Chapter 5

Conclusion

This study creates a new knowledge in developing a natural product available in Prince of Songkla University to construct scaffolds being applicable in bone tissue engineