CHAPTER I

INTRODUCTION

1.1 Background

Three-dimensional scaffolds are required in tissue engineering to support for the formation of tissue as well as to promote cell migration, adherence, and formation of new extracellular matrix and to foster the transport of nutrients and metabolic wastes. Porous structure with interconnected pores and appropriated pore size is usually requested for scaffolds. Sufficient mechanical properties of the scaffolds are also necessary to support tissue function and integration. Scaffolds also have to biodegrade at a rate comparable with new tissue growth [1]. As a result, types of biodegradable materials suitable to fabricate such scaffolds are widely explored. Typical material used is collagen.

As silk fibroin has been used commercially as biomedical sutures for decades, recently it has been explored for many biomedical applications, because of its impressive biological compatibility and mechanical properties. For example, silk fibroin from *Bombyx mori* silkworm is reported to support matrixes and ligament using osteoblast, hepatocyte and fibroblast cell for tissue engineering [2-5]. Therefore, the preparation of silk fibroin porous scaffolds, having high porosity and interconnected pores, has become one of the major challenges in tissue engineering.

In Thailand, Thai native silkworms have been long cultivated and Thai silk is well known in textile industry for many decades. Distinct characteristics of cocoon Thai silk are its yellow color and coarse filament. There is more sericin or silk gum in Thai silk (e.g. up to 38%) than in normal *Bombyx mori* silk (e.g. 20-25%) [6]. These characteristics provide Thai silk a unique texture for textile industry. However, Thai silk fibers have not much been reported for biomedical uses, especially in tissue engineering application. It is therefore of our interest to explore the uses of Thai silk fibroin as three-dimensional scaffolds for tissue engineering applications.

In addition, to enhance the biological properties of Thai silk fibroin-based scaffolds, the concept of blending with gelatin is employed. This due to the fact that gelatin is a derivative of collagen which is a major constituent of skin, bones and connective tissue. Gelatin contains arginine-glycine-aspartic acid (RGD)-like sequence that promotes cell adhesion and migration [7]. Particularly, it does not exhibit antigenicity and it is widely applied in many industries such as pharmaceutical, cosmetics, food and tissue engineering. Recently, crosslinked gelatin-based scaffolds have been reported as a potential wound substitute [8].

Moreover, as known that natural bone comprises about 70wt% of a mineral phase, primarily hydroxyapatite, and 30wt% of an organic matrix [9]. Hydroxyapatite was employed to strengthen scaffolds used for bone tissue engineering [10-12].

The purpose of this study is to develop silk fibroin and gelatin/silk fibroin scaffolds using silk fibroin extracted from cocoon of Thai silkworm. The influence of the type of gelatin (i.e. type A and type B) and hydroxyapatite on scaffold fabrication are studied. Chemical, physical, and biological properties of scaffolds will be investigated in order to evaluate the applicability of Thai silk fibroin and gelatin/Thai silk fibroin scaffolds as a biomaterial for tissue engineering.

1.2 Objectives

1.2.1 To develop three-dimensional Thai silk fibroin and gelatin/Thai silk fibroin scaffolds for tissue engineering applications.

1.2.2 To study chemical, physical, and biological properties of Thai silk fibroin and gelatin/Thai silk fibroin scaffolds.

1.3 Scopes of Research

1.3.1 Prepare silk fibroin solution from cocoons of Thai silkworm.

1.3.2 Prepare Thai silk fibroin and gelatin/Thai silk fibroin scaffolds via freeze-drying.

1.3.3 Prepare Thai silk fibroin, conjugated gelatin/Thai silk fibroin, and hydroxyapatite-conjugated gelatin/Thai silk fibroin scaffolds via salt-leaching.

1.3.4 Characterize the chemical properties of Thai silk fibroin scaffolds including:

1.3.4.1 Attenuated total reflection fourier transforms infrared (ATR-FTIR) spectroscopy

1.3.4.2 X-ray diffraction (XRD)

1.3.5 Characterize the physical properties of Thai silk fibroin, gelatin/Thai silk fibroin and hydroxyapatite-conjugated gelatin/Thai silk fibroin scaffolds including:

1.3.5.1 Morphology (porous structure analysis)

1.3.5.2 Compression modulus

1.3.5.3 Water swelling

1.3.6 Characterize the biological properties of Thai silk fibroin and gelatin/ Thai silk fibroin scaffolds including *in vitro* biocompatibility of bone-marrow derived mesenchymal stem cells (MSCs) and mouse osteoblasts-like cells (MC3T3-E1).