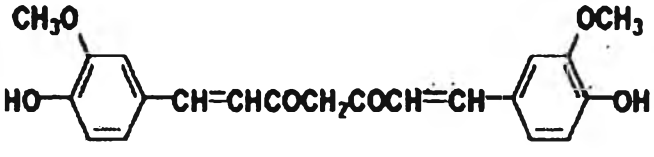
สำนักงานวิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

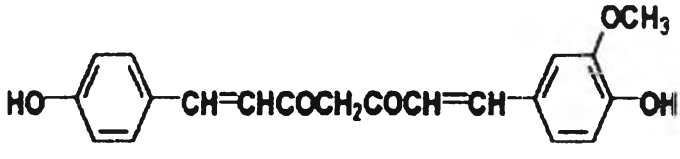
CHAPTER II
LITERATURE REVIEW

2.1 Curcumin

The coloring principle isolated from the rhizomes of *Curcuma longa* (turmeric) in the 19th century was named curcumin. Jayaprakasha *et al.* (2005) reported that curcuminoids corresponding to a group of phenolic compounds present in turmeric are chemically related to its principal ingredient curcumin. Curcuminoid content in turmeric is about 1-5% containing three major pigments of curcuminoids, curcumin, demethoxycurcumin, and bisdemethoxycurcumin (**Figure 2.1**).



Curcumin
(Diferuloyl methane)



Demethoxy curcumin
(*p*-Hydroxy-cinnamoyl-feruloyl-methane)

Bisdemethoxy curcumin
(*pp'*-Dihydroxy-dicinnamoyl-methane)

Figure 2.1 Structures of curcuminoids from *C. longa* (Jayaprakasha *et al.*, 2005).

Curcumin (diferuloyl methane) or 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a bis-α,β-unsaturated β-diketone that exists in equilibrium with its enol tautomer. The bis-keto form predominates in acidic and neutral aqueous

solutions (Wang *et al.*,1997). Jovanovic *et al.* (1999) suggested that the keto form of curcumin composes of the heptadienone linkage between the two methoxyphenol rings contains a highly activated carbon atom, and the C–H carbon bonds on this carbon are very weak due to delocalisation of the unpaired electron on the adjacent oxygens, as shown in **Figure 2.2**. Therefore, curcumin acts as an extraordinarily potent H-atom donor at pH 3–7. In contrast, above pH 8, the enolate form of the heptadienone chain predominates, and curcumin acts mainly as an electron donor, a mechanism more typical for the scavenging activity of phenolic antioxidants (Jovanovic *et al.*,1999). Curcumin is relatively insoluble in water, but dissolves in acetone, dimethylsulphoxide and ethanol.

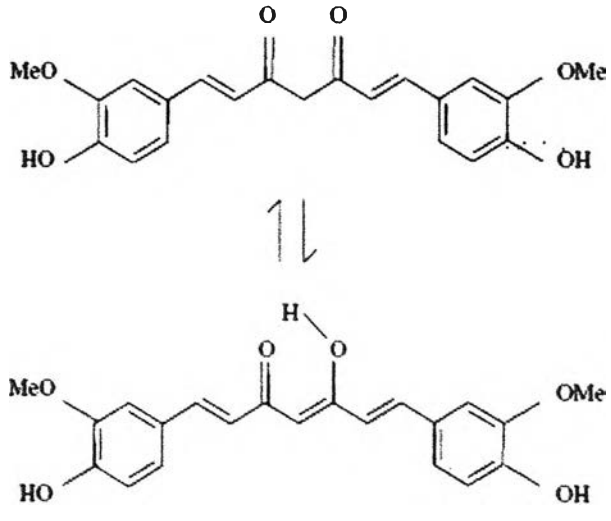


Figure 2.2 Tautomerism of curcumin under physiological conditions under acidic and neutral conditions, the bis-keto form (top) predominates, whereas the enolate form is found above pH 8 (Sharma *et al.*, 2005).

It has been suggested that the hydroxyl groups on the benzene rings, double bonds in the alkene portion of the molecule and/or the central β-diketone moiety could be responsible for the high biological activity of curcumin (Ruby *et al.*, 1995).

