo(ester) blocks also reduced the viscosity of the gel. An increase in the oligo(ester) block length resulted in a decrease in the sol–gel transition. The limit is the presence of the hydrophobic degradable blocks, directly linked to the hydrophilic PEO segments, led to destabilizing the aggregates generated in water, generation of larger nanostructures.

Heparin-immobilized Pluronic (F-68)/Polyvinylalcohol (PVA) composite microparticles were prepared for the sustained drug delivery of ionic drug (Park et al., 2008). Venlafaxine, antidepressant medication, was used as a model drug. SEM showed that the microparticles have two layers, which indicates that the aggregation of polymer matrix by tetraglycol occurred at the outer layer of microparticles. Interaction between heparin and Pluronic improved elastic rheological properties, which exhibited an infinite network structure. DSC indicated the formation of intra/ intermolecular interactions and also tetraglycol enhanced the intra/intermolecular interactions. The immobilization of heparin into the gel network led to the formation of stable microparticles with ionic functional groups, which enable the efficient loading and sustained release of ionic drug. The composite with 10 weight% of heparin was used for the release application, mechanical properties, so it useful for a delivery system of ionic drug.

2.4 Chitin

Chitin is the second most abundant natural polysaccharide in the world next to cellulose. The predominant sources exploited are in the shells of crustaceans such as crabs, shrimps and squid pens, the cuticles of insects, and the cell walls of fungi (Jayakumar et al., 2010). Chitin is a polysaccharide contaning of 2-acetamido-2-deoxy-D-glucopyranose while chitosan is an *N*-deacetylated derivative of chitin as shown in figure 2.3. Chitin may be observed as cellulose with hydroxyl at position C-2 replaced by an acetamido group.

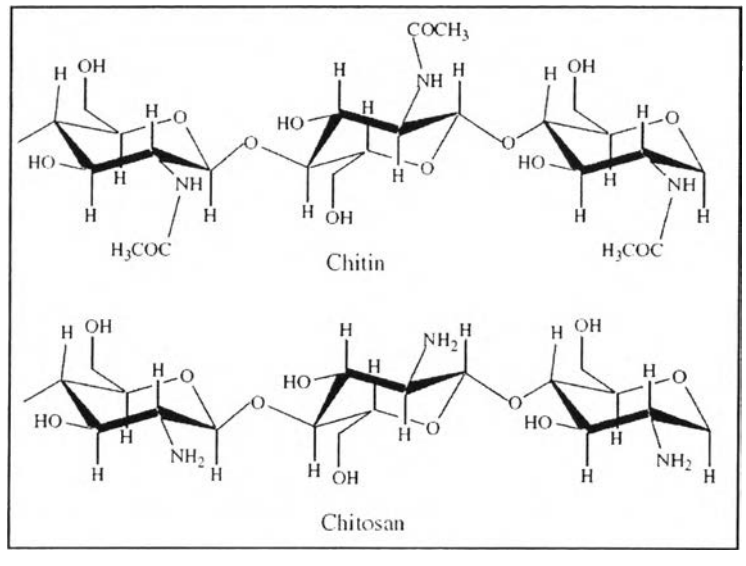

Figure 2.3 Structure of chitin and chitosan (Jayakumar et al., 2010)

In the case of crabs or shrimp shells, the chitin production is associated with food industries such as shrimp canning. The typical proceture for the processing of chitin from the shells as follows: the shells of crab or shirmp were first cleaned and treated with diluted hydrochloric acid at room temperature to remove calcium carbonate. The decalcified shells were then cut into small flakes and heated in sodium hydroxide at 100 °C to decompose the proteins and pigments. This α-Chitin extracted from these shells was obtained as colorless to off-white powdery materials.

However, in cause of squid pens, due to ist loose in the molecular packing, β-chitin were easily received by by treating squid pens with hydrochloric acid and sodium hydroxide under mild conditions to give β-chitin (Jayakumar et al., 2010).

Chitin possess great biological properties, such as antiviral activity, low-toxicity, low-allergy, high radiation resistance, biocompatibility, biodegradability, and etc. which made it attractive to use as biomaterial. However, the existence of hydroxyl and amino groups in the monomer unit of chitin produce strong hydrogen bonds provided highly crystalline structure, then chitin presents a problem in solubility as shown in figure 2.4.

