CHAPTER II
LITERATURE SURVEY

2.1 Carboxymethyl-Chitin Based Material for Drug Delivery Studies

Nishimura *et al.* (1983) reported that the effect of chitin and its derivatives on the activation of peritoneal macrophages in *vivo*, on the suppression of tumour growth in syngeneic mice and on the protection of the host against bacterial infection was examined. Thirty percent deacetylated chitin (30 % DA-chitin), 70 % DA-chitin and carboxymethyl-chitin (CM-chitin) induced cytotoxic macrophages most effectively.

Tokura *et al.* (1990) studied the sustain-release of methamphetamine-bound CM-chitin. After methamphetamine-bound CM-chitin (MAEA-CM-chitin) was injected subcutaneously in rabbits, it was found that the introduction of antibody production was significantly low. The MAEA oligosaccharide was released slowly after the biodegradation of MAEA-CM-chitin and maintained at a significant level in serum for more than 120 h, while the blood level of MAEA was out of the range of detection within 7 h after the injection, probably owing to rapid metabolism, CM-chitin was reveled to be a suitable drug carrier for controlled release.

Tokura *et al.* (1990) reported that CM-chitin was employed as a sustained-release drug carrier for the subcutaneous injection. The induced specific antibody was applied to the titration of methamphetamine secreted into the blood of rabbits. The methamphetamine concentration in blood serum was maintained for 120 h at fairly high levels. Methamphetamine was also excreted into urine at high levels with a similar time course to that in blood serum.

Tokura *et al.* (1992) studied the two-step release of drug by a covalently drug-pendented CM-chitin in which the drug was couple through an enzyme-susceptible bond. In these conjugates, the drug will be released through several hydrolysis processes, since oligomerization of CM-chitin by lysozymic hydrolysis is the first step, and the release of parent active drug is followed by the cleavage of spacer-drug linkage with proteolytic hydrolysis as a second step. The release of active drug from polymeric drug can be inhibited by the protection of cleavage site

with CM-chitin-calcium complex when molecular weight of polymeric drug is high enough.

Watanabe *et al.* (1992) studied antimetastatic activity of neocarzinostatin that incorporated into controlled release gels of CM-chitin. 6-O-carboxymethyl-chitin (6-O-CM-chitin), a water-soluble chitin derivative, was gelled in the presence of 15 to 30 mM iron (III) chloride. At the time of gel formation, a peptidic anticancer drug neocarzinostatin (NCS), was efficiently (>50%) incorporated into the gel in the co-presence of 25 to 50 mM calcium chloride and iron (III) chloride. 6-O-CM-chitin gel containing NCS was digested by lysozyme in *vivo* and NCS was released from the gel in both a time- and dose-dependent manner. Antimetastatic effects of the CM-chitin gel containing NCS were studied in the spontaneous pulmonary metastasis model using B16-BL6 melanoma. It was concluded that CM-chitin gels are useful as a sustained-release drug carrier.

Miura *et al.* (1992) studied 6-O- carboxymethyl-chitin (6-O-CM-chitin) as a carrier for sustained release of drugs. The peptides containing neutral amino residue, especially phenylalanine (Phe), were found to be adsorbed specifically and stabilized by entrapping until 6-O-CM-chitin was biodegraded to oligomers of small sizes.

Uraki *et al.* (1993) studied site specific binding of calcium ions to anionic chitin derivatives. 6-O-carboxymethyl-chitin (6-O-CM-chitin) and *N*-succinylated chitosan (Suc-chitosan) are shown to have a high affinity for calcium ions on a 10^5 mol^{-1} dm^3 order of adsorption constants. It is suggested that 6-O-CM-chitin especially forms a tight complex with calcium ions.

Tokura *et al.* (1994) studied drug delivery system by using biodegradable carrier. Methamphetamine (MA) was used as a model drug. A prodrug was found to be released slowly into blood following the subcutaneous injection of polymeric drugs, in which the prodrug was either pendanted through a covalent bond to 6-O-carboxymethyl-chitin CM-chitin or entrapped within (6-O-CM-chitin) matrix in the presence of Fe^{3+}. The prodrug was then hydrolyzed, to become the active form, by enzymes in the blood.

