CHAPTER I
INTRODUCTION

Chitin or poly(β(1-4)-N-acetyl-D-glucosamine) is one of the most abundant polysaccharides found in nature. It can be found in skeletal materials of crustaceans, cuticles of insects, and cell walls of various fungi. Chitosan is prepared by chemical N-deacetylation of chitin. Both of them are observed to have biological functions. In biomedical applications, chitin and chitosan were both found to have an accelerating effect on wound healing process (Mori *et al.*, 1997; Lloyd *et al.*, 1998; Stone *et al.*, 2000; and Ueno *et al.*, 2001).

It is well known that chitin cannot be dissolved in common solvents. This obviously limits practical utilization of this material. Additionally, even though the N-deacetylated derivatives of chitin, i.e. chitosan, can be dissolved in aqueous solutions with a pH < 6.5, removal of acid salt prior to actual uses is required which could result in the change in the shape and size of the material. Therefore, chemical modification of both chitin and chitosan to render their solubility in common solvents is necessary so the materials can be utilized to their fullest.

Carboxymethyl derivatives of both chitin and chitosan are water-soluble and exhibit a low toxicity (Tokura *et al.*, 1996). Chen *et al.* (2002) reported that carboxymethyl chitosan (CM-chitosan) promoted the proliferation of normal skin fibroblast cells *in vitro*, while Ragnhild *et al.* (1997) showed that the biodegradability of these water-soluble polymers depended strongly on the degree of deacetylation (DD), the degree of substitution, and the substitution site. For wound dressing or scaffolding applications, these water-soluble polymers must be crosslinked to prolong their dimensional integrity during use.

Normally, chemical crosslinking is sought for, but possible presence of toxic residues, either from the unreacted chemicals or unwanted side reactions, that could be released during *in vivo* applications may present a restriction on such a chemical pathway. Alternatively, these derivatives can

be crosslinked by a physical means, e.g. thermal treatment in a dry oven or an autoclave (Janvikul *et al.*, 2003; and Muramatsu *et al.*, 2003). However, use of these conventional heating techniques results in long crosslinking time and low thermal homogeneity can be achieved.

In the past couple of years, the use of microwave energy in chemical synthesis has received considerable attention. Microwave heating, as an alternative to the conventional heating techniques, has been proved to be more rapid and efficient for several chemical reactions, e.g. sol-gel synthesis (Lee *et al.*, 2001), esterification of cellulose (Stange *et al.*, 2002), and N-phthaloylation of chitosan (Liu *et al.*, 2004). Use of the microwave energy as a source of heat to crosslink some biopolymers, e.g. collagen (Visser *et al.*, 1992) and gelatine (Vandelli *et al.*, 2004), was proven to be successful.

In wound healing applications, positive interaction and good response of fibroblast cells towards the materials of interest are of the prime concern. A number of researchers (Mori *et al.*, 1997; Chen *et al.*, 2002) adopted the promotion of fibroblast cell growth as a criterion for evaluating the wound healing ability of a polymeric material. In the actual would healing process, it is well accepted that fibroblast cells follow inflammatory cells into the site of the injured tissue and contribute to the wound healing process through the synthesis of structural proteins.

The objectives of the present contribution were to determine the effects of microwave heating temperature and time on the crosslinking of carboxymethyl chitin (CM-chitin) and CM-chitosan and to investigate the possibility for the use of such materials as wound care materials by investigating some biological response of model fibroblast cells towards the as-crosslinked films.

1.i Theoretical Background

Annually, several thousand people need skin grafts due to dermal wounds. Trauma to the skin can be caused by heat, chemical, electricity, ultraviolet or nuclear energy. According the National Institute of General Medical Sciences, 1.25 million burn-related injuries require medical attention annually in the US alone. Roughly 50,000 of these patients require hospitalization and 25,000 are admitted to special burn units. Infection leads to approximately 10,000 deaths (Seal et al., 2001). Trauma can cause several degrees of skin damage. First degree, the loss of the epidermal layer. Second degree, the loss of epidermal layer and a portion of the dermis. Third degree, the loss of tissue through the dermis, including the hair follicles and sweat glands, and extending into the hyperdermis (subcutaneous) layer. Finally, full-thickness wound is refered to as a fourth degree which is defined as extending downward through the subcutaneous tissues to involve tendon, bone, muscle, and other deep structures (Ratner et al., 1996).

1.1.1 Wound Dressing

A wound, whether it is the result of an incision during surgery, or an injury, or a burn, requires a dressing to protect it from infection and help it heal.

Factors affecting performance

A) Environment

Until recently, wounds were throught to heal quicker under drier condition. Now a moist environment is considered ideal. Assuming that exudate production has already caused, a dry dressing will lead to a scab, which may anchor some of the dressing fibers and cause pain and reopen the wound when the dresssing is removed. This scab also preventd the migrating epidermal cells fom reforming as fibrous layers of skin and, therefore, lengthens the healing time. An occlusive dresing, which keeps the wwound moist, does not allow a scab to form, thus the cells are free

to move through the exudate at the wound-dressing interface. A totally occlusive or semipermeable also preventd secordary damage due to dehydration. Loss of moisture can lead to the drying out of epidermal cells in the hair follicles ans sweat glands located in the skin's base layer and is a determiningfactor for the time of healing. This moisture loss produces formation of a hard eschar or scab (excess plasma combined with necrotic cells). Allowing exudate to pass through the dressing while keeping celluiar material at the wound surface increases the healing rate, provided that the fluid transfer rate of exudate through the dressing is approximately equal to the rate of exudate production (Edwards and Vigo, 2001).

