

## A Preliminary Study of the *In Vitro* Biocompatibility Testing of Silk Fibroin/Alpha Tricalcium Phosphate Composite Scaffolds for Bone Tissue Engineering

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**Abstract.** Silk fibroin is a natural biodegradable polymer that has been demonstrated for use as scaffolds for bone tissue engineering. To improve the osteoconductivity and the osteoinductivity of silk fibroin scaffolds, ceramics were added.  $\alpha$ -tricalcium phosphate ( $\alpha$ -TCP) is the expected ceramic that useful for scaffolds for bone tissue engineering either alone or blended with silk fibroin. From the previous study, we evaluated the mechanical properties of three-dimensional porous silk fibroin/ $\alpha$ -TCP scaffolds and concluded that the scaffolds containing 8% (w/w)  $\alpha$ -TCP exhibited the highest compressive modulus. The objective of this study was to evaluate the biological properties of three-dimensional porous silk fibroin/ $\alpha$ -TCP scaffolds. The scaffolds were constructed using a solvent casting and salt leaching technique. The hybrid strain of degummed Thai silk fibroin, Nangnoi Srisaket 1 x Mor, was dissolved in hexafluoroisopropanol at 16% (w/v).  $\alpha$ -TCP was incorporated to produce 4, 8, 12, and 16 wt% solution. Sucrose (particle size 250-450  $\mu$ m; sucrose/silk fibroin = 8.5/1 w/w) was used as a porogen. Human gingival fibroblasts (passage 5) were cultured in these scaffolds. After 72 h, the biocompatibility of seeded scaffolds was evaluated under the inverted phase contrast microscopy. Cell proliferation was determined by DNA assays and scanning electron microscopy. The images from inverted phase contrast microscopy revealed the human gingival fibroblasts can be attached at the surface of scaffolds in all groups. The results from the DNA assays showed that the number of human gingival fibroblasts was increased as the culture period was prolonged but was not as the increasing of  $\alpha$ -TCP. At 120 h, the scaffolds containing 8% (w/w)  $\alpha$ -TCP exhibited the highest cell number. The scanning electron microscope images at 24, 72, and 120 h after cell culturing presented human gingival fibroblasts can be expanded well and exhibited the normal morphology. The results suggested that the scaffolds containing 8% (w/w)  $\alpha$ -TCP may be a potential candidate for bone tissue engineering applications.

### Introduction

Silk fibroin (SF) is a natural fiber that derived from a cocoon of *Bombyx mori* silkworm. It has been used in textile industry for thousands of year. In 1993, US Food and Drug Administration (FDA) has recognized SF as a biomaterial and is widely use as suture material [1]. Moreover, SF is one of considerable material that use in biomedical application because of many studies proved the biocompatibility of the SF and can be used in tissue engineering [2] [3]. Bone tissue engineering is a highly challenge field and a promising strategy to improve the current treatment for bone injury. So that, the scaffolds should have the osteoconductive and osteoinductive properties. These properties can be accomplished by incorporate the minerals into a scaffolds such as hydroxyapatite, calcium phosphate, bioactive glass, and  $\alpha$ -tricalcium phosphate ( $\alpha$ -TCP).

$\alpha$ -TCP is biocompatible and biodegradable bioceramics that hydrolyses rapidly to calcium-deficient hydroxyapatite, which makes  $\alpha$ -TCP use as a raw material for injectable hydraulic bone