CHAPTER I

INTRODUCTION

1.1 Background

As known that the natural healing of large dermal defects does not perfectly occur, scar formation would be inevitable unless some skin substitutes were used. In the past decades, many skin substitutes such as xenografts, allografts and autografts have been employed for wound healing. However, because of the antigenicity, the limitation of donors and donor sites, they cannot accomplish the complete recovery of the skin and yet not be widely used. Therefore, many studies are turning toward tissue engineering approach, which utilizes both engineering and life science discipline to promote tissue regeneration and to sustain and regain organ functions. One crucial factor in skin tissue engineering is the construction of a scaffold. A three-dimensional scaffold provides an extracellular matrix (ECM) analog which functions as a necessary template for host infiltration and a physical support to guide the proliferation and differentiation of cells into the targeted functional tissues or organs [1].

Porous three-dimensional scaffolds have been used extensively as biomaterials in the field of tissue engineering for *in vitro* study of cell–scaffold interactions and tissue synthesis and *in vivo* study of induced tissue and organ regeneration. Regardless of the application, the scaffold material, as well as the three-dimensional structure of the scaffold, have a significant effect on cellular activity. They act as a physical support structure and as an insoluble regulator of biological activity that affects cell response such as migration, contraction, and division. Collagen is a significant constituent of the natural extracellular matrix. Scaffolds made of collagen have been used in a variety of applications due to a number of useful properties; such as hemostatic effect, low antigenicity, and appropriate mechanical characteristics for

use in soft tissue engineering applications [2]. In addition, collagen scaffolds have been observed to promote cell and tissue attachment and growth [2, 3]. Collagen contains basic residues, such as lysine and arginine, and specific cell adhesion sites such as arginine-glycine-aspartate (RGD) groups. The RGD group actively induces cellular adhesion by binding to integrin receptors, and this interaction plays an important role in cell growth, and in the differentiation and overall regulation of cell functions [4]. For a biologically active scaffold to promote cell adhesion and growth, it must satisfy a number of constraints. It must be biocompatible and can be degraded in human body at a compatible rate to cellular processes. The products of degradation must also be nontoxic. The chemical composition must incorporate ligands appropriate for the binding of cells specific to each application. The average pore diameter must be large enough for cells to migrate through the pores and small enough to retain a critical total surface area for appropriate cell binding. To allow cell transportation and metabolites the scaffold must have a high specific surface and large pore volume fraction (generally greater than 90%) as well as an interconnected pore network. Scaffold pore size, pore shape, and pore volume fraction are especially critical as they define the total surface area and special distribution of ligands presented to cells [5]. Scaffolds manufactured from a copolymer of collagen and glycosaminoglycan (GAG) possess a number of useful qualities for uses as tissue engineering constructs: they can be sterilized by both dry heat and chemical treatments, they have degradation rates that can be adjusted within a wide range, and they can be fabricated from a number of macromolecular constituents with a variety of pore structures [2, 3].

Collagen is known to be the most promising material in tissue engineering applications for their excellent biocompatibility and biodegradability. However, its fast biodegradation restricts further usage of this material [1]. For this reason, the blending of biodegradable polymers has been employed to produce desirable collagen-based scaffolds. Chitosan, an amino polysaccharide (poly-1,4-D-glucosamine) derived from chitin by deacetylation, has been widely applied in biomedical applications, such as wound dressings and drug delivery systems on account of its nontoxic and biocompatible nature [6, 7]. Since chitosan composes of

both reactive amino and hydroxyl groups that can be chemically modified and, physically, is relatively easy to manipulate for different pore structures. It has a high potential in tissue engineering applications. One of the most interesting effects of chitosan on wound healing is the formation of granulation tissue with angiogenesis. It is reported that chitosan induces fibroblasts to release interleukin, which is involved in migration and proliferation of fibroblasts [5]. In experimental animal models, chitosan was shown to influence all stages of wound repair. In inflammatory phase, chitosan has unique haemostatic properties that are independent of the normal clotting cascades [8]. In addition, chitosan can be served as GAG analog in stimulating cellular processes of dermal tissue regeneration [9]. Therefore, chitosan, a novel property biomaterial, was introduced for fabricating collagen/chitosan scaffolds. However, these previous studies provided at least in part to the results from using chitosan in wound healing applications. It is still not clear about the relationship between the molecular weight of chitosan and its effect on fibroblast growth and physical properties of collagen/chitosan scaffolds. This is the first report focused on the physical and biological properties of hybrid scaffolds fabricated from porcine skin type I collagen and chitosan with various molecular weight. The morphology as well as biodegradability of the hybrid scaffolds was investigated.

1.2 Objectives

To study the effects of molecular weight of chitosan and blending compositions on physical and biological properties of collagen/chitosan scaffolds for fibroblast cells.

1.3 Scope of work

1. Develop collagen/chitosan scaffolds by using type I collagen and chitosan having different molecular weight.
2. Vary the blending composition ratio of collagen to chitosan: 100/0, 90/10; 70/30; 50/50; 30/70; 10/90; and 0/100.

3. Characterize the chemical and physical properties of collagen/chitosan polymeric scaffolds including:
   3.1 Fourier transform infrared (FT-IR) spectroscopy.
   3.2 Differential scanning calorimeter (DSC)
   3.3 Compressive modulus.
   3.4 Swelling ratios.
   3.5 Morphology.
4. Characterize the biological properties including:
   4.1 Biodegradation.
   4.2 Cell adhesion.
   4.3 Cell proliferation.