B) Permeability and pH

The partial pressure of oxygen (pO_2) at the wound plays an important role. By reducing pO_2, an increase in fibroblast growth and production of angiogenic factors from tissues on macrophages occurs in vitro. On the other hand, with an increase in pO_2, the growth of epidermal cells increases. Accordingly, a pO_2 balance is needed to promote healing at an optimum rate. A healthy, non-infected wound needs a more hypoxic environment (low pO_2) that favors angiogenesis and formation of granulation tissue. Once the wound has stopped exuding, a more permeable dressing should be used to increase epithelial growth and discourage production of excess granulation tissue. The conclusion of most studies conducted on the effects of pH on healing is that a consistent, chemically mild, acidic environment at the wound will lead to an increase in healing rate (Edwards and Vigo, 2001).

C) Adherence

The problems caused by a wound contacting a dressing have been examined since 1913 and continue to be studied. The formation of a granulation caused by fibers entering the body is of great concern. Fibers from dressing, disposable paper gowns, gauze, and polyurethane foams can become part of the eschar or even the granulation tissue. Blood or exudate seeping into the dressing hardens and forms a scab. This causes the dressing to become stiffene and part of the scab, which can

disruot normal healing and lead to keloids, wound dehiscence, incisional hernias, intestinal obstructions, and bed sores. A foam can cause hypertrophy of the epidermis and inflammatory cell reaction of connective tissues which can result in infection. Removing the dressing can reopen the wound, disturbing the formation of new epithelium, and cause pain. The dressing, accordingly, should have little or no adherence to a wound and should not lint. For a non-adherent dressing to function, however, it should have small pores in contact with the tissue so that the exudate will pass through and not collect on the wound surface causing inflammation (Edwards and Vigo, 2001).

Functions of ideal dressing

The main purpose of a dressing is to protect the wound from further damage as well as to alleviate the pain, absorb exudate, and curb bleeding. An ideal dressing, in order to accelerate healing, would keep the wound at an optimum temperature and pH level, moist, free of infection and excessive slough, free of toxic chemical, particles, or fibers that could be released from a dressing, and undisturbed from dressing changes (Edwards and Vigo, 2001).

1.1.2 Skin Grafting

Second degree burns can generally be classified as either superficial or deep. In superficial injury, enough of the deep epidermal or superficial dermal layers may remain to allow spontaneous healing of the wound by reepithelialization. Other sources of epidermal cells are the epidermal appendages, including the hair follicles and sweat and sebaceous glands. Deep second degree burns have completely destroyed the epimermis and extent further into the dermis, with large amount of necrotic tissue being present. Both fluid and bacterial barriers are severely compromised, putting the patient at a much higher risk. These wounds, if allowed to heal on their own, result in hypertrophic scarring, with nonoptimal cosmetic results. These types of wounds should be treated as if they were third degree burns to allow for faster and better healing as well as to prevent infection (Ratner et al., 1996).

Clearly the best coverage for the wound is natural skin taken from the individual himself (an autograft) to avoid specific immunological incompatibility. If the burn injury is anywhere from 35 to 50% of the total surface area of the body, it is frequently possible to transplant partial thickness skin grafts from other noninjuried areas of the pateint. However, for burns that encompass more than 50% of the body's surface area, obtaining enough autograft becomes difficult because the pateints have a limited area available for donor sites. Using skin grafts from cadavers (allograft) or porcine (xenograft) provide some help, but as with any tissue transplanted between individuals, allograft invoke an immune response from the patient and cause eventual rejection of the grafted skin. Allograft at least allow temporary wound closure and time for re-epithialization of a donor site, but all allografts must eventually be replaced by autograft from the regemerating donor sites. For xenograft have been used after preservation but problems with storage as well as limited biocompatibility restrict their usefulness. It is important to keep in mind that all natural grafts other than autografts must eventually be replaced with the patient's own skin (Ratner et al., 1996).

Because of the limitation of using autograft, allograft, and xenograft and advance of tissue engineering. Several commerial products of engineered skin substitutes and experimental models for dermal and/or epimermal are being presented.