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2.1.1 Biological Activities of Curcumin

Over the past three decades, curcumin has been shown to possess wide range of pharmacological activities including antioxidant, antimicrobial effect, anti-cancer, anti-inflammatory (Jayaprakasha *et al.*, 2005), as well as wound healing (Sidhu *et al.*, 1998).

2.1.1.1 Antioxidant Activity of Curcumin

In 1992, Pulla Reddy and Lokesh observed that curcumin is capable of scavenging oxygen free radicals such as superoxide anions and hydroxyl radicals, which are the initiators of lipid peroxidation. It was found that curcumin inhibits lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates (Pulla Reddy *et al.*, 1994). The lipid peroxidation has a main role in the inflammation, in heart diseases, and in cancer. The effect of curcumin on lipid peroxidation was also studied in various models by several authors. In 2004, Gopinath *et al.* prepared curcumin-incorporated collagen (CICM) films. The antioxidant activity of CICM was monitored by inhibition of oleic acid peroxidation using 2,2'-azobisisobutyronitrile (AIBN). Following the addition of radical initiator (AIBN), the absorbance steadily increased resulting in conjugated diene (oxidized form) that absorbs at 234 nm. In the presence of CICM, the absorbance began to decrease steadily due to the scavenging action of curcumin (**Figure 2.3a**). Curcumin itself showed similar antioxidant activity as that of CICM (**Figure 2.3b**). This indicated that the scavenging action of curcumin is not hindered by collagen as the curcumin without collagen shows similar scavenging action. Recently, Suwantong *et al.* (2007) investigated the antioxidant activity of the as-loaded curcumin in the curcumin-loaded e-spun cellulose acetate (CA) fiber mats and corresponding as-cast CA films by the 1,1'-diphenyl-2-picrylhydrazy (DPPH) assay. The results were summarized in **Table 2.1**. The antioxidant activity of curcumin relates to the ability of curcumin in de-activating the DPPH radicals, which could be detected photometrically. They indicated that the free radical scavenging ability of the as-loaded curcumin, even after it had been subjected to a high electrical potential during e-spinning, was retained.

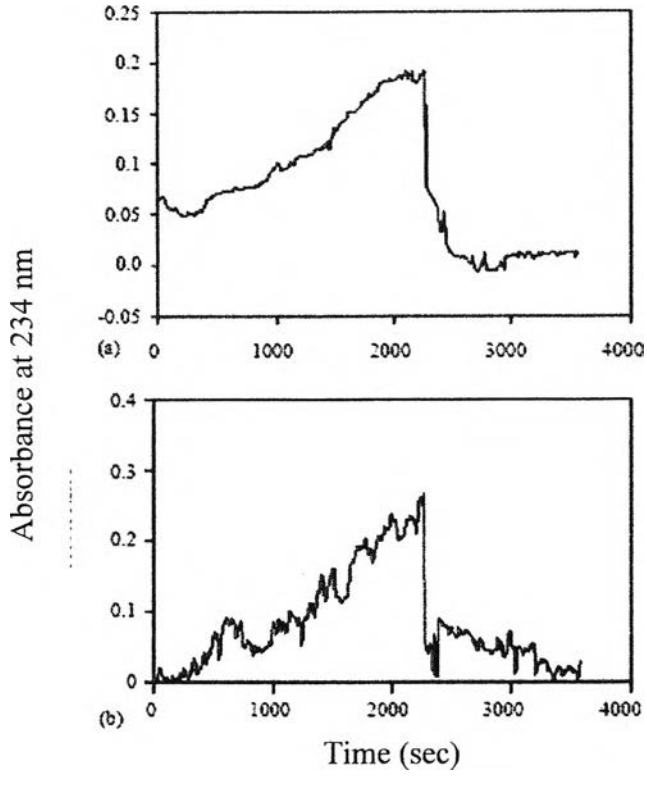

Figure 2.3 Lipid peroxidation inhibition studies: (a) CICM, (b) curcumin in solution, respectively (Gopinath *et al.* 2004).

2.1.1.2 Anti-cancer Activity of Curcumin

In the past few decades, anti-cancer properties of curcumin have been the subject of several articles. Although the exact mechanism is unclear, several modes of action have been proposed. In 1996, Singh *et al.* studied the effect of curcumin on the proliferation and cell cycle progression of human umbilical vein endothelial cells (HUVEC). They found that curcumin inhibited the DNA synthesis of HUVEC without significantly affecting the viability of the cells. Pulse labeling studies with [^{3}H]thymidine demonstrated that curcumin affected cells that were actively undergoing DNA synthesis activated by thymidine kinase (TK) enzyme. A significant loss of TK activity might be one of the possible mechanism(s) for the inhibition of DNA synthesis activity of HUVEC by curcumin. A unique mode of action of curcumin in blocking the cell cycle progression by inhibiting the activity of TK enzyme during S-phase was also revealed.

Table 2.1 Antioxidant activity of curcumin from curcumin-loaded electrospun CA fiber mats and corresponding solvent-cast CA films (Suwantong *et al.*, 2007)

Type of sample	The antioxidant activity (%)
Curcumin-loaded electrospun CA fiber mats	
With 5 wt.% curcumin	46.1 ± 5.5
With 10 wt.% curcumin	74.8 ± 2.7
With 15 wt.% curcumin	68.4 ± 1.9
With 20 wt.% curcumin	65.7 ± 1.7
Curcumin-loaded solvent-cast CA films	
With 5 wt.% curcumin	85.4 ± 2.0
With 10 wt.% curcumin	64.4 ± 5.8
With 15 wt.% curcumin	92.0 ± 6.7
With 20 wt.% curcumin	89.5 ± 5.6

The migration, proliferation and differentiation of HUVEC lead to angiogenesis, which facilitates the tumor initiation and promotion. Since curcumin inhibited the proliferation of HUVEC, it could turn out to be a very useful compound for the development of novel anti-cancer therapy. Khar *et al.* (1999) suggested anti-cancer activity of curcumin could be mainly due to its ability to induce apoptosis in tumor cells. Furthermore, Ozaki *et al.* (2000) studied the action of curcumin on rabbit osteoclast apoptosis and demonstrated that curcumin drastically inhibits bone resorption and stimulation of apoptosis in the cells. Since, cancer and bone inflammation are diseases that increase bone resorption, the authors suggested that curcumin may be useful in the therapy of these diseases. The anti-cancer action of curcumin has also been studied in a standard model of radiation-induced tumour in rat mammary gland (Inano *et al.*, 2000). They suggested that curcumin has the potential to be an effective agent for chemoprevention of radiation-induced initiation stage of mammary tumourogenesis. Curcumin was found to decrease the Ehrlich's ascites carcinoma (EAC) cell number by the induction of apoptosis in the tumor cells reported by Pal *et al.* (2001). An apoptosis enhancing capability of curcumin in EAC by modulating the cell cycle progression, as well as the cross talk of various pro- and

antiapoptotic factors have implications for the clinical use. The apoptotic response of EAC cells suggested the efficacy and possible application of curcumin in cancer prevention, and perhaps also in cancer therapy.

2.1.1.3 Anti- inflammatory Activity of Curcumin

A number of articals have studied the effect of oral curcumin on inflammatory diseases in humans. In 1980, Deodhar *et al.* administered 1200 mg curcumin four times daily to 18 patients with rheumatoid arthritis for 2 weeks; they reported a significant improvement in the patients' inflammatory symptomology without apparent toxicity. Similarly, Satoskar *et al.* (1986) found a significant anti-inflammatory effect objectively and subjectively from 400 mg thrice daily for 5 days in post-operative patients. The effects of oral curcumin on ophthalmological conditions were reported by Lal *et al.* (1999). Curcumin, 375 mg, administered thrice daily to patients with chronic anterior uveitis within 12 weeks resulted in a suggestion of improvement in the condition. In a subsequent study, the same dose of curcumin was administered to eight patients with idiopathic inflammatory orbital pseudotumours for 6–22 months. Complete response was observed in half the patients up to 2 years of follow-up. More recently, Chuang *et al.* (2000) have shown that administration of 200 mg of curcumin suppresses diethyl nitrosamine-induced inflammation and hyperplasia in rats, as shown by histopathological examination. Apparently, this effect is due to the fact that curcumin can act as an inhibitor of the inflammation-factor lipoxygenase reported by Skrzypezac-Jankun *et al.* (2000). Curcumin was reported to suppress activation of nuclear factor-kappa B NF-KB by repression of degradation of the inhibitory unit IKBa, which hampers subsequent nuclear translocation of the functionally active subunit of NF-KB (Surh *et al.*, 2001). Furthermore, curcumin was reported as a lead candidate for anti-inflammatory agent as it inhibits Protease-activated receptors (PAR2- and PAR4) mediated mast cell activation through a block of extracellular signal-regulated kinase (ERK) pathway.

