CHAPTER I
INTRODUCTION

1.1 Chitin and Chitosan

Chitin and chitosan are the kind of amino polysaccharide; they are presented in enormous things in the environment. Chitin and chitosan was found in the shells of crustaceans, the exoskeletons of insects, the cell walls of fungi, mushroom and lichen where it plays a structural role, chitin is counted among the most plentiful, renewable organic resources in nature. The chemical structure of them, chitin is composed of β(1→4) linked 2-acetamido-2-deoxy-β-d-glucose units (or *N*-acetylglucosamine) forming a long chain linear polymer and chitosan is the primary derivative of chitin, is obtained by *N*-deacetylation to a varying extent that is characterized by the degree of deacetylation (Krajewska, 2005).

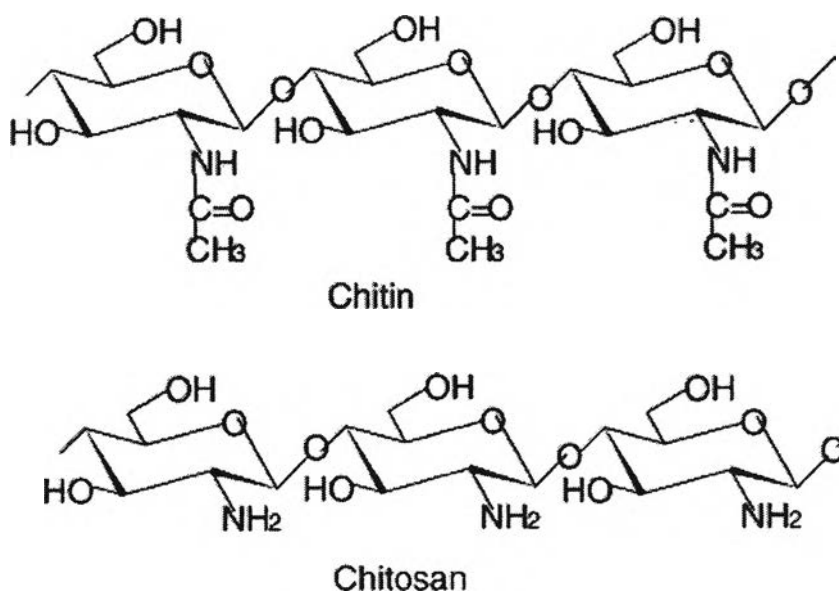

Figure 1.1 Molecular structure of chitin and chitosan (Krajewska, 2005).

The properties of chitin are hydrophilic, tough and inert solid, insoluble in water and most ordinary solvents. In contrast, chitosan can be dissolved in water and dilute acid solution. The procedure of isolated chitin and chitosan from the shells of crustaceans and the exoskeletons of insects is showed following the chart.

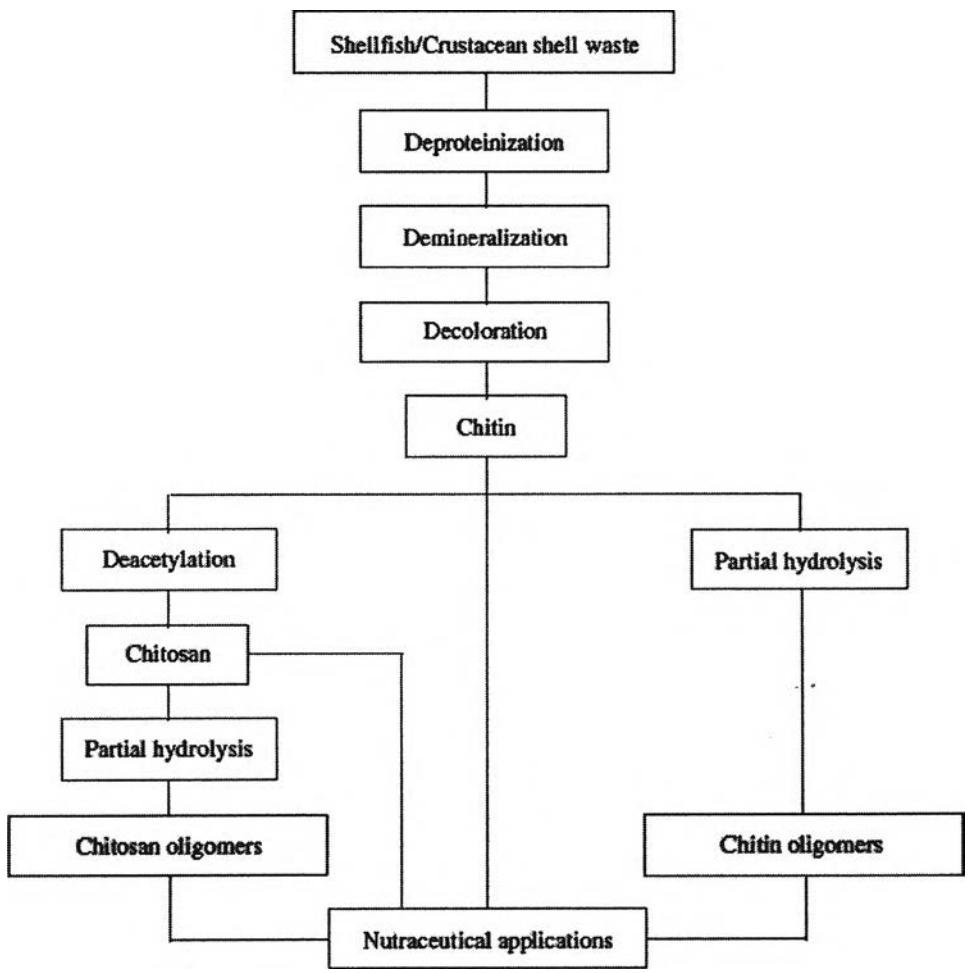

Figure 1.2 Schematic represent of the isolation of chitin, chitosan and their oligomers (Vasnev *et al.*, 2006).

The principles of chitin extraction are relatively simple. The proteins are removed by the treatment in a dilute solution of sodium hydroxide (1-10%) at high

temperature (85-100°C). Shells are then demineralized to remove calcium carbonate. This is done by treating in the dilute solution of hydrochloric acid (1-10%) at room temperature. The decolorization is done by soaking in organic solvents or in a very dilute solution of sodium hypochlorite. Chitosan can be prepared by the treatment of chitin with concentrated sodium hydroxide (40%) at high temperature (85-100°C).

1.1.1 Chitin in Metabolism in Insects

Formation of the different chitin forms is catalyzed by chitin synthase (UDP-*N*-acetyl-D-glucosamine:chitin 4-β-*N*-acetylglucosaminyltransferase; EC 2.4.1.16), a highly conserved enzyme found in every chitin-synthesizing organism. It utilizes UDP-*N*-acetylglucosamine (UDPGlcNAc) as the activated sugar donor to form the chitin polymer. The mechanism following the figure 1.3.

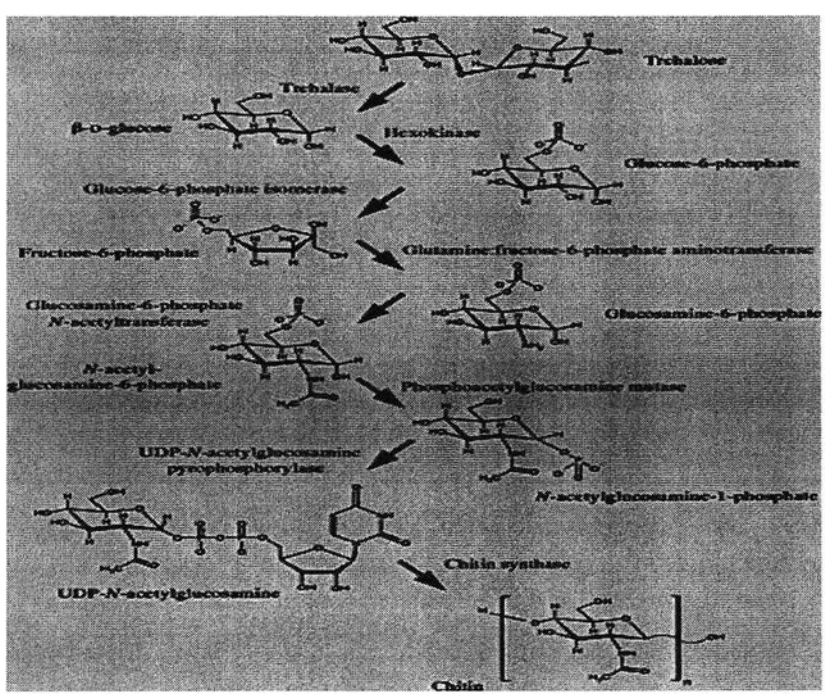

Figure 1.3 Mechanism of the synthesis of chitin in insects (Merzendorfer and Zimoch, 2003).

1.1.2 Chitin and Chitosan in Fungi

Chitin is widely distributed in fungi, occurring in *Basidiomycetes*, *Ascomycetes*, and *Phycomycetes*, where it is a component of the cell walls and structural membranes of mycelia, stalks, and spores. The amounts vary between traces and up to 45% of the organic fraction, the rest being mostly proteins, glucans and mannans. However, not all fungi contain chitin, and the polymer may be absent in one species that is closely related to another. Variations in the amounts of chitin may depend on physiological parameters in natural environments as well as on the fermentation conditions in biotechnological processing or in cultures of fungi. Chitin is the major component in primary septa between mother and daughter cells of *S. cerevisiae*, and also one of the main components of the hyaline outer wall of spores of four arbuscular mycorrhizal *Glomus* species. Hyphal walls of the Oomycete *Pythium ultimum* contain cellulose and chitin, whereas only cellulose is present in another Oomycete, *Phythophthora parasitica*. Both polysaccharides are present in cell walls of the Ascomycetes *Ophiostoma ulmi* and *Colletotrichum lindemuthianum*, whereas the Ascomycete *Fusarium oxysporum* and the Basidiomycete *Rhizoctonia solani* contain only chitin. The zoopathogenic fungi *Cryptococcus neoformans*, *Pityrosporum canis* and *Rhizopus oryzae* contain chitin, but not β-(1,3)-glucan.

The chitin of the cell wall of the white-rotfungus *Rigidoporus lignosus* is degraded by enzymes excreted as a defense response by the host cell, and therefore is not detectable during the process of infection. The fungal sheaths of another white-rot fungus, *Phellinus noxius*, do not contain chitin. The mycelia, and the caps and stalks of fruiting bodies of four edible mushrooms, *Lentinus edodes, Lycophyllum shimeji, Pleurotussajor-caju*, and *Volvariella volvacea* contain chitin as a minor component.

Chitosan occurs naturally in the Mucorales, in particular *Mucor, Absidia*, and *Rhizopus* species. There is apparently only one report on the presence of chitosan in a Basidiomycete, *Lentinus edodes* (Shiitake mushroom).

Table 1.1 Chitin and chitosan in fungi

Organism	**Gene**	**Comments**
Agaricus bisporus	CHS1 CS AqCHSA	class III; 2727 bp (ORF); 909 AA;
Ampelomyces quisqualis	CS	class I; 2786 bp; 910 AA
Aspergillus fumigatus	CHSD	CS-like; low but significant similarity to other CS
Aspergillus nidulans	CHSA CHSB	1013 AA 916 AA
A. nidulans	CHSD CHSE	CS class V and CS class IV; high sequence identity to ScCHS3 and CaCHS3
Beauveria brongniartii	BbCHS1	Fragment; CS class II; 95.8% similarity with CHS2 of Metarhizium anisopliae
Candida albicans	CaCHS1A	775 AA
Fonsecaea pedrosoi	FpCHS1 FpCHS2 FpCHS3	600 bp and 366 bp; CS class I and II; homology to S. cerevisiae CS
Metarhizium	MaCHS1	CS class I
anisopliae	MaCHS2 MaCHS3	CS class I CS class III
Mucor circinelloides	McCHS1	CS class TI; expressed during exponentially growing hyphal stage
Neurospora crassa	CHS2	Similar to CHS from other fungi
Paracoccidioides brasiliensis	CHS2	CS class II; 1043 AA
Penicillium chrysogenum	PcCHS1	CS class I

	PcCHS2 PcCHS3 PcCHS4	CS class II CS class II CS class III
P. chrysogenum	CHS4	CS class III; 915 AA (ORF); close relationship between P. chrysogenum and Aspergillus CHS
Phialophora verrucosa	PvCHS1 PvCHS2 PvCHS3	CS class I and II; 614 bp CS class III; 366 bp; 88.2% similarity and 78.4% identity; with
		the S. cerevisiae enzyme
Pyricularia oryzae	Fragment	340 bp; 86% homologous to A. fumigatus CHSE
Rhizopus oligosporus	CHS3	CS class IV; sequence similarity to CHS3 of S. cerevisiae; 46.7% identity with class IV CS of N. crassa
Saccharomyces cerevisiae	CHS4	696 AA
S. cerevisiae	CHS5	671 AA
S. cerevisiae	CHS6	See text
S. cerevisiae	CHS7	See text
Saprolegnia monoica	CHS2	Oomycetes and chitinous fungi have conserved CS
Tuber borchii		CS class II; ca. 600 bp
T. magnatum	TmCHS4	1230 AA; 62% homology to class IV CHS of N. crassa
Ustilago maydis	UmCHS1 UmCHS2	See text
U. maydis	UmCHS5	Predicted CHS class IV; high similarity
		CHS3 from S. cerevisiae and C. albicans,

		CHS4 from N. crassa, CHSE from A. nidulans
Wangiella dermatitidis	WdCHS4	High homology with CS class IV (Chs3p) of S. cerevisiae

1.1.3 Chitin and chitosan application (Majeti and Kumar, 2000)

1.1.3.1 Photography

Chitosan has important this spin system is the removal and recovery of applications in photography due to its resistance to abrasion. Silver complexes are not appreciably retained by chitosan and therefore can easily be penetrated from one layer to another of a film by diffusion.

1.1.3.2 Cosmetic

Chitin and chitosan have fungicidal and fungistatic 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.

1.1.3.3 An Artificial Skin

It appears that chitosan, having structural characteristics similar to glycosaminoglycans, could be considered for developing such substratum for skin replacement.

1.1.3.4 Chitin- and Chitosan- Based Dressings

Chitosan–gelatin complex claimed that, in contrast to conventional biological dressings, this experimental dressing displayed excellent adhesion to subcutaneous fat. Wound dressing comprising a nonwoven fabric composed of chitin fibres made by the wet spinning technique. Chitin-based commercial wound dressings

are concerned, one product (BeschitinÒ, Unitika) is commercially available in Japan, which is a nonwoven fabric manufactured from chitin filaments.

1.1.3.5 Food and Nutrition

The N-acetylglucosamine (NAG) moiety present in human milk promotes the growth of bifido bacteria, which block other types of microorganism and generate the lactase required for digestion of milk lactose. Animal nutritional studies have shown that the utilization of whey may be improved if the diet contains small amounts of chitinous material. This improvement is attributed to the change in the intestinal microflora brought about by the chitinous supplement.

1.1.3.6 Opthalmology

Chitosan possesses all the characteristics required for making an ideal contact lens: optical clarity, mechanical stability, sufficient optical correction, gas permeability, particularly towards oxygen, wettability and immunological compatibility. Contact lenses are made from partially depolymerized and purified squid pen chitosan by spin casting technology and these contact lenses are clear, tough and possess other required physical properties such as modulus, tensile strength, tear strength, elongation, water content and oxygen permeability. The antimicrobial and wound healing properties of chitosan along with an excellent film capability make chitosan suitable for development of ocular bandage lenses.

1.1.3.7 Water Engineering

Chitosan and modified chitin can be used in metal capture from wastewater and color removal from textile mill effluents.

1.1.3.8 Paper Finishing

Chitosan has been reported to impart wet strength to paper. Hydroxymethyl chitin and other water-soluble derivatives are useful end additives in paper making.

1.1.3.9 Solid-state Batteries

Chitosan is insoluble in water. This poses a problem in the fabrication of solid-state proton conducting batteries because there will not be any water present in the chitosan which can act as a source of hydrogen ions. Chitosan is a biopolymer which can provide ionic conductivity when dissolved in acetic acid.

1.1.3.10 Drug-delivery Systems

Hydrogels based on chitin and chitosan

i. Chitosan/polyether interpenetrating polymer network (IPN) hydrogel
ii. Semi-IPN hydrogel polymer networks of b-chitin and poly(ethylene glycol) macromer
iii. Hydrogels of poly(ethylene glycol)- poly(lactone) diacrylate macromers and β-chitin
iv. Hydrogels of poly(ethylene glycol) macromer / β-chitosan
v. Hydrogels of chitosan /gelatin hybrid polymer network
vi. Chitosan–amine oxide gel

