

CHAPTER II

LITERATURE SURVEY

2.1 Scaffold

Chen *et al.* (2001) developed a new method of preparing porous scaffolds composed of synthetic biodegradable polymers by combining porogen leaching and freeze-drying techniques using prepared ice particulates as the porogen material. The pore structures of the polymer sponges could be manipulated by controlling processing variables such as the size and weight fraction of the ice particulates and the polymer concentration.

Shanmugasundaram *et al.* (2001) developed a polymer scaffold using collagen and chitosan, in the form of interpenetrating polymeric network (IPN), for *in vitro* culture of human epidermoid carcinoma cells (Hep-2, Cincinnati) by using glutaraldehyde as cross-linking agent. The result suggested that the scaffolds prepared from collagen and chitosan can be utilized as a substrate to culture Hep-2 cells and can also be used as an *in vitro* model to test anticancerous drug.

Ameer *et al.* (2002) developed a biodegradable composite system as a way to rapidly entrap cells within a support of predefined shape to potentially facilitate cell delivery into a target site. The composite construct consisted of freshly isolated cells, entrapped in a fibrin gel phase and dispersed throughout the void volume of a polyglycolic acid (PGA) non-woven mesh. *In vitro* degradation of fibrin gel was evaluated via gel-entrapped urokinase. Varying the concentration of entrapped urokinase could affect controlled degradation of fibrin gel.

Lee *et al.* (2002) prepared a porous chitosan matrices, chitosan-poly(L-lactide) (PLLA) composite matrices, and chitosan coated on PLLA matrices for obtaining high bone forming efficiency. All chitosan based devices demonstrated improve bone forming capacity by increasing mechanical stability and biocompatibility. Release of platelet-derived growth factor-BB (PDGF-BB) from these matrices exerted significant osteoinductive effect in addition to the high osteoconducting capacity of the porous chitosan matrices. The hydrophobic surface

of PLLA matrices was modified by chitosan to enhance cell affinity and wettability. The results showed that chitosan enhance the tissue regeneration efficiency.

Park *et al.* (2002) fabricated porous matrices containing collagen and hyaluronic acid by freeze drying at -20°C , -70°C or -196°C . The matrices were crosslinked using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EMC). The results demonstrated that the matrices obtained before cross-linking process had interconnected pores with mean diameters of 40, 90, or 230 μm and porosity of 58-66% and also the porous structures after cross linking were retained.

Rocha *et al.* (2002) synthesized anionic collagen with enhanced piezoelectric properties can be obtained through hydrolysis of carboxyamides groups of asparagine and glutamine residues from collagen in carboxylic. The low cost of production associated to the biocompatibility and osteoconductivity performance make the anionic collagen matrices promising for bone defects treatment.

2.2 Chitosan in Medical Applications

Taravel *et al.* (1995) studied the interaction which can occur when chitosan is in contact with bovine anticollagen. There exists a competition between collagen gelation and the formation of a pure polyanion-polycation complex with chitosan. They attempted to achieve the 1:1 stoichiometry by two different methods. When collagen is denatured, the polyanion-polycation complex is improved, but the theoretical stoichiometry is not achieved. The presence of a large excess of chitosan gives rise to a formation of a pure polyanion-polycation complex which chitosan seems to induce the denaturation of collagen.

Thacharodi *et al.* (1995) prepared composite membrane consisting of collagen and chitosan in various proportions. The permeability properties of these membranes for propranolol hydrochloride were studied and compared with those of collagen and chitosan membranes. The permeability properties of the composite membranes were found to depend on the concentration of collagen and chitosan in the membrane.

Taravel *et al.* (1996) studied collagen and its interactions with chitosan. Bovine atelocollagen and high molecular weight fully deacetylated chitosan can form complexes whether by means of purely electrostatics interactions or by hydrogen bonding. In the purely electrostatics interactions, it is difficult to conclude whether chitosan prevents collagen digestion by collagenase or not. The other one, chitosan induces a strong protection toward specific enzyme. In case of the mechanical properties of polyanion/polycation complexes, chitosan brings softening rather than hardening to the system and the complex behaves like some polymer blends.

Thacharodi *et al.* (1996) fabricated membrane-permeation-controlled transdermal devices for the controlled delivery of lipophilic drug (nifedipine) and propranolol hydrochloride using composite membranes made of chitosan and collagen. These devices were evaluated in modified Franz glass diffusion cells using rabbit pinna skin. The data showed that the devices effectively controlled the transdermal of both drugs.

Kojima *et al.* (1998) evaluated the granulation tissue induced by chitin- or chitosan-coated polyester nonwoven fabric (chitin-NWF, chitosan-NWF) and uncoated nonwoven fabric (control) implanted in the feline abdominal wall by macroscopic and microscopic observation. Tissue growth around the implant was most marked with chitosan-NWF and least with chitin-NWF. The inflammatory reaction was much stronger with chitosan-NWF.

Madihally *et al.* (1999) examined the use of chitosan for formation of porous scaffolds of controlled microstructure in several tissue-relevant geometrics. Porous chitosan materials were prepared by controlled freezing and lyophilization of chitosan and gels. Hydrated porous chitosan membranes were at least twice as extensible as non-porous chitosan membranes, but their elastic moduli and tensile strengths were about tenfold lower than non-porous controls.

Francis *et al.* (2000) studied the chitosan-based polysaccharide biomaterials in cartilage tissue engineering. Chondrocytes cultured on chondroitin 4-sulfate/chitosan may be well suitable as a carrier-material for the transplant of autologous chondrocytes.

Hirano *et al.* (2000) studied the improvements of blood compatibility of chitin and their chemical N-modified fibers by blending with tropocollagen. The

tropocollagen in the blended fiber affected little their tenacity and elongation values. A transparent blended hydrogel of N-acetylchitosan (chitin) with tropocollagen was produced from mixed solution by treatment with acetic anhydride, and its membrane and sponge sheet were also prepared from the hydrogel.

Ma *et al.* (2001) designed the bilayer structure of chitosan film and sponge as a scaffold for skin tissue engineering and a dermis substitute. It was processed successively via the formation of a dense chitosan film by casting method and a porous chitosan sponge by lyophilization. The results suggested that the bilayer chitosan material remained stable in shape and size during the cell culture and it would be an alternative of collagen materials which was obviously contracted during cell culture.

Shanmugasundaram *et al.* (2001) studied various types of scaffolds using collagen and chitosan in the form of interpenetrating network (IPN) for in vitro culture of human epidermoid carcinoma cells (HEp-2, Cineinnati) by using glutaraldehyde as a crosslinking agent. The scaffold that showed optimum swelling property was selected as the best scaffold for performing in vitro culture studies. The scaffolds prepared from collagen and chitosan can be utilized as a substrate to culture HEp-2 cells and can also be used as an in vitro model to test anticancerous drugs.

Zhao *et al.* (2002) prepared hydroxyapatite/chitosan-gelatin network (HA/CS-Gel) composite of similar composition to that of normal human bone as a three-dimensional biomimetic scaffold by phase separation method. Changing the solid content and the compositional variables of the original mixtures allowed control of the porosities and densities of the scaffolds. The result indicated that the osteoblasts attached to and proliferated on the scaffolds.

Gingras *et al.* (2003) developed a reconstructed skin made of a collagen-chitosan sponge seeded with human fibroblasts and keratinocytes and grown in vitro for 31 days for the treatment of deep and extensive burns. The human reconstructed skin was transplanted on the back of nude mice and the growth of nerve fibers. They concluded that the three-dimensional architecture of the collagen-chitosan tissue-engineered skin sponge encourages nerve growth.

Li *et al.* (2003) studied cultures of hepatocytes on fructose-modified chitosan scaffolds prepared by lyophilization with average pore size 50-200 μm . The scaffold modified with fructose accommodated a much large number of hepatocytes than only chitosan due to the specific interaction between seeded hepatocytes and fructose moieties conjugated onto the surface of the scaffold.

Mao *et al.* (2003) prepared chitosan-gelatin hybrid polymer network scaffolds via the freeze-drying technique by using the ice-microparticle as a porogen. Monolayer and bilayer scaffolds were obtained by using different pre-freezing methods. The results suggested that the porosity and pore size of the scaffolds could be modulated with thermodynamic and kinetic parameters of ice formation.

2.3 Carboxymethyl Chitin (CM-chitin) in Medical Applications

Nishimura *et al.* (1984) reported that chitin could interact with bovine blood proteins and the affinities of these proteins for chitin tended to be decreased by introduction of O-carboxymethyl groups onto chitin surface. As the adsorption of blood proteins to the CM-chitin (d.s. 0.35) was assumed to follow an isothermal adsorption curve, the adsorption coefficients were estimated by applying the Langmuir equation. The binding site of bovine serum albumin (BSA) for CM-chitin was assumed to be regulated not only by cationic groups of BSA but also by other factors such as the recognition capacity of BSA to bind to N-acetylglucosamine in CM-chitin.

Nishimura *et al.* (1986) studied the effect of chitin and its derivatives on the activation of peritoneal macrophages *in vivo*, on the suppression of tumor growth in syngeneic mice and on the protection of the host against bacterial infection. Thirty percent deacylated chitin (30% DA-chitin), 70% DA-chitin and carboxymethyl-chitin (CM-chitin) induced cytotoxic macrophages most effectively.

Tokura *et al.* (1995) 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.

Tokura *et al.* (2001) studied on peroral and intravenous administration of ^{14}C -labeled 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, and it was found that control of degree of deacetylation was a very important factor in using CM-chitin as bone repairing and as bone material.

2.4 Carboxymethyl Chitosan (CM-chitosan) in Medical Applications

Chen *et al.* (2002) prepared different molecular weight CM-chitosans and investigated the effects on the growth and collagen secretion of normal skin fibroblasts and keloids fibroblasts. CM-chitosan promoted the proliferation of the normal skin fibroblast significantly but inhibited the proliferation of keloid fibroblast. For high concentration of CM-chitosan concentration, the effects in the growth promotion decreased earlier and faster than the lower concentration. And the lower CM-chitosan concentration had a longer affecting time to the normal skin fibroblast.

Krause *et al.* (2001) demonstrated that the ability of N,O-carboxymethylchitosan markedly reduced the formation of postsurgical pericardial adhesion in the rabbit model without untoward cardiac side effects. This hydrogel derivative may prove to be of great therapeutic value when used prophylactically in the setting of cardiac surgery.

Janvikul *et al.* (2003) prepared CM-chitosan hydrogels by steam. The steam induced crosslinking of CM-chitosan sodium salt involved the $-\text{NH}_2$ and $-\text{COONa}$ groups and formed amide linkages ($-\text{CONH}-$). The overall efficiency of the steam method for the crosslinking of CM-chitosan sodium salt was quite high. No weight

loss in the dry weight was observed when the samples was steam at 115°C or higher for 15 min or longer.

2.5 Synthesizing Carboxymethyl Chitosan (CM-chitosan)

Guan *et al.* (1998) synthesized a ternary blend of chitosan/N,O-dicarboxymethyl chitosan/viscose rayon. The phase behavior of the blends is influenced by the presence of N,O-dicarboxymethyl chitosan. Morphology observations of the blends disclosed that chitosan microparticles are distributed over the viscose rayon phase. The addition of N,O-carboxymethyl chitosan into the blend can improve the compatibility of chitosan with viscose rayon.

Zhang *et al.* (2000) reported that the blend membranes were satisfactorily prepared by coagulating a mixture of O-carboxymethylated chitosan and alginate in aqueous solution with 5 wt% CaCl₂ and then by treating with 1 wt% HCl aqueous solution. The results indicated that the blends were miscible, when the weight ratio of CM-chitosan to alginate was in the range from 1:1 to 1:5. The polymer interpenetration including a Ca²⁺ crosslinked bridge occurred in the blend membrane and led to high separation factor for pervaporation separation of alcohol/water and low permeation.

Thanou *et al.* (2001) synthesized trimethyl chitosan choride (TMC) and mono-carboxymethylated chitosan (MCC) to overcome chitosan's limited solubility and effectiveness as absorption enhancer at neutral pH values such as those found in the intestinal tract. TMC has been shown to considerably increase the permeation and absorption of neutral and cationic peptide analogs across intestinal epithelia. MCC is a polyampholytic polymer, able to form visco-elastic gels in aqueous environments or with anionic macromolecules at neutral pH values. MCC appeared to be less potent compared to TMC. Nevertheless, MCC was found to increase the permeation and absorption of low molecular weight heparin (LMWH; an anionic polysaccharide) across intestinal epithelia.

Chen *et al.* (2002) prepared carboxymethyl chitins and chitosan (CM-chitins, CM-chitosans) of different substitution sites under different reaction conditions and partially depolymerized carboxymethyl chitins of various molecular

weights from 24.8×10^4 to 0.26×10^4 . Moisture-absorption and retention abilities of these compounds were tested in comparison with those of hyaluronic acid (HA). The results revealed that 6-carboxymethyl groups in the molecular structures of CM-chitin and CM-chitosan were main active sites responsible for moisture retention. Although carboxymethylation at OH-3 and N position were not essential, they contributed to the moisture-absorption and retention abilities. Moisture retention ability was also related to molecular weight; higher molecular weight helped to improve moisture-retention ability.