2.1.1.4 Wound Healing Enhancement of Curcumin

The healing of wounds has always been a central consideration in medical practice. Wound healing involves a series of well-organized cellular and molecular events, including inflammation, angiogenesis, fibroplasia, wound contraction, epithelialization, as well as matrix remodeling (Cohen *et al.*,

1992). Since wound healing abnormalities cause great physical and psychological stress to affected patients that are extremely expensive to treat, the use of herbal products in the reconstruction of irradiated wounds is an attractive proposition. They have wide acceptability, are well tolerated, are economical, and can be safely manipulated for human use (Ammon *et al.*, 1991). Based on many studies, curcumin has been reported to be effective in wound repair in normal and diabetic-impaired healing, used both orally and topically (Sidhu *et al.*, 1998; Mani *et al.*, 2002). Phan *et al.* (2001) demonstrated that curcumin has inhibitory activity against hydrogen peroxide–induced oxidative damage in human keratinocytes and fibroblasts, suggesting the antioxidant role in enhanced wound repair. In 2004, Gopinath *et al.* indicated that curcumin incorporated collagen matrix exhibited wound reduction, enhanced cell proliferation of adult male Wistar rats as compared with control. In the same year, Jagetia *et al.* suggested that curcumin may be able to improve radiation-induced delay in wound repair. More recently, Maheshwari *et al.* (2006) stated that curcumin treatment resulted in faster closure of wounds, better regulation of granulation tissue formation and induction of growth factors, indicating that curcumin acts at different levels of wound repair.

2.1.2 Drawbacks of Curcumin for Its Clinical Applications

Although curcumin exhibits a wide range of pharmacological activities as described above, some drawbacks have been observed. Curcumin is a fat soluble pigment and it is insoluble in aqueous medium. It was found that curcumin undergoes photodegradation when exposed to light in solution as well as in solid form. It is moderately stable to heat, but unstable at basic pH and undergoes alkaline hydrolysis in alkali/higher pH solution. Hydrolytic decomposition of curcumin is reported even in *in vitro* physiological condition, isotonic phosphate buffer pH 7.2 (Wang *et al.*, 1997). Regarding to these disadvantages of curcumin, several attempts have been done to improve and/or solve the problems.

2.1.2.1 Improvement of Stability and Solubility of Curcumin

The complex kinetic of pH-dependent degradation of curcumin in aqueous solution was first reported by Tonnesen *et al.*, 1985. It was found that curcumin is unstable and degrades within 30 min at basic pH (Lin *et al.*, 2000). The presence of foetal calf serum or human blood, or addition of antioxidants

such as ascorbic acid and *N*-acetylcysteine or glutathione completely blocks this degradation in culture media or phosphate buffer above pH 7. Under acidic conditions, the degradation of curcumin is much slower, with less than 20% of total curcumin decomposed at 1 h. It have also found that curcumin is more stable in cell culture medium containing 10% foetal calf serum or in human blood, with less that 20% decomposition within 1 h compared to 90% within 30 min in serum-free medium, as shown in **Figure 2.4** (Wang *et al.*, 1997).