Chitin and chitosan tablets

vii. Directly compressed tablets chitin or chitosan in addition to lactose or potato starch

viii. Chitosan tablets for controlled release: anionic–cationic interpolymer complex

ix. Microcapsules/microspheres of chitosan

1. Crosslinked chitosan microspheres coated with polysaccharides or lipid
2. Chitosan/gelatin network microspheres
3. Chitosan microspheres for controlled release of diclofenac sodium
4. Chitosan–polyethylene oxide nanoparticles as protein carriers
5. Chitosan/calcium alginate beads
6. Multiporous beads of chitosan

x. Chitosan-based transdermal drug delivery systems

1.1.3.11 Biotechnology

1. Preparation of biotechnological materials

2. Cell-stimulating materials

3. Antibacterial agents

4. Blood anti-coagulants (heparinoids)

5. Anti-throbogenic and haemostatic materials

1.1.3.12 Chitosan as Fat Trapper

1.1.4 Chitin derivatives and their applications

Chitin and chitosan can be modified the functional group for applied in various application. N-acetylglucosamine of chitin and amino of chitosan are expected to change for applications. The example of chitin derivatives and their proposed uses is showed following the table:

Table 1.2 Chitin derivatives, the examples and their applications (Majeti and Kumar, 2000)

Derivative	Examples	Potential uses
N-Acyl chitosans	Formyl, acetyl, propionyl, butyryl, hexanoyl, octanoyl, decanoyl, dodecanoyl, tetradecanoyl, lauroyl, myristoyl, palmitoyl, stearoyl, benzoyl, monochloroacetoyl, dichloroacetyl, trifluoroacetyl, carbamoyl, succinyl, acetoxybenzoyl	Textiles, membranes and medical aids
N-Carboxyalkyl (aryl) chitosans	N-Carboxybenzyl, glycine-glucan (N-carboxy-methyl chitosan), alanine glucan, phenylalanine glucan, tyrosine glucan, serine glucan, glutamic acid glucan, methionine glucan, leucine glucan	Chromatographic media and metal ion collection
N-Carboxyacyl chitosans	From anhydrides such as maleic, itaconic, acetyl-thiosuccinic, glutaric, cyclohexane 1,2-dicarboxylic, phthalic, cis-tetrahydrophthalic, 5-norbornene-2,3-dicarboxylic, diphenic, salicylic, trimellitic, pyromellitic anhydride	?
o-Carboxyalkyl chitosans	o-Carboxymethyl, crosslinked o-carboxymethyl	Molecular sieves, viscosity builders, and metal ion collection

Sugar derivatives	1-Deoxygalactic-1-yl-, 1-deoxyglucit-1-yl-, 1-deoxymelibiit-1-yl-, 1-deoxylactit-1-yl-, 1-deoxylactit-1-yl-4(2,2,6,6-tetramethylpiperidine--1-oxyl)-, 1-deoxy-69-aldehydolactit-1-yl-, 1-deoxy-69-aldehydomelibiit-1-yl-,cellobiit-1-ylchitosans, products obtained from ascorbic acid	?
Metal ion chelates	Palladium, copper, silver, iodine	Catalyst, photography, health products, and insecticides
Semisynthetic resins of chitosan	Copolymer of chitosan with methyl methacrylate, polyurea-urethane,poly(amideester), acrylamidemaleic anhydride	Textiles
Natural polysac--charide complexes, miscellaneous	Chitosan glucans from various organisms	Flocculation and metal ion chelation
	Alkyl chitin, benzyl chitin	Intermediate, serine protease purification
	Hydroxy butyl chitin, cyanoethyl chitosan	Desalting filtration, dialysis and insulating papers
	Hydroxy ethyl glycol chitosan	Enzymology, dialysis and special papers
	Glutaraldehyde chitosan	Enzyme immobilization
	Linoelic acid–chitosan complex	Food additive and anticholesterolemic
	Uracylchitosan,theophylline chitosan, adeninechitosan, chitosan salts of acid polysaccharides, chitosan streptomycin, 2-amido-2,6-diaminoheptanoic acid chitosan	