Kittur *et al.* (2002) characterized chitin, chitosan and their O,N-carboxymethyl derivatives by differential scanning calorimetry (DSC) mainly focusing on changes in physical and chemical structure at different levels of acetyl and carboxymethyl contents. The thermograms were characterized by endo- and exotherms corresponding to water evaporation and decomposition of the polymer. Each endo- or exothermic peak temperature and area changed as a function of primary and higher order structures of the macromolecules. It was found that the enthalpy value for endotherms increased with increase in amino and carboxymethyl contents. In case of carboxymethyl derivatives, no glass transition was observed despite the presence of substantial amount of amorphous content. The decomposition peak temperature and area changed as a function of molecular weight (MW), acetyl and carboxymethyl contents. A theoretical basis was adopted to correlate the heat of the reaction, ΔH , to the degree of deacetylation (%DD) and carboxymethylation (DS). A good correlation was obtained when the corresponding peak area and peak height were plotted against %DD and DS.

Xie *et al.* (2002) synthesized carboxymethyl chitosan sodium (CMCTS) by using chitosan and chloroacetic acid and an alkaline catalyst. Acrylic acid sodium salt and methylacrylic acid sodium salt were grafted onto CMCTS to obtain copolymers with good water solubility. The graft reaction was carried out at 70°C for 2 h and ammonium persulfate was used as an initiator. The antibacterial activity of chitosan derivatives against *Staphylococcus aureus* and *Escherichia coli* were explored by the viable cell counting method.

Li *et al.* (2002) prepared chitosan/N,O-carboxymethylated chitosan/ viscose rayon antibacterial fibers (CNVFs) by blending chitosan emulsion, N,O-

carboxymethylated chitosan (NOCMC), and viscose rayon together for spinning. Although the addition of chitosan slightly reduced the mechanical properties, the antibacterial fibers properties were obtained and were found to meet commercial requirements. The antibacterial activity increased along with the chitosan concentration and was not greatly affected by 15 washing of water.

Li *et al.* (2002) prepared two types of O-carboxymethylated chitosan (O-CMCh)/cellulose polyblends by mixing cellulose LiCl/N,N-dimethylacetamide (DMAc) solution with O-CMCh aqueous solution (I) or DMC emulsion (II) and their corresponding films (I and II) and their corresponding were generated in water. Both blend films I and II exhibited satisfying antibacterial activity against *E.coli*, even the O-CMCh concentration was only 2 wt%. Due to the coagulation effect of water on the polyblend, O-CMCh water solution is suitable for the preparation of the blend film with low O-CMCh concentration, while O-CMCh /DMAc emulsion should be selected when high O-CMCh concentration is needed

Tang *et al.* (2001) studied the chelation mechanism of zinc ions onto O,N-carboxymethyl chitosan (ONCMCh). The chelation sites took place at the carboxyl group rather than at the -OH and NH₂ groups. The results were provided evidence to distinguish different chelating mechanisms between water-soluble and water-insoluble complexes. Water-insoluble chelates, which were formed through the Zn-O and Zn-N bonds, presented a tetrahedral structure. The water-soluble complexes where zinc ions connected with oxygen of C=O and water molecules were only due to electron attraction.

Sun *et al.* (2003) prepared carboxymethyl chitosan (CMCTS). The graft copolymerization of methacrylic acid (MAA) onto CMCTS using ammonium persulfate (APS) as an initiator was carried out in an aqueous solution. The effect of APS, MMA, reaction temperature and time on graft copolymerization were studied by determining the grafting parameters such as grafting efficiency.

Zhao *et al.* (2003) irradiated carboxymethylchitin (CM-chitin) and carboxymethylchitosan (CM-chitosan) in aqueous solutions at various irradiation dose by high-energy radiation electronic accelerator in vacuum. High concentrated paste-like conditions of CM-chitin derivatives were favorable for crosslinking structure. CM-chitosan that high degree of deacetylation negatively correlates to the

ability to crosslinking even if it has high degree of substitution. The hydrogels produced from CM-chitin and CM-chitosan exhibit excellent mechanical properties, good swelling in water and pH sensitivity in swelling behavior. The gel of CM-chitin with a high DS (0.91) swelled in acid ($\text{pH} < 3.5$) and alkaline ($\text{pH} > 6$) conditions and deswelled between pH 3.5 and 6.0 due to the ionic composition changes of the gel networks.