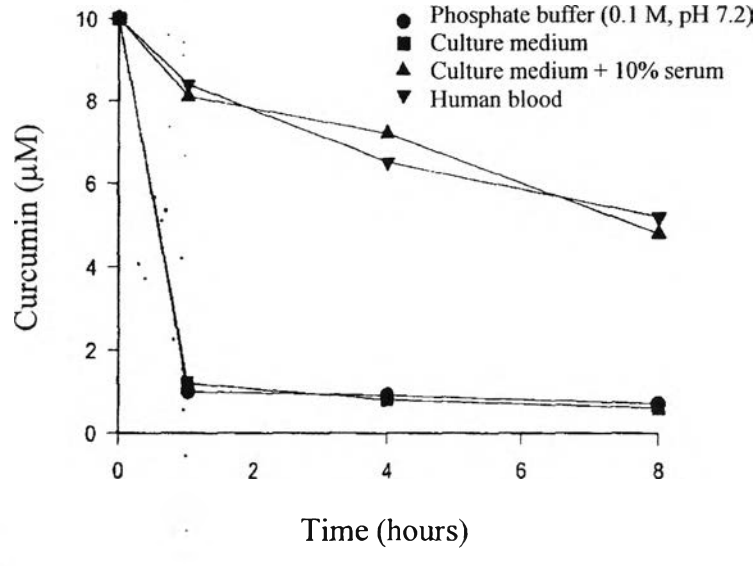

Figure 2.4 Effect of different physiological conditions *in vitro* on the stability of curcumin incubated at 37°C for 1, 4 and 8 h (Wang *et al.*, 1997).

Due to the hydrophobic nature of curcumin, its biological functions are limited after oral administration. Many authors suggested incorporation curcumin into micelles leading to enhancement of solubility as well as stability of curcumin in micellar systems. Iwunze *et al.* (2004) suggested thermodynamic stability of curcumin solubulized in the micellar system using cetyltrimethylammonium bromide (CTAB) surfactant. In 2006, Kunwar *et al.* studied binding constants of curcumin with phosphatidylcholine (PC) liposomes. The liposomal vehicle was examined for the delivery of curcumin to mouse spleen lymphocyte cells and EL4 lymphoma cells. It was found that liposomal vehicle is capable of loading more curcumin in to the cell

lines. Furthermore, emulsion-based systems have been widely used in food industry to protect active ingredients against extreme conditions, to enhance their stability as well as maintain their effectiveness (Madene *et al.*, 2006). Recently, Wang *et al.* (2008) prepared oil-in-water (O/W) nanoemulsions of different sizes using a high-speed homogenizer. Tween 20 was used as an emulsifier, to encapsulate curcumin. They reported that, in the presence of O/W emulsions, anti-inflammation activity of encapsulated curcumin was improved evidenced by the inhibition on the edema of mouse ear. **Figure 2.5** showed the UV–vis spectra of O/W emulsion with curcumin homogenized by the high-speed homogenizer as a function of storage times. The nearly-unchanged absorption peak of curcumin indicated that at a pH between 5.0 and 5.5, the stability of curcumin can be maintained in O/W nanoemulsions.

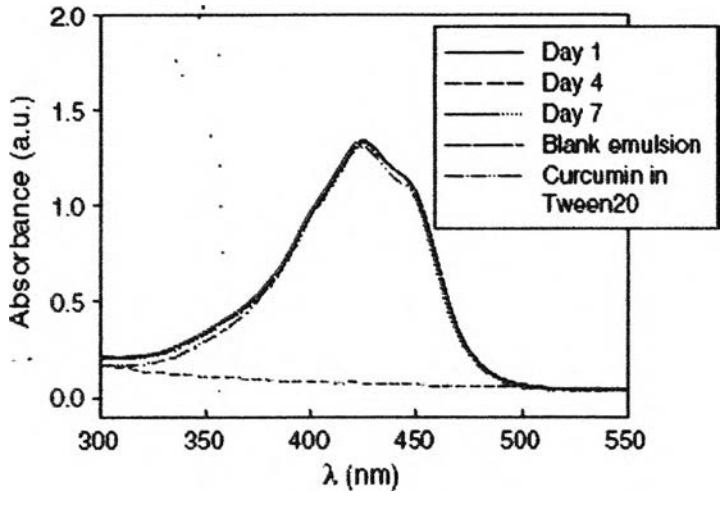

Figure 2.5 UV–Vis spectra of 1% curcumin nanoemulsion prepared by high-pressure homogenization after 1, 4, 7 days, as well as the blank O/W emulsion and curcumin in 10% Tween 20 water solution (Wang *et al.*, 2008).