1.1.5 Chitin whisker

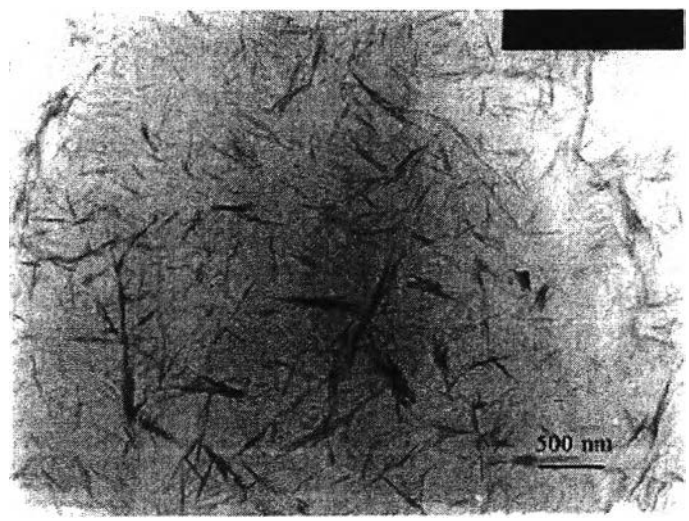

Figure 1.4 Transmission electron microscope picture (TEM) of α-chitin whisker (Sriupayo *et al.*, 2005).

When chitin passes through the acid hydrolysis process, nanofibrous structure can be formed as called chitin whisker (fig 1.4). Chitin whisker is the nano-scale fiber which can be used for reinforcement some material to improve the properties such as tensile strength, tensile modulus or stiffness. In case of biomaterials field, reinforced materials by chitin whisker can found the delay of biodegradation. Thus, chitin whisker can enhance biodegradability property due to chitin whisker has some interaction with the matrix; hydrogen bonding is one of example of interaction between chitosan and chitin whisker that disperse in chitosan matrix (Sriupayo *et al.*, 2005). Polyvinyl alcohol and polycarprolactone were reinforced with chitin whisker also showed better of some properties (Sriupayo *et al.*, 2005, Yánez *et al.*, 2005). On the other hand, the limitation of materials which reinforced by chitin whisker was showed lower elongation or fragile when apply them into some application such as wound dressing that it need this property to resist the expansion and contraction.

1.1.6 Wound dressing

The dressings have many functions and play an important role on a wound. They act as a physical barrier against the outside environment to prevent wound infection; and also protect the wound from injuries. Normally, the dressing should also remove exudates and toxic components on a wound; provide thermal insulation; maintain high enough humidity at the wound; allow gases exchange and removal without trauma at dressing change. Dressings can provide homeostasis of the wound by applying proper pressure. They also provide comfortable to the patient by simply masking wounds and allowing them to interact with the public during the healing period. The type of the dressings depends on the nature of the wound, cause, depth, and stage in wound healing. The cause of wound, especially for burn skin wound, can be classified in three main types (fig 1.5). First degree is not crucial wound or the mildest of the three, it just loss a top layer of skin or epidermis losing. Signs and symptoms of first degree burn are redness, pain, and minor swelling. The skin is dry without blisters. For healing time, Healing time is about 3 to 6 days; the superficial skin layer over the burn may peel off in 1 or 2 days. The wound dressing can be applied into the wound to relief painful. Second-degree burns are more serious and involve the skin layers beneath the top layer including epidermis and some of the part of dermis. Signs and symptoms, these burns produce blisters, severe pain, and redness. The blisters sometimes break open and the area is wet looking with a bright pink to cherry red color. For healing time, Healing time varies depending on the severity of the burn. In order to relief painful and protect the wound against outer environment of this burn, the wound dressing is necessary to use. For the last thing, third-degree burns are the most serious type of burn and involve all the layers of the skin and underlying tissue. Signs and symptoms of burn, the surface appears dry and can look waxy white, leathery, brown, or charred. There may be little or no pain or the area may feel numb at first because of nerve damage. The healing time depends on the severity of the burn. Deep second- and third-degree burns (called full-thickness burns) will likely need to be treated with skin grafts, in which healthy skin is taken from

another part of the body such as top of legs and surgically placed over the burn wound to help the area heal.

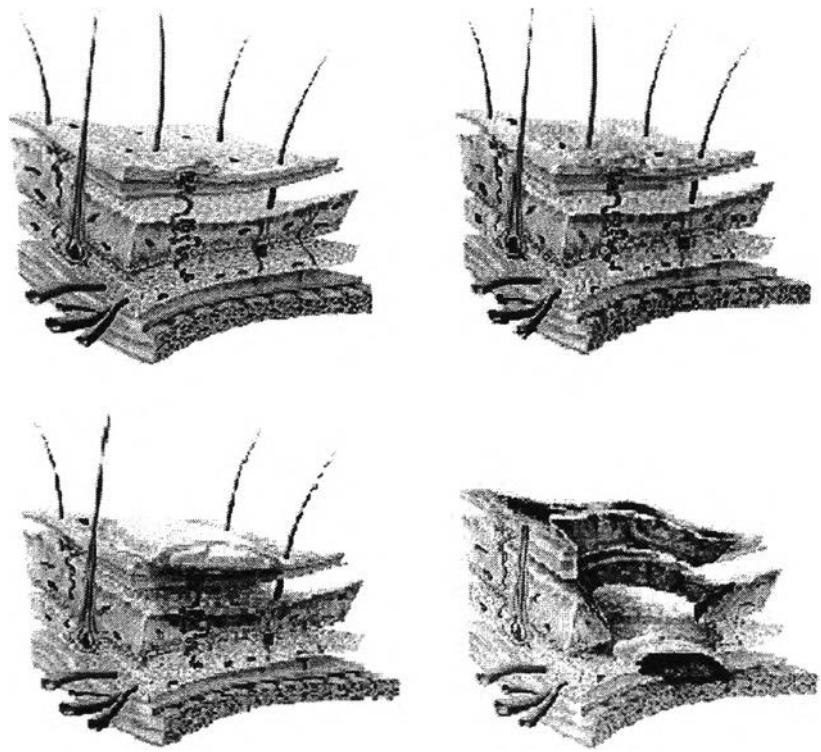

Figure 1.5 The degree of burns, normal skin (a), first degree burn (b), second degree burn (c) and third degree burn (d).

Dressings can be open or occlusive. There is also the term semi permeable, which means that they allow gases and water vapor throughout them. However, when it is first applied, the presence of exudates may block the pores.

1.1.6.1 Occlusive Dressings

Occlusion creates a moist environment that helps to maintain an electrical gradient between the wound and surrounding skin, which may stimulate epidermal cell migration. Occlusive dressings increase re-epithelization rates and reduce scarring while enhancing re-epithelization. Fluid from occluded wounds has been shown to contain proteinase that can lyse the fibrin plugs formed during hemostasis, a necessary step before healing can progress. Occlusion of a wound prevents excessive crust formation, excludes exogenous infection agents, reduces postoperative pain, decreases erythema and inflammation, simplifies wound management, and increases patient comfort with an acceptable appearance. The increased cost of this type of dressing, and the inability to visually inspect the wound with the dressing in place, are some of its disadvantages. There are studies reporting that the occlusive dressing needs to be applied within 2 hours after wounding and left in place for at least 24 hours for optimal healing to result. In ischemic wounds an impermeable occlusive dressing can severely impair the healing process. It will create a claustrophobic and constraining environment, and will increase susceptibility of infection. On the other hand, some studies did reveal that this increased bacterial growth has not translated into a higher rate of infection in clinically non infected wounds.

1.1.6.2 Open Dressings

Open dressings have many advantages: the monitorization of the wound throughout all the healing process thus avoiding complication factors; lower cost for the physician who will require less staff time; and involvement of the patients in their wound care. As disadvantages the patients must apply an ointment regularly to avoid risks of complications, there is increased pain and erythema during the recovery phase, and slower reepithelization. 16 Open dressings do not provide the beneficial moist environment beneficial to wound

healing; 14 as well as bacterial infections can develop unnoticed. This could lead to the development of persistent erythema or loss of skin texture.

OBJECTIVES

1. Preparation of β-chitin film and α-chitin whisker reinforced β-chitin films nanocomposite
2. Study on the effect α-chitin whisker reinforced material in β-chitin matrix in mechanical and thermal properties, water adsorption ability, *in vitro* biodegradation, oxygen permeability, crystallinity and cytotoxicity
3. Study on the possibility usage of nanocomposite films for wound healing application

SCOPE OF RESEARCH WORK

1. Preparation of α and β-chitins from shell of shrimps and squid pens
2. Preparation of β-chitin gel from squid pens chitin
3. Preparation of α-chitin whisker from shrimp shells chitin
4. Preparation of pure β-chitin and α-chitin whisker reinforced β-chitin films nanocomposite by using solvent casting
5. Characterization of films in mechanical and thermal properties, water adsorption ability, *in vitro* biodegradation, oxygen permeability, crystallinity and cytotoxicity