CHAPTER I
INTRODUCTION

Scaffold for tissue engineering is the three-dimensional support for cell attachment and tissue organization. Scaffold requires both biodegradable and biocompatible properties in order to promote cell growth, allow cell to attach the surface, and promote cell differentiation and proliferation. The periodontal disease is the one of oral disease which the tissue engineering technique is required for severe patient in order to graft and regenerate the new bone or gingival tissues to replace the losing one. Nowadays, researchers attempt to synthesize scaffold from natural protein based materials such as gelatin, collagen, chitosan and silk because they are closely simulate the native cellular surroundings and accepted by many cell types. Likely, attractive feature of silk protein derived from silk worm has an interesting efficiency to be use in cell culture application.

Natural silk is composed of two major proteins named fibroin and sericin. Fibroin is the core protein hold together by the glue like protein called "sericin". In silk manufacturing, silk sericin must be removed from the raw silk by "degumming process" and discarded in processing wasted water lead to water contamination. It is estimated that 400,000 tons of dry cocoons in the worldwide production, around 50,000 tons of silk sericin could be recovered (Zhang, 2002). If silk sericin in silk waste water could recover, it could be substantial for economy and environmental benefits. Silk sericin is an invaluable by product but to be useful in many applications range from the cosmetics to biomedical application including anticancer drugs, anticoagulants, wound dressing, and cell culture additives. Many researchers suggested silk sericin has the potential to enhance cell growth such as Takeuchi *et al.,* 2005 used silk sericin coated on the porous structure of alpha-tricalcium phosphate to enhance bone regeneration and bone density in rabbit tibia.

Over the past decade, the layered silicate which also known as clay such as sodium montmorillonite, sodium bentonite and laponite are extensively used to develop polymer/clay nanocomposites due to their properties such as high surface area, high aspect ratio and relatively low cost. Interestingly, layered silicate clay pass through the environmental friendly process called freeze-drying; it can be rearranged from granular particles to the lamella structure or the "house of cards" structure, this material is widely known as "clay aerogel". Clay aerogel is low-density and highly porous material, which similar to the typical polymeric foams. When consider deeply into the structure of clay aerogel, the porous structure makes it suitable for a variety of biomedical application such as drug delivery system and three-dimensional scaffold. Many researchers interested in combined clay aerogel into biotechnological application including three-dimensional scaffold for tissue engineering. In 2009, Haroun *et al.* developed the three-dimensional gelatin–montmorillonite (MMT)/cellulose scaffold by using freeze-drying process. They proposed that using MMT aerogel can improve cytocompatibility between cell and biocomposite. Because of the lamella structure of clay aerogel occurred from the interaction between opposite electrostatic charge of face and edge of clay platelets, which is not strong enough to maintain the construction under high stress level. Thus, the combination of others materials like polymer is required in order to facilitate the mechanical properties.

Since the properties of both silk sericin and clay aerogel are appropriated for medical used. It has the possibility to cooperate silk sericin and clay aerogel for medical application including scaffold for tissue engineering because porous structure of clay aerogel is convenient for the regeneration of cell and silk sericin has the potential to enhance cell growth. A few researchers have been developed the biopolymer or natural polymer/clay aerogel but the silk sericin/clay aerogel has not been studied so far.

In this study, the silk sericin/clay aerogels were prepared by freeze-drying process. Silk sericin was extracted from different species of Thai silk cocoons; Nang Noi, Nang Lai, Dok Bua and Luang Pairote and freeze-dried to obtain the silk sericin powder. In each species, the silk sericin would be studied on the solid contents, amino acid compositions and thermal properties. After that, the silk sericin/clay aerogels were prepared by varied of silk sericin from Nang Noi species, clay and also the crosslinking agent contents in order to study the influences of content on the properties of the aerogels. Because of the fragility of neat silk sericin/clay aerogel hence poly(vinyl alcohol) was employed in order to improve the mechanical properties of silk sericin/clay aerogel. The aerogels were studied on morphology, mechanical and thermal properties. Moreover, silk sericin/clay aerogels would be studied on the possibility to use as the 3D scaffold for tissue engineering using *in vitro* direct contact test and MTT assay.

OBJECTIVES

1. To prepare the silk sericin/clay aerogels by freeze-drying process.
2. To determine the silk sericin contents in different species of Thai silk cocoons; Nang Noi, Nang Lai, Dok Bua, and Luang Pirote.
3. To study the effects of silk sericin, clay, and cross-linked agent contents on the morphology and properties of aerogels.
4. To investigate the morphology, mechanical and thermal properties of silk sericin/clay aerogels.
5. To study the possibility to use silk sericin/clay aerogels as the scaffold for tissue engineering.

SCOPE OF RESEARCH WORK

The scopes of this research were cover following:

1. Extraction of silk sericin from the different species of Thai silk cocoons; Nang Noi, Nang Lai, Dok Bua and Luang Pirote and investigation of the silk sericin contents in each species.
2. Preparation of the silk sericin/clay aerogels via freeze-drying by varied the silk sericin content as 1, 2, 3 and 4 wt%, the clay content as 2, 4, 6 and 8 wt% and the amount of cross-linked agent in 3, 5 and 7 μl/ml.
3. Study of morphology and identify structure of the silk sericin/clay aerogels by using FE-SEM and XRD, respectively.
4. Investigation of mechanical properties of silk sericin/clay aerogels such as compressive modulus by using the universal testing machine.
5. Investigation of thermal properties of silk sericin/clay aerogels by using TG-DTA.
6. Study of the possibility to use silk sericin/clay aerogels as the scaffold for tissue engineering based on *in vitro* direct contact test, MTT assay and swelling behavior.