CHAPTER II
LITERATURE REVIEW

Vasnev *et al.* (2006) designed efficient methods for acylation of chitin (1) and chitosan (2) using acid chlorides of p-nitrobenzoic acid (NBAC) and myristic acid (MAC). The acylation of chitin and chitosan included acylation of chitin at the solid–liquid interface, acylation of chitin in a DMA–LiCl solution, acylation of chitosan at the liquid–liquid interface,and acylation of chitosan in a DMA solution. The modification of chitin and chitosan obtained for improvement of water-resistant and water-stable properties of their.

Wang *et al.* (2006) isolated and identified a protease-producing bacterium as Bacillus sp. TKU004. It can be used for deproteinization of squid pen in the preparation of β-chitin. The optimized condition for protease production was found when the culture was shaken at 30 °C for 4 days in 100mL of medium containing 2% squid pen powder (SPP) (w/v), 0.1% K_2HPO_4, and 0.05% $MgSO_4$. After isolated Bacillus sp. TKU004, sodium dodecyl sulfate-polyacrylamide gel electrophoresis and gel filtration were examined the molecular wejght, was 27 and 57 kDa, respectively.

Paul and Sharma (2004) reviewed on the chitosan and alginate wound dressing. Calcium alginate was suitable for wound dressing application because calcium alginate is a natural hemostat, alginate based dressings are indicated for bleeding wounds. The gel forming property of alginate helps in removing the dressing without much trauma, and reduces the pain experienced by the patient during dressing changes. It provides a moist environment that leads to rapid granulation and reepithelialization. And chitosan had the various properties such as haemostatic properties, bacteriostatic and fungistatic properties. In addition to chitosan provides a non-protein matrix for 3D tissue growth and activates macrophages for tumoricidal activity. It stimulates cell proliferation and histoarchitectural tissue organization. Chitosan is a hemostat, which helps in natural blood clotting and blocks nerve endings reducing pain (figure 3). Chitosan will gradually depolymerize to release N-acetyl-β-D-glucosamine, which initiates fibroblast

proliferation and helps in ordered collagen deposition and stimulates increased level of natural hyaluronic acid synthesis at the wound site. It helps in faster wound healing and scar prevention.

Merzendorfer and Zimoch (2003) reviewed on Chitin metabolism in insects; the chitin content constitutes up to 40% of the exuvial dry mass depending on the insect species and varies considerably with the different cuticle types even in a single organism. The structure of chitin is N-acetyl-β-D-glucosamine and chitin can be degraded by chitinases enzyme, the degrading enzymes include the chitinases {poly[1,4-(N-acetyl-β-D-glucosaminide)] glycanohydrolase; EC 3.2.1.14} and β-N-acetylglucosaminidases (β-N-acetyl-β-D-hexosaminide N-acetylhexosaminohydrolase; EC 3.2.1.52). All of them catalyze the hydrolysis of β-(1-4)-glycosidic bonds of chitin polymers and oligomers. Some of them, including one insect enzyme, additionally catalyze transglycosylation reactions. Since chitin-degrading enzymes can be used to convert chitin-containing raw material into biotechnologically utilizable components, they are of significant interest for the chemical and pharmaceutical industry.

Yamashita *et al.* (2003) investigated the effect of chitin hydrolysate made from crustacean shells and cephalopod cartilage on the denaturation and state of water in lizard fish myofibrillar protein (Mf) during frozen storage at 225 °C for 120 days. The amount of unfrozen water in frozen-stored myofibrils was also measured. The results indicated that chitin hydrolysates increased the amount of unfrozen water in myofibrils and suppressed freeze-denaturation.

Jayakumara *et al.* (2005) intended to systematize related issues that include: various methods of chitosan grafting; the effects of reaction variables such as type and concentration of initiator, monomer concentration, reaction temperature and time on the grafting efficiency; and the physicochemical properties of grafted chitosan. Moreover, the potential applications of grafted chitosan in the various fields such as drug delivery, biomedical; tissue engineering and environmental is also discussed. Finally, an attempt is made to discuss some of the current applications and future prospects of grafted chitosan. The way of grafting copolymerization, Grafting initiated by free radicals,

Grafting by using radiation, enzymatic grafting and Cationic graft polymerization. The application of graft copolymerized chitosan include drug delivery systems, tissue engineering, anti-microbial agents, Other biomedical applications, Adsorption of metal ions and Dyes removal.

