CHAPTER I

INTRODUCTION

1.1 Background

An important purpose of tissue engineering is to regenerate new tissues using biomaterials as scaffolds or templates. The biomaterials used as scaffolds for *in vivo* tissue engineering in the form of gels, sponges, and woven meshes are required to disappear by resorption into the body after accomplishment of tissue regeneration. Different tissues required biodegradable scaffolds with different physical and chemical characteristics. Collagen and silk fibroin are examples of natural biodegradable materials that have been used as tissue engineered scaffolds due to their biocompatible and biodegradable characteristics.

Silk fibroin is a major constituent of raw silk fiber, which has been widely explored for many biomedical applications, because of its impressive biocompatibility and biodegradability, minimal inflammatory reactions, and favorable mechanical properties. For example, silk fibroin from *Bombyx mori* silkworms is reported to support matrixes and ligament using osteoblast, hepatocyte and fibroblast cell for tissue engineering [1]. Thai silk is one of *Bombyx mori* silkworms. Characteristics of cocoon Thai silk are its yellow color and coarse filament. In the recent years, there are a few reports of Thai silk scaffolds for tissue engineering such as preparation of electrospun silk fibroin fiber mats as bone scaffolds [2] and preparation of freeze-dried and salt-leached Thai silk fibroin scaffolds [3]. To enhance the biological properties of Thai silk fibroin-based scaffolds, Chamchongkaset *et.al.* [3] has introduced the concept of blending Thai silk fibroin with gelatin. This was 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. Particularly, it does not exhibit antigenicity, and inherent biocompatibility and biodegradability. Unfortunately, upon hydration in any aqueous solution, gelatin immediately shrinks and disintegrates. Thus, crosslinking is necessity in order to maintain the structural integrity of the scaffolds. However, typical protocols for crosslinking have used glutaraldehyde and

formaldehyde, which might be cytotoxic. An effective method to circumvent this problem is to introduce a safe crosslinker, that do not release any toxic byproducts, such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, hydrochloride (EDC) [4].

Recently, Gil, E.S. *et.al.* [5] reported the production of some stable silk fibroin/gelatin hydrogels with excellent mechanical properties and thermal stability by blending type A gelatin with amorphous *Bombyx mori* silk fibroin. The formation of β-sheet crystals in silk fibroin was subsequently induced upon exposure to an aqueous methanol solution. Lv, Q. *et.al.* [6] used EDC to crosslink collagen gel (type I from bovine skin) with silk fibroin solution to form a stable silk fibroin/collagen hydrogels at body temperature without methanol treatment. In this work, the direct crosslinking by adding EDC in Thai silk fibroin/gelatin solution to stabilize gelatin in the desired scaffolds is of our interest, as well as the study on *in vitro* cell culture of these scaffolds.

It is therefore the aim of this research to focus on the chemical crosslinking of Thai silk fibroin/gelatin solution by using EDC as a crosslinker. Silk fibroin extracted from cocoon of Nangnoi Srisaket 1 Thai silkworm, and type A gelatin will be used in this study. The effect of the weight blending ratios of Thai silk fibroin/gelatin will be investigated. Furthermore, the addition of hydroxyapatite particle, which is reported to enhance mechanical properties, mineralization, and bone formation, was studied. The morphology, weight loss (%), compressive modulus, *in vitro* biodegradability, in *vitro* biocompatibility using bone marrow-derived stem cells (MSCs) will be investigated in order to evaluate the applicability of Thai silk fibroin/gelatin scaffolds for tissue engineering.

1.2 Objectives

To investigate the effects of chemical crosslinking and hydroxyapatite on the properties of Thai silk fibroin/gelatin scaffolds.

1.3 Scopes of Research

1.3.1 Prepare non-crosslinked and crosslinked Thai silk fibroin/gelatin scaffolds using 1-Ethyl-3-(3 dimethylaminopropyl)carbodiimide, hydrochloride (EDC).
Total solid weight used is 4wt%.
Parameter to be investigated is:
- The weight blending ratios of Thai silk fibroin/gelatin, (SF/G): 0/100, 20/80, 40/60, 50/50, 60/40, 80/20, and 100/0

1.3.2 Prepare homogenized Thai silk fibroin/gelatin scaffolds with and without hydroxyapatite incorporation.
The weight blending ratio of organic (Thai silk fibroin and gelatin) to inorganic (hydroxyapatite) parts used is 30/70.

1.3.3 Characterize non-crosslinked and crosslinked Thai silk fibroin/gelatin scaffolds including:
1.3.3.1 Morphology by scanning electron microscope (SEM)
1.3.3.2 Weight loss (%)
1.3.3.3 Compressive modulus (dry and wet condition)
1.3.3.4 *In vitro* biodegradability using 1 U/ml collagenase at 37°C, pH 7.4.
The characteristics of degraded scaffolds were examined as follows:
(Incubation periods: 15 min, 30 min, 1 h, 6 h, 12 h, 1, 3, 5, and 7 days)
- Remaining weight (%)
- Conformational structure by X-Ray Diffraction (XRD)

1.3.3.5 *In vitro* biocompatibility using bone marrow-derived stem cells (MSCs)
- MSCs initial attachment and proliferation tests by DNA assay
- MSCs morphological observation

1.3.4 Characterize homogenized Thai silk fibroin/gelatin scaffolds with and without hydroxyapatite incorporation including:
1.3.4.1 Morphology by scanning electron microscope (SEM)
1.3.4.2 *In vitro* biocompatibility using bone marrow-derived stem cells (MSCs)
- MSCs initial attachment and proliferation tests by DNA assay
- Osteogenic differentiation test by ALP activity and calcium content
- MSCs morphological observation