CHAPTER I

INTRODUCTION

1.1 Statement of Problem

The advent of tissue engineering has been motivated by the challenge of producing tissue substitutes that can restore the structural features and physiological functions of natural tissues *in vivo*. A number of strategies can be engineered tissues and organs, but the most appealing strategy is the approach to combine a patient's own cells with polymer scaffolds. The tissue-specific cells are seeded into the scaffold with nutrients. Then, the cells proliferate, migrate and differentiate into the specific tissue while secreting the extra cellular matrix components (ECM) required to create the tissue. Porous scaffolds are essential and widely used to provide a three-dimensional structure.

In order to achieve cell growing in the scaffold, the porosity of a scaffold must be large and pores must be interconnected for cells and nutrients migration into the scaffold. Meanwhile, the pore size must be proper to the size of each cell type for appropriate cell binding [1]. Therefore, designing and controlling pore size and morphology are important. Moreover, scaffold should be made from biodegradable and biocompatible materials avoiding toxic agent occurring its degradation. A number of synthetic (poly (glycolic acid) (PGA) [2], poly (L-lactic acid) (PLLA) [3, 4], and poly (DL-lactic-co-glycolic acid) (PLGA) [5,6]) and natural (collagen [7,8], gelatin [9,10], and chitosan [11,12]) degradable polymers are currently being employed as tissue scaffolds. According to the lack of cell adhesion which is the important factor for success implantation of synthetic polymer scaffold, natural polymer has been attractive candidates to numerous researchers to use it in tissue engineering as possible.

Chitosan is a copolymer of glucosamine and *N*-acetylglucosamine. It is a derivative of chitin, a biopolymer extracted from an exoskeleton of crustacean, cuticle of insects, and cell wall of fungi. Interestingly, many advantage properties make it suitable for biomedical application such as biodegradable, biocompatible, non-toxicity, cell-stimulating [13], blood anticoagulability, wound healing [14], and hemostatic potential. Furthermore, *N*-acetylglucosamine is a compound found in ECM, affecting cellular movement shape, proliferation and differentiation. Due to this property, chitosan, presently, has been increasingly studied in tissue engineering application of cartilage [15,16], bone [17], liver [18] and nerve [19]. Several scaffolds fabrication methods have been employed to achieve ideally scaffolds, but none of them can be used for all tissues. Freeze-drying technique is a gentle dry method since it can produce an uniform interconnected pore using water as porogen. Therefore, this technique has been used widely to prepare natural polymer scaffolds.

A critical factor for scaffold fabrication design is shape retention. Principally, pore size is one important factor for cell implantation. Pore size of scaffold, before and after usage, should not be changing in their sizes. A proper pore size to each cell size gives a greater number of cells [20]. However, biopolymer generally swells a great deal in fluid leading to an increase of the pore size. Furthermore, the scaffold needs to be maintaining their stability for surgical handling during implantation. Crosslinking the polymer matrix is one method that has been used to improve these properties. Both physical and chemical treatment to create crosslinking were introduced, *e.g.* dehydrothermal treatments, ultraviolet radiation [21], ionic crosslinking [22], and chemical treatment [23,24].

Glutaraldehyde is mostly used because it is inexpensive and water-soluble. However, due to its cytotoxicity showing cell-growth inhibition even at low concentration [25] and a potential for calcification in applications, many researchers attempt to use other reagents instead. Among various crosslinking reagents, azide group is an attractive crosslinker since it gives highly reactive nitrene groups after UV exposure which many reactions can be occurred and led to crosslinking.

1.2 Objectives

1. To investigate the morphology and properties of non-photo-crosslinked and photo-crosslinked chitosan scaffolds fabricated by lyophilization (freeze-drying) process.

2. To study the factors affected the pore morphology, and properties of non-photo-crosslinked and photo-crosslinked chitosan scaffolds.

1.3 Scope of the Investigation

In this study, porous chitosan scaffolds were fabricated using freeze-drying technique. In the first part, the type of chitosan, concentration of chitosan solution, and freezing temperature on pore morphology and size were studied. In the second part, a UV irradiation was applied to create crosslinking in the scaffold by using an azide as a crosslinker. The morphology of pores, shape retention in aqueous media and mechanical properties of the photo-crosslinked chitosan and original chitosan scaffolds were compared. Finally, the cytotoxicity of this photo-crosslinked chitosan scaffolds were tested.