Tokura *et al.* (1983a) studied biopolymeric properties of a water-soluble and biodegradable chitin derivative, 6-O-carboxymethyl-chitin (6-O-CM-chitin), these properties have been investigated to demonstrate the immunological function serving

to induce a hapten-specific antibody and the chemotherapeutic function as a drug carrier of controlled release. When 6-O-CM-chitin was linked by methamphetamine (MA) through a nonbiodegradable spacer, 1-aminobutane (MABA-CM-chitin), MA-specific antibody was produced by the subcutaneous injection, in rabbits, of MABA-CM-chitin in combination with Freund's complete adjuvant. When injected without intense immunoajuvant, MABA-CM-chitin oligomer was secreted into blood for more than 120 h. Two-step hydrolysis of polymeris drug was also investigated in order to design a more sophisticated drug delivery system. Phenylalanine-containing peptide spacers were designed to be hydrolysised by emzymes other than lysozyme as a model drug. The chromophore-bound phenylalanine peptide spacer was stabilized against enzymatic hydrolysis untill 6-O-CM-chitin was hydrolysed by lysozyme.

Tokura *et al.* (1983) reported that a porous chitin foam was regenerated from chitin dope in calcium chloride dihydrate saturated methanol. The porous chitin foam was shown to have cationic property, because chitin foam tended to adsorb anionic dyes through ionic binding and hydrophobic interaction. A pendant type of polymeric drug was prepared by applying peptide spacer composed of phenylalanine at amino end and two step hydrolyses of polymeric drug were shown to release active drug at the final step using lysozyme and chymotrypsin in vitro.

Khor *et al.* (1997) reported that the reversible water-swellable chitin gel has been produced by the carboxymethylation of a dry chitin film. It took up water but was not soluble and retained a degree of rigidity even when wet. The degree of swelling depended on the reaction conditions and alkali (sodium or potassium hydroxide) used as a co-reactant during the carboxymethylation. Upon drying, the gel returned to its dry film form. This water uptake and loss could occur in cyclic, which is a desirable property in certain applications and is a tremendous advantage in the handling of this material.

Wan *et al.* (1997) reported that a chitin hydrogel was modified to give a bilayer structure comprised of a surface layer of carboxymethyl-chitin and bulk chitin within. By gradually increasing the sodium hydroxide concentration used in the activation step of the reaction, thickness of the carboxymethyl layer with accompanying swellability was varied. These bilayer hydrogels showed distinct

morphological differences between the surface and bulk regions, visualized with a basic dye test. Carboxymethylation was found to increase the lysozyme susceptibility of these hydrogels. These modified hydrogels have potential in orthopedics applications where enhanced water swellability and calcium affinity imparted by surface carboxymethylation are desirable properties.

Hata *et al.* (2000) examined the effect of the deacetylation degree on the biodegradability and biodisposition characteristics of 6-O-carboxymethyl chitin (6-O-CM-chitin). The degree of deacetylation of 6-O-CM- chitin was controlled by changing the alkaline treatment time. The less biodegradability of carboxymethyl chitin was observed as the degree of deacetylation increased. The elimination from the bloodstream and the excretion into urine became slower as the degree of deacetylation increased. The body retention of 6-O-CM-chitin may be controlled by changing the degree of deacetylation.

Chen *et al.* (2000) reported that for the complex films composed of various molar (repeat unit) ratios of chitosan and carboxymethyl chitin (CM-chitin) moisture sorptions were measured at 20 degrees C, 65% RH and 20 degrees C, 90% RH and water sorptions were measured in 5,000-fold as much water as complex film after 24 h at 30 degrees C. Hygroscopicities of complex films became higher as the increase of the content of CM-chitin irrespective of relative humidity and they were higher independent of the composition of complex film at 90% RH compared with those at 65% RH. Water sorptions of complex films were lower as molar (repeat unit) ratios of chitosan and CM-chitin were in the range of 0.4/0.6 to 0.3/0.7.

To overcome current limitations in wound dressings for treating mustard-burn induced septic wound injuries, Loke *et al.* (2000) developed a nonadherent wound dressing with sustained anti-microbial capability. The wound dressing consists of two layers: the upper layer is a carboxymethyl-chitin hydrogel material, while the lower layer is an anti-microbial impregnated biomaterial. The hydrogel layer acts as a mechanical and microbial barrier, and is capable of absorbing wound exudate. In physiological fluid, the carboxymethyl-chitin hydrogel could swell considerably, imbibing water up to 4 times of its own weight and was also highly porous to water vapor.

Tokura *et al.* (2001) studied peroral and intravenous administrations of ^{14}C-labeled carboxymethyl-chitin (CM-chitin). It was revealed that CM-chitin accumulated in bone marrow. Thus, a composite of CM-chitin with hydroxyapatite (HA) was prepared to examine the bone repairing properties by animal and cell line experiments. The new bone formation of CM-chitin·/HA composites was superior to that of CM-chitin, HA, and blank. A porous CM-chitin·/HA composite was a functional material which could act as a scaffolding of osteoblast-like cells, a barrier to ingrowth of fibrous connective tissues. The cytotoxicity of CM-chitin was evaluated using the MC3T3-E1 cell line. It was found that control of degree of deacetylation is a very important factor in using CM-chitin as bone repairing material.

2.2 Silk Fibroin Based Blend Films

Yamaura *et al.* (1990) studied the properties of the blend film of silk fibroin and syndiotactic rich poly(vinyl alcohol). It was found that mechanical properties did not change. However, the presence of silk fibroin in the blend film promoted the permeation of neutral salts and ions.

Liang *et al.* (1992) improved physical properties of silk fibroin membrane by blending with sodium alginate, a natural polymer generally found in red algae. The addition of sodium alginate to fibroin showed that water absorbability, mechanical properties and thermal stability of fibroin membranes were improved. The water content of the membrane containing 50% by weight sodium alginate was 66% higher than that of pure fibroin. Because alginate is an ionic polymer, so the hydrophilicity is high. Furthermore, the tensile strength and thermal stability were also improved.

Freddi *et al.* (1995) prepared the blend films of silk and cellulose by Both its strength and elongation at break were improved. The addition of cellulose to silk fibroin permited the preparation of membrane with excellent elastic behavior. Moreover hydrogen bonding was found between fibroin and cellulose by analyzing with Fourier Transform Infrared Spectroscopy (FTIR).

Liu *et al.* (1996) prepared the blend of silk fibroin and poly(vinyl alcohol) in order to obtain a good matrix for enzyme immobilization. It was found that the blend membrane retained the merit of silk fibroin and it posed better mechanical strength and higher water absorbance. The glucose biosensor could be prepared by immobilizing GOD (glucose peroxidase) in the blend membrane of silk fibroin and poly(vinyl alcohol) coupling the Clark oxygen electrode. The response time of biosensor was shortened by preparing porous blend membrane with poly(ethylene glycol) as a removable component. The glucose sensor had the ability of resistance over a broad range of pH and temperature and had good stability.

Freddi *et al.* (1999) studied on the preparation and characterization of silk fibroin (*Bombyx mori*)/cellulose blend films. By dissolution with a metal complex solution, the average molecular weight of silk fibroin slightly decreased, while cellulose was almost unaffected. After coagulation and washing, transparent films were obtained for all blend proportions. The crystalline structures of regenerated fibroin and cellulose were beta-form and cellulose II, respectively. Density values increased with increasing cellulose content, though less than expected from a pure additive behavior. Moisture regain increased following the addition of a small amount of cellulose to silk fibroin. The mechanical properties showed that both strength and elongation at break of silk fibroin films were improved by blending with cellulose, suggesting the occurrence of intermolecular interactions between fibroin and cellulose through hydrogen bond formation.

Chen *et al.* (1997) reported that a novel natural polymer blend, namely, a semi-interpenetrating polymer network (semi-IPN) composed of crosslinked chitosan with glutaraldehyde and silk fibroin was prepared. It was found that chitosan and silk fibroin had a strong hydrogen-bond interaction and formed an interpolymer complex. The semi-IPN showed good pH and ion sensitivity

Chen *et al.* (1997) studied on the conformation of silk fibroin in silk fibroin/chitosan (SF/CS) blend. The results demonstrated that the SF showed β-sheet conformation when the SF content in blend membranes was 10% (w/w) and 60-80% (w/w), while the pure SF membrane showed random coil conformation. A mechanism of the conformation could be explained the SF chain could use the rigid CS chain as a mold plate to stretch itself to form a β-sheet structure according to the

strong hydrogen bond between CS and SF. Therefore, a new concept, named 'Polymer-Induced Conformation Transition was proposed.