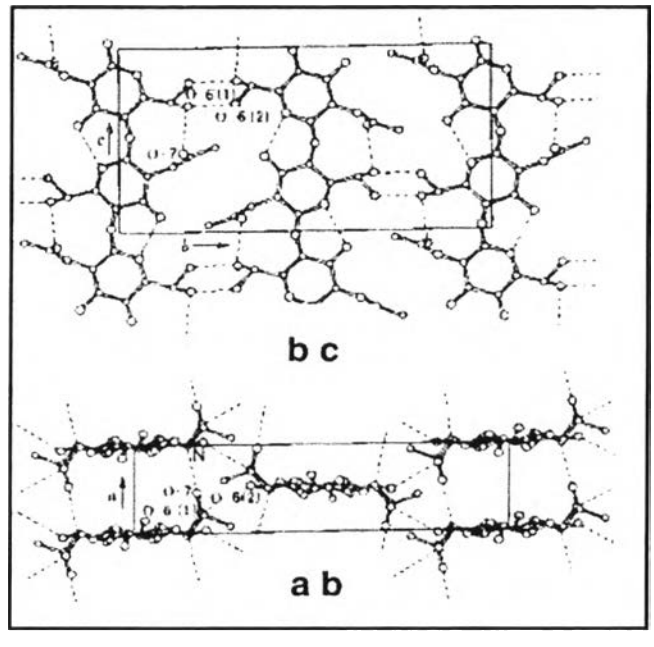

Figure 2.4 Crystalline structure of α-chitin (Tamura et al., 2006)

2.4.1 Chitin whiskers

Whiskers are very hopeful reinforcing materials for composites, because of their high stiffness and strength (Tjong et al., 1999). Owing to their small diameter, whiskers are nearly free of internal defects, thereby yielding strength near to the maximum theoretical value predicted by the theory of elasticity (Courtney et

al., 1990). It was found that the enhancement of their reinforcement depends on such factors (Chazeau et al., 2000) such as the nature of the matrix, the generation of a strong fiber-matrix interface through physicochemical bonding, the aspect ratio, and dispersion of the whiskers in the matrix. Moreover, whiskers from renewable resources have many advantages such as renewability, low cost, easy availability, good biocompatibility, and easy modification chemically and mechanically, compared with inorganic whiskers (Zinai et al.,1996).

In 2001, Paillet & Dufresne prepared the suspensions of chitin crystallites as described in the research of Li *et al.* (1996) by acid hydrolysis of chitin obtained from squid pen. The propose of this treatment was to dissolve away regions of low order so that the water-insoluble of highly crystalline residue may be converted into a stable suspensoid by subsequently vigorous mechanical shearing action. Samples were firstly boiled and then stirred in a KOH solution for 6 hours to remove most of the proteins. This suspension was subsequently kept at room temperature overnight under stirring, filtered, and washed several times with distilled water. Chitin samples were then bleached with $NaClO_2$ solution which containing sodium acetate buffer for 6 h at 80 °C. The bleaching solution was changed every 2 hr followed by abundant rinsing the sample with distilled water. After bleaching, the suspension was kept in KOH solution for 72 hours to remove residual protein. The resulting suspension was centrifuged to separate the product.

In another study, chitin whisker suspensions were prepared by hydrolyzing the purified chitin sample with 3 N HCl at the boil for 1.5 hours under stirring (Paillet & Dufresne, 2001). The ratio of 3 N HCl to chitin was 30 mL/g. After acid hydrolysis, the suspensions were diluted with distilled water followed by centrifugation (10,000 rpm for 5 min). This process was repeated three times. and then, the suspensions were transferred to a dialysis bag and dialyzed for 24 h against distilled water until pH 6. The pH was subsequently adjusted to 3.5 by adding HCl. It was subsequently filtered to remove residual aggregates and kept in a refrigerator until used after adding sodium azide as protectant against microorganisms. A typical

transmission electron micrograph obtained from a dilute suspension of hydrolyzed squid pen chitin was shown in figure 2.5.

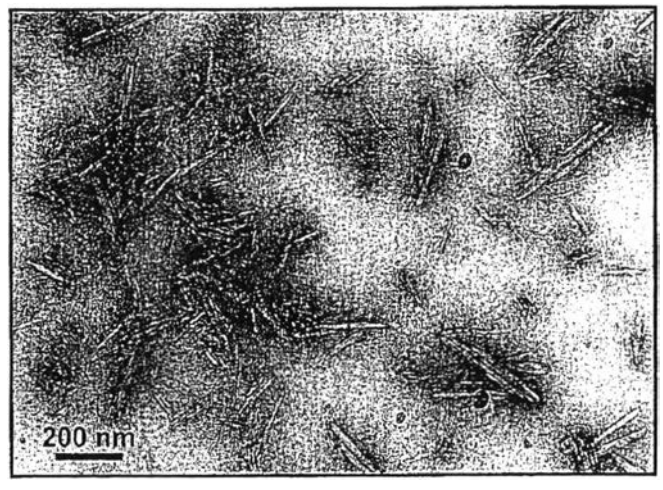

Figure 2.5 Transmission electron micrograph of a dispersion of hydrolyzed squid pen chitin (Paillet et al., 2001)