2.2 Chitin

Chitin is the second most abundant natural polymer in the world next to cellulose. The main sources exploited are two marine crustaceans, shrimp and crabs (Rinaudo *et al.*, 2006). Chitin is an aminopolysaccharide that composes of β(1→4)

linked 2-acetamido-2-deoxy-β-D-glucose units (or *N*-acetylglucosamine) forming a long linear polymer chain. Therefore, it is highly ordered with extensive hydrogen bonding between adjacent polymer chains (Yusof *et al.*, 2003) resulting in high rigidity, poor solubility, low swellability, and low processability of chitin. However, there are several attempts suggesting formation of chitin. It was firstly transform into solutions using solvent systems (**Table 2.2**) follwed by various forming techniques; for eamples, solvent casting, wet-spinning, salt leaching, as well as coagulant techniques resulting in various products. The transformation and/or fabrication of this alternative biomaterial contributed to the applications development is interesting, since it possesses many advantages such as non-toxicity, biodegradability, and biocompatibility. Chitin can be degraded, owing to the presence of chitinases widely distributed in nature and found in bacteria, fungi and plants, and in the digestive systems of many animals, and subsequently generates harmless products (Kurita *et al.*, 2000).

2.2.1 Chitin with Biological Properties.

The main development of chitin film and fiber is in medical and pharmaceutical applications as wound-dressing material (Rathke *et al.*, 1994; Hudson *et al.*, 1998,1999; Yusof *et al.*, 2003) and controlled drug release (Kanke *et al.*, 1989; Kato *et al.*, 2003). Chitin is also used as an excipient as well as drug carrier in film, gel or powder form for applications. In the presence of the acetamide group which is similar to the amide linkage of protein in living tissues, chitin is biocompatible and safe to be utilized for human (Muzzarelli, 1997). In 1985, Nishimura *et al.* fabricated adjuvant from chitin derivatives to circulate antibody. Chitin has been reported to be used as skin substitutes (Su *et al.*, 1997). Okamoto *et al.* (2002) studied the analgesic effect of chitin and chitosan. They found that chitin particles can absorb bradykinin, resulting in reliving of the pain from wound. Subsequently, Okamoto *et al.* (2003) studied the effect of chitin and chitosan on blood coagulation. It was investigated that that both chitin and chitosan enhanced the release of the platelet to stop the blood. In the same year, chitin/PLGA microspheres were fabricated to be used as drug delivery system (Mi *et al*).

Table 2.2 Solvent systems for chitin solution forming various products

Solvent systems	products	Literature review
5% LiCl/ *N,N* dimethylacetamide	microsphere	Mi F.L., *et al.* (2003)
	gel and bead	Yilmaz E., *et al.* (2003)
	scaffold	Lee S.B., *et al.* (2004)
	film and scaffold	Ge Z., *et al.* (2004)
NaOH (alkaline chitin)	fiber	Hirano S., *et al.* (2002)
	bead	Zhou D., *et al.* (2005)
1,1,1,3,3,3-hexafluoro-2 propanol (HFIP)	nanofiber	Min B.M., *et al.* (2004)
		Park K.E., *et al.* (2006)
		Noh H.K., *et al.* (2006)
Tetrabutylammonium fluoride/ Dimethyl sulfoxide	film	Yoshimura T., *et al.* (2005)
Formic acid	nanofiber	Min B.M., *et al.* (2004)
	film	Peesan M., *et al.* (2003)
Sat $CaCl_2$ in methanol	hydrogel	Tamura *et al.* (2006)

2.2.1.1 Chitin and Cell Behaviors

In 1997, Mori *et al.* studied the effect of chitin and its derivatives on the proliferation of fibroblast. They found that chitin accelerated the proliferation of fibroblast. Okamoto *et al.* (2002) studied the effect of chitin/chitosan and their oligomers/monomers on fibroblast and vascular endothelium migration. It was investigated that chitin affected to the vascular endothelium migration, and the

chitin monomer had a high effect with fibroblast migration. More recently, Noh *et al.* (2006) developed a chitin scaffold or wound dressing for tissue engineering composed of electrospun chitin nanofibers (Chi-N) and microfibers (Chi-M).