Krajewska (2005) prepared membrane of chitin/chitosan and looks at how chitin/chitosan materials can be exploited in membrane-based processes and if they can help advance their applications. The membrane processes classified by Howell for such future applications include among others:

1. Health care: controlled release, biosensors, bioartificial organs, tissue engineering.
2. Environment: industrial membrane bioreactors, and cleaner industrial processes.
3. Energy: oxygen enrichment, fuel cells, hydrogen economy.
4. Water: virus-free supply, water reuse, micropollutant-free water.
5. Food: barrier technologies, beverage filtration, functional food processes.

Khora and Lim (2003) reviewed overview of the more recent research undertaken in the area of implantable applications of chitin and chitosan highlighting orthopedic/ periodontal, tissue engineering, wound healing and drug/gene delivery applications, and some biocompatibility and sterility issues published in the major biomaterials journal and select conference proceedings. For the conclusion, this review has proved the utility of chitin and chitosan as potential materials for various implant applications and some of the challenges in demonstrating biocompatibility. The eventual realization of real implants awaits the take-up of these materials on a more commercial basis that would see the introduction of chitin-based implantable devices.

Majeti N.V and Kumar (2000) reviewed closer look at chitin and chitosan applications that based on current research and existing products, some new and futuristic approaches in this fascinating area are thoroughly discussed. The content of this review included processing of chitin and chitosan, economic aspects, properties of chitin and chitosan, chitin and its derivatives in fibre formation, and application. For the applications can be divided into various application such as photography, cosmetic, artificial skin, wound dressing, food and nutrition, ophthalmology, water engineering

(metal capture from wastewater), paper finishing, solid-state battery, drug delivery system, biotechnology and fat trapper.

Somashekar and Joseph (1995) reviewed the properties and application of chitosanases enzyme. Chitosanases (EC 3.2-l-99), which represent a class of hydrolytic enzymes, are found in bacteria, fungi and plants. Chitosanases occur widely in soil microorganisms and in some plants, fulfilling a possible defense role in the 1atteE. Chitosanases may find important industrial application in the utilization of the enormous chitosan and chitin substrates, available from sea-food-processing units, for the generation of the size-speciJic chitosan oligomers required particularly in pharmaceutical industries. The distinction between chitosanase and chitinase is not sharp, as both enzymes have the ability to degrade a variety of chitosans with different degrees of acetylation. Chitinases which preferentially attack highly N-acetylated polymers, and which are more efficient on chitosan having low N-acetyl content, is known. Chitosanases are also distinguished from chitinases by their lower apparent molecular mass. The numerous applications of chitosanases are in the fields of agriculture, food and the pharmaceutical industries.

Ohshima Y. *et al.* (1987) studied the healing of wounds in 91 patients when applied chitin non-woven as a wound dressing in various wounds. Chitin non-woven was fabricated by the same way used in paper fabrication. This material was applied to 199 areas, which site of limb, head and neck, and trunk. The cause of wound was indicated from donor site, burn, graft site, skin abrasion, other skin ulcer and hemostasis. The results showed that chitin dressing provide rapid epithelization and normal granulation that can acceleration of wound healing. Moreover, the advantages of chitin dressing are reduce pain and suitable for superficial burns, deep dermal burns, donor sites, raw areas after dermabrasion, deep ulcers and skin defects following flap elevation.

London A.P *et al.* (1995) fabricated the composite that composed of poly(lactic acid), polycaprolactone and chitosan for biodegradable wound dressing. Some properties such as topography, water vapor permeability, biodegradation and bacterial permeation

were investigated. Topography of the films exhibited few pores. The chitin films were allowed to water permeation but provided a barrier to bacteria. For biodegradation, the chitin film can be degraded in 10-14 days but poly(lactic acid) and polycaprolactone cannot be degraded in that time. Normally, they can be degraded approximately in 1 year. However, the films used in this study are very thin, the degradation time in the presence of enzymes and bacteria should be in the range of 2-6 weeks.

Lee *et al.* (2000) prepared the wound dressing which was made from β-chitin containing with silver sulfurdiazine as antibacterial agent. The dressing can be fabricated by casting technique of blend solution of β-chitin and poly(ethylene glycol) macromer. The UV irradiation was used for crosslink step. The water vapor transmission rate, in vitro biodegradation, antibacterial test and animal test were investigated. The result found that transmission rate of water was calculated to be 2400-2900 gm^{-2} day^{-1}. Most of the hydrogels can be degraded in PBS lysozyme for one week. The wound dressing materials impregnating AgSD had complete bactericidal capacity against Pseudomonas aeruginosa. Histological studies of the novel spongetype materials containing AgSD confirm a proliferation of fibroblast in the wound bed of a wistar rat after 12 days and a reduction of infectious cells.