As Figure 2.6, transmission electron micrograph represents chitin whiskers which obtained from a dilute suspension. The suspension was constituted of individual chitin fragments consisting of slender parallelepiped rods that had a broad distribution in size. These fragments had a length ranging from 500 nm up to 10 μm, and they were weakly distributed in width (around 18 nm). The dimensions of the whiskers were averaged on 240 representative items.

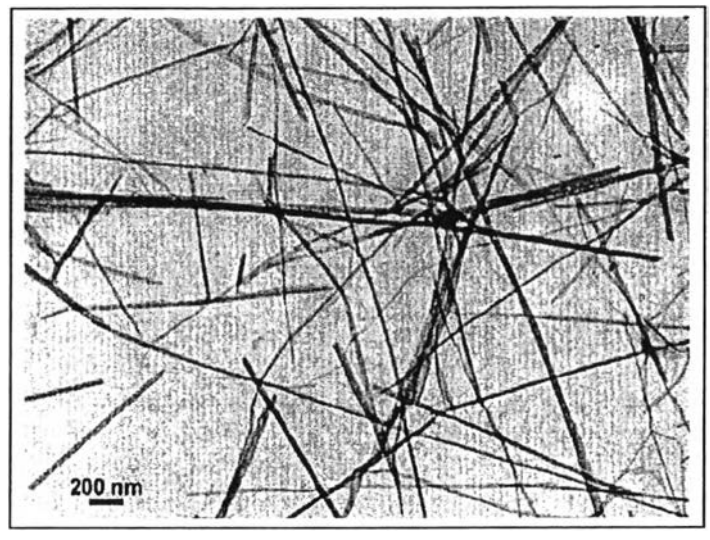

Figure 2.6 Transmission electron micrograph from a dilute suspension of chitin whiskers from *Riftia* tubes (Morin & Dufresne, 2002)

Because of their mechanical and biological properties, chitin whiskers has been extensively incoporated in various interesting biomaterials such as soy protein (Lu et al., 2004), silk fibroin (Wongpanit et al., 2007), and chitosan (Sriupayo et al., 2005).

Nanocomposite sponges at various chitin whiskers as nanofiller to silk fibroin as a matrix were prepared by using a freeze-drying technique (Wongpanit et al., 2007). Chitin whiskers exhibited the average length and width of 427 and 43 nm, respectively. The presence of chitin whiskers embedded into silk fibroin sponge not only improved its dimensional stability due to the β- sheet formation of silk fibroin the but also enhanced its compression strength. The percentage of the shrinkage of chitin whiskers-reinforced silk fibroin sponges were illustrated in figure 2.7. It was found that the percent shrinkage decreased with an increasing of the whisker content.

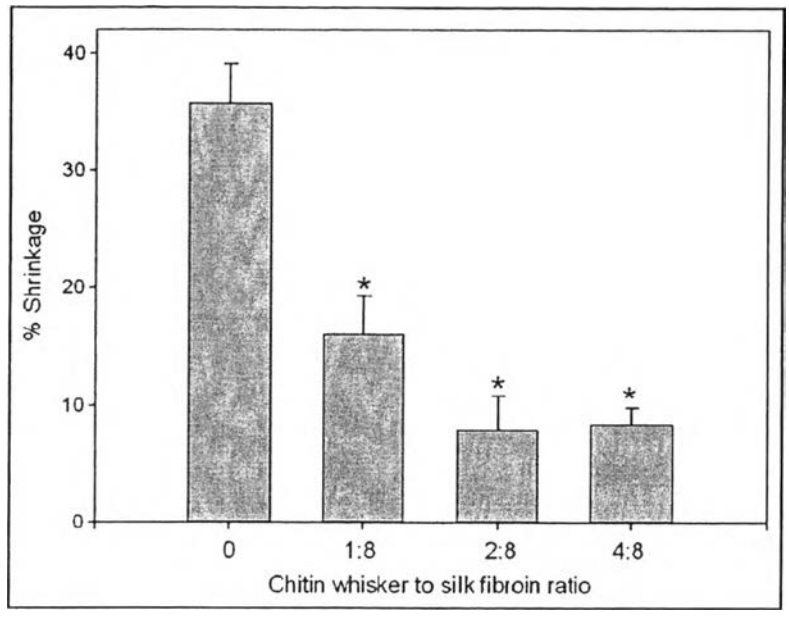

Figure 2.7 Percent shrinkages of chitin whisker/silk fibroin sponges at various C/S ratio (Wongpanit et al., 2007).