Park *et al.* (1999) prepared silk fibroin/chitosan blend films by the solvent casting method. The conformational transition of silk fibroin from random coil form to β-sheet structure was induced by blending with chitosan resulting in the increase of crystallinity and density of the blend films. The blend film containing 30 wt% chitosan exhibited a maximum increase in crystallinity and density. It was found that the tensile strength and initial tensile modulus of blend films were greatly enhanced with increasing the chitosan content and showed a maximum value at the composition of 30 wt% chitosan.

Freddi *et al.* (1997) characterized blend films obtained by mixing silk fibroin (SF) and polyacrylamide (PAAm). The peak of dynamic loss modulus of silk fibroin at 193 degrees C gradually shifted to lower temperature in the blend films, suggesting an enhancement of the molecular motion of the fibroin chains induced by the presence of PAAm. Changes in the NH stretching region of silk fibroin are attributed to disturbance of the hydrogen bond pattern of silk fibroin and formation of new hydrogen bonds with PAAm. The values of strength and elongation at break of blend films slightly improved at 20-25% PAAm content.

Yang *et al.* (2000) prepared two kinds of blend membranes of regenerated cellulose and silk fibroin (SF) by coagulating the mixture solution of cellulose and silk fibroin in cuoxam with acetone-acetic acid (4:1 by volume) and 10 wt% NaOH aqueous solution, respectively. The compositions, miscibility, structure, water permeability of the blend membranes were measured, and the effects of various coagulants and the weight percent of SF on the structure and microporous formation of the blend membranes were investigated and discussed.

Kweon *et al.* (2001) studied on the conformational changes of silk fibroin in silk fibroin/chitosan blend films. The effects of fibroin/chitosan blend ratios (chitosan content) on the physical and mechanical properties were investigated to evaluate the feasibility of using these films as biomedical materials such as artificial skin and wound dressing. The mechanical properties of the blend films containing 10-40% chitosan were found to be excellent. The chitosan contents of the blend films, which were also related to the density and degree of swelling, affected the

tensile strength, breaking elongation, and Young's modulus. The coefficient of water vapor permeability of the blend films increased linearly with increasing the chitosan content, the blend film containing 40-50% chitosan showed very high oxygen permeability.

2.3 Silk Fibroin Based Material for Controlled Release Studies

Chen *et al.* (1994) investigated the transport of pharmaceuticals through silk fibroin membranes prepared from Chinese cocoon. The permeability coefficient of 5 kinds of pharmaceuticals, i.e. 5 fluorouracil (5FU), L-(+)-ascorbic acid (Vc), resorcinal (res.), sodium phenolsulpahte (SPS), an benzyltrimethyammonium chloride (BTAC), could be regulated by changing the pH value of the external solution. The silk fibroin membrane was an amphoteric ion exchange membrane composed of both weak acidic and weak basic groups and it was expected to be used as the matrix of the drug delivery system with pH-responsive function.

Tsukada *et al.* (1994) studied the preparation and morphological characterization of porous materials obtained by freezing and lyophilizing silk fibroin solutions. When an aqueous silk solution was frozen at different temperatures (-18, -45, and -80 °C), the average pore size decreased with lowering of the freezing temperature. Silk fibroin aggregates obtained in these conditions exhibited a sheetlike structure. By lowering the pH from neutrality to 4.01 and 2.65, the morphology of the solid phase changed from a sheet to a fiber structure. The average pore size was smaller at pH 4.01 than is the former value corresponding to the isoelectric point of silk fibroin. The addition of different amounts of methanol to the silk solution resulted in a sharp fall of the average pore size and hardened the material, as a consequence of the high packing density of the fibroin molecules. Silk fibroin aggregates prepared in these conditions exhibited a typical fibrous structure. A drug-delivery system was prepared by incorporating acetylsalicyclic acid into a porous silk fibroin carrier, and the kinetics of the drug release were determined.

Katayama *et al.* (2000) investigated the applicability of silk fibroin to controlled release type dosage tablets by using theophylline as model drug. The drug release form silk fibroin tablets was not affected by the pH of the release medium.

The greater the fibroin content in the tablets, the lower the percentage released of theophylline. Furthermore, it was found that the drug release from the fibroin tablets was diffusion-controlled through the matrix.