Khan, T.A., Peh, K.K., and Ch'ng, H.S. (2000) studied the mechanical, bioadhesive strength and biological evaluations of chitosan wound dressing. This work have been done to investigate the proper of chitosan films which prepared by dissolve in acetic acid and lactic acid for comparison with a commercial dressing, Omiderm. The characterization of films was undergoing for mechanical and in vitro bioadhesive strength properties. In addition, the vapor permeability, primary skin irritation test and intracutaneous test also investigated. From the result of mechanical and vitro bioadhesive strength properties showed that chitosan in lactic acid exhibited a lower tensile strength; on the other hand, it exhibited more flexible and better bioadhesive than chitosan in acetic acid. All of chitosan films allowed the permeability of water. For skin irritation test, chitosan in lactic acid and Omiderm had not irritant and did not promote any skin allergic reaction. In case of chitosan in acetic acid inflicted adverse skin effect.

Tanodekaew *et al.* (2004) prepared the grafting of acrylic on chitin for wound dressing application. The various contents of acrylic acid that used for grafting were investigated for water adsorption ability. The chitin grafted poly(acrylic acid) was made from the polymerization by using potassium peroxodisulfate as initiator. The thermal property, swelling property, the surface analysis and cell response of films were studied. Moreover, the evidences of grafting were found from FTIR and solid state ^{13}C NMR. For FTIR and NMR exhibited the new signals that were interpreted of the carbonyl of acrylic when compare with the original chitin. The thermal result, chitin grated poly(acrylic acid) was found a lower thermal stability than pure chitin and poly(acrylic acid). Chitin grated poly(acrylic acid) was higher degree of swelling, especially, when the amount of poly(acrylic acid) increased. Surface morphology of the chitin–PAA film, the surface of the dry film appeared to be irregularly rough or cobble stone-like and rather dense while the hydrated film swelled in the growth medium resulting in more stretched pattern. The L929 cells can contacted and grown on films in 24 hr. They also proliferated on films without an inhibition zone.

Yusof, N.L., Lim, L.Y., and Khor, E. (2004) fabricated the chitin film from chitin/ 5% LiCl-DMAc solution system in flexible form. The minimal dimensional shrinkage and maximum flexibility were considered. A cold-press method was to fabricate this film. The transparency by UV-vis spectrophotometer, the crystallinity by X-ray diffractometer, topographical imaging by AFM, mechanical behavior by INSTRON universal testing machine and surface morphology by SEM were investigated. Films were passing through the cold-press process, were transparent and translucent. The crystallinity of the flexible chitin films were found to be a function of the amount of shrinkage from the gel to the final film that was obtained. The sharper XRD showed the result which depend on the increased shrinkage of film after washing. AFM and SEM were essentially to obtain surface topology and morphology, they found that the structural morphology of the films to the formation of sub-microscopic micelles. In addition, the mechanical properties depended on the film shrinkage; a higher shrinkage was provided a higher mechanical properties.

Muzzarelli *et al.* (2005) modified chitin at 3 and 6 positions by using butyric anhydride to form dibutyryl chitin. Dibutyryl chitin was dissolved easily in common solvents but was not dissolved in aqueous solvent. These films and non-wovens were demonstrated by the Viability/Cytotoxicity assay, In situ Cell Proliferation assay, Neutral Red Retention assay, Lactate Dehydrogenase Release assay, MTS cytotoxicity assay, and scanning electron microscopy. Biocompatible results indicated that dibutyryl chitin was not toxic with fibroblasts and keratinocytes. The SEM data on the colonization of non-woven dibutyryl chitin by rat fibroblast-like cells confirmed the biocompatibility of dibutyryl chitin. The healing of wounds after dibutyryl chitin implanted in rats followed the regular course; thus, the presence of dibutyryl chitin did not delay the early stages of the healing process.

Draczynski Zbigniew, Szosland Lidia (2005) prepared soluble copolyesters of honeybee chitin for using as dressing materials. Chitin was isolated from honeybee bodies and was reacted in mixture of acetic acid and butyric anhydride to promote copolymer between polyester and chitin. Intrinsic viscosity of chitin was determined in dilute solutions of chitin in 5% LiCl- DMAc. FTIR spectra of chitin, chitin/ polyester copolymers and dibutyryl chitin were recorded by using thin films of the samples. For measuring of the tensile strength and elongation at-break, films of the chitin derivatives in form of strips of 5 mm wide and 10 mm long were used tensile testing machine of ZWICK (Germany). Intrinsic viscosity of chitin solution was 19.94 dL/g corresponding to viscosity average molar mass of the polymer of ca 454×10^3 g/mol (Daltons). The FT-IR spectra of chitin copolyester were presented no band of absorption at ca. 3450 cm^{-1} due to hydroxyl groups present in IR spectra of pure chitin, and there are new bands of strong absorption at 1741 cm^{-1} characteristic for ester group.