Moreover, α-chitin whiskers as reinforcement in chitosan films was studied on with and without heat treatment (Sriupayo et al., 2005). The length of the as-prepared whiskers obtained from shrimp shells ranged between 150 and 800 nm, while the width ranged between 5 and 70 nm, with the average values being about 417 and 33 nm, respectively. The addition of chitin whiskers did not affect much the thermal stability and the apparent degree of crystallinity of the chitosan matrix. The tensile strength of α-chitin whisker reinforced chitosan films increased from that of the pure chitosan film with initial increase in the whisker content and decreased gradually with further increase in the whisker content, while the percentage of elongation at break decreased from that of the pure chitosan with initial increase in the whisker content and leveled off when the whisker content was greater than or equal to 2.96 wt%. The addition of α-chitin whiskers as well as heat treatment can improve the water resistance, leading to decrease the percentage of weight loss and the percentage of degree of swelling, of the nanocomposite films.

2.5 Virgin coconut oil (VCO)

The coconut palm (Cocos nucifera, L.) is one of the most useful plants (Elias et al., 2005). For many years, tropical communities have used coconut oil in key areas of their lives, such as cooking, to cure and prevent disease, and for hair- and skin-care. Coconut oil has extensive usage and effects on various diseases. Moreover, there have been various studied on its antiviral and antimicrobial benefits. It has been speculated by various researchers that some of benefits of coconut oil can be attributed to the presence of lauric acid, capric acid, and caprylic acid. Lauric acid in coconut oil has antibacterial. Monoglyceride monolaurin is the substance that keeps infants from getting viral or bacterial or protozoal infections (Agyemang-Yeboah, 2011).

Coconut oil can extract by two method, dry process and wet process. Dry process is done by crushing copra in an expeller. The meal (or cake) may be further treated with solvents to extract residual oil. Conversely, the wet-process feedstock is fresh kernel instead of copra. The extracted oil does not have to be refined, unlike the oil from copra. The coproducts of oil from the wet process are edible (Agyemang-Yeboah, 2011). This process is more desirable as no chemical or high heat treatment is imposed on the oil. The coconut oil produced through the wet method is known as virgin coconut oil (VCO). The term VCO refers to an oil that is obtained from fresh, mature kernel of the coconut by mechanical or natural means, with or without the use of heat and without undergoing chemical refining (Marina et al., 2009). Thus, the wet process is more environmental friendly than the solvent extraction, dry process. This extraction process avoids the loss of minor components like provitamin A and vitamin E and polyphenols due to UV irradiation from sunlight during drying of copra. (Nevin et al., 2008). The wet process is also much simpler, which can be carried out at home by anyone who are interested in producing their own natural oil. (Nevin et al., 2004)

The beneficial properties of VCO are fast spreading. The availability of VCO is increasing in the market especially in South East Asia involving the

Philippine, Thailand, Indonesia and Malaysia. The continued rise in demand for VCO can be attributed not only to its superior flavor, but also to reports of its potential health benefits. Because no chemical or high heat treatment is imposed on the oil, the beneficial minor components in the oil are retained. VCO is reported to lower the lipid levels in serum and tissues, and possesses high potential in protecting low-density lipoproteins against oxidative stress induced by physiological oxidants. Apart from that, the oil is also well known for its high content of medium-chain triacylglycerols (MCT), which are used in medical and cosmetic applications (Ghazali et al., 2009). MCT is unique due to its physicochemical properties such as having shorter chain length and smaller molecules compared to long chain triglyceride (LCT), making them more rapidly absorb and hydrolyze in the body. (Seneviratne et al., 2009).

Coconut oil is also rich in lauric acid; a fatty acid with strong antimicrobial property which inhibited various pathogenic bacteria such as Listeria monocytogenes (Wang et al., 1992).

Virgin coconut oil has several benefits such as immune system, cardiovascular health, thyroid support, weight loss, staph fighter, wound healing, stretch mark prevention, skin benefits, healthy hair and digestive system.