Table 1.1 commerial products and experimental models (Boyce, S.T., 2001)

Tradmark Name	Source	'Dermis'	'Epidermis'
EpiCel™	Genzyme biosurgery	Allodermis	cultured auto HK
Integra™	Integra life sciences	collagen-GAG & silicone	thin autograft
AlloDerm™	Lifecell corporation	acellular dermal matrix	thin autograft
DermaGraft™	Advanced tissue sciences	PGA/PLA+allo HF	thin autograft
n/a	Univ Cincinnati/Shriners	collagen-GAG+auto HF	cultured auto HK
LaserSkin™	Fidia biopolymers (Italy)	hyaluronic acid	cultured auto HK
PolyActive™	HC implants (Netherlands)	PEO/PBT+auto HF	cultured auto HK
ApliGraf™	Organogenesis, Inc.	collagen gel+allo HF	cultured allo HK
Comp Cult Skin™	Ortec international, Inc.	collagen + allo HF	cultured allo HK
TransCyte™	Advanced tissue sciences	Allo HF	BioBrane™

The list of products in this table is presented as neither all-inclusive, exclusive, or an endorsement. GAG, glycosaminoglycan; PGA, poly-glycolic acid; PLA, poly-lactic acid; PEO, polybethylene oxide; PBT, polybutylene terephthalate; HF, human fibroblasts; HK, human keratinocytes.

1.1.3 Tissue Engineering

Tissue engineering is a field that has truly emerged in the last decade. It has brought together diverse technologies, e.g. cell culture, polymer chemistry and transplantation. The creation of matrices to guide tissue regeneration allows manipulation at several levels, i.e. the cells employed, the choice of polymer and the design of construct assembly methods. In typical application, donnor tissue is harvested from the patient and dissociated into individual cells using enzymes. The cells are then seeded onto a porous scaffold, in vitro, in a cell culture medium. The disease or damaged tissue is removed and the cell/polymer scaffold constructoin is then implanted in the patient (Fig. 1.1). Over time, the synthetic matrix resorbs into the body and the cells produce their own natural extracellular matrix.

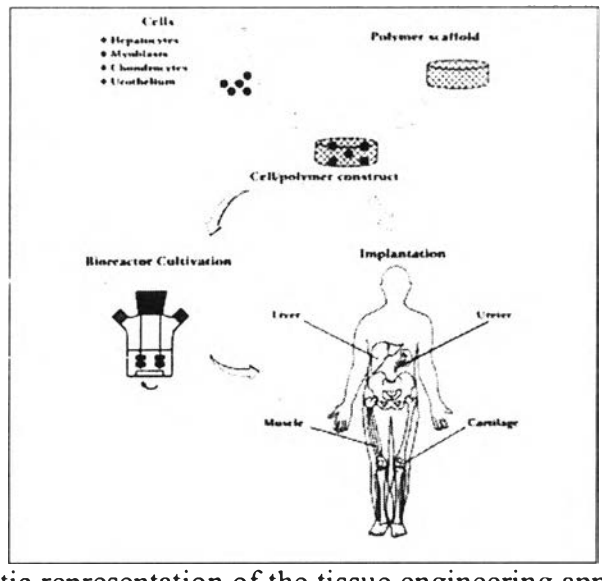

Figure 1.1 Schematic representation of the tissue engineering approach. Specific cell populations are harvested from the appropriate tissue and seeded on a biodegradable polymer scaffold. The cell /polymer constructs may undergo a period of dynamic tissue culture in a bioreactor prior to implantation. Organized structural and functional tissues may be produced in this way (Marler et al., 1998).

Cells used in tissue engineering may be drawn from a variety of sources, including primary tissues and cell lines. Primary tissues may be xenogeneic (from different species), allogeneic (from different members of the same species), syngeneic (from a genetically identically individual) or autologous from the same individual. Currently, the use of xenogeneic and allogeneic cells in the setting of 'open' cell /polymer constructs is limited by the need for host immunosuppression. However, with the advent of techniques to render cells immunologically transparent, the use of banked xeno-/ allogeneic cells may become a clinical reality (Marler et al., 1998).

Polymer scaffolds can be constructed from natural or synthetic biomaterials. While naturally occurring biomaterials may most closely simulate the native cellular milieu, their limitations include large batch-to-batch variations upon isolation from biological tissues, as well as restricted versatility in designing devices with specific biomechanical properties. Regardless of the sources, the scaffold material, as well as the three dimensional structure of the scaffold, have a significant effect on cellular activity. For a biologically active scaffold to promote cell adhesion and growth, it

must satisfy a number of requirements. It must be biocompatible and degrade in the body at a rate that allows the scaffold to remain insoluble just for the duration of the critical cellular processes; the products of degradation must also be biocompatible.

Construct assembly methods refer to techniques that (a) deliver cells to polymer scaffolds, (b) optimize their attachment following polymer processing and (c) initiate tissue formation where a period of in vitro tissue cultivation may yield an optimal construct (Marler et al., 1998).

The cellular structure of the scaffold must also be designed to satisfy several requirements. High porosity is needed for cell seeding and ingrowth (typical porosities are greater than 90%). Pore size must be a critical range (usually 100-200 μm): the lower bound is controlled by the size of the cell (~20 μm) while the upper bound is related to the specific area through the availability of binding sites. Porosity must be interconnected to allow ingrowth of cells, vascularization and diffusion of nutrients. And the material has to have sufficient mechanical integrity to resist handling during implantation and in vivo loading (Freyman et al., 2001).