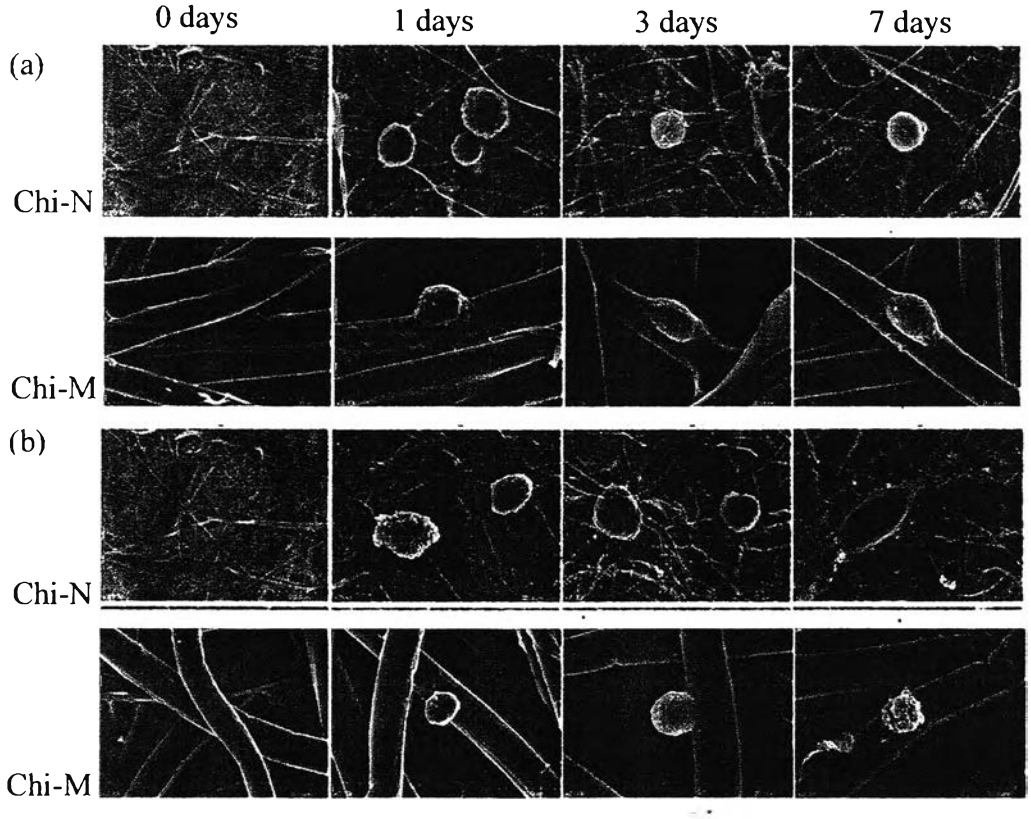

Figure 2.6 SEM micrographs of the interaction between chitin and (a) normal human oral keratinocytes, and (b) normal human epidermal keratinocytes after 0, 1, 3, and 7 days of culture (Noh *et al.*, 2006).

The cellular response to chitin in normal human keratinocytes and fibroblasts, as well as the interaction between cells were determined. In the cytocompatibility assessment, chitin nanofibers were found to promote cell attachment and spreading of normal human keratinocytes and fibroblasts compared to chitin microfibers, as shown in **Figure 2.6**.

2.2.1.2 Wound Healing Enhancement of Chitin

Minagawa *et al.* (2007) examined the effects of chitin, chitosan, on wound healing using a linear incisional wound model in rats. They found that not only chitin/chitosan but also their oligomers as well as monomers enhanced wound healing acceleration. Histologically, it was notable that collagen fibers run perpendicular to the wound line in the oligomers groups, which suggests wound healing acceleration. It was suggested that when each sample with different molecular size was administered to the wound, the degree of biodegradability of each sample was different. Therefore, in chitin/chitosan, it takes more time to be biodegraded and absorbed in the wound due to their high molecular weight, while monomers are absorbed quickly due to low molecular weight. In addition, oligomers are also suitable in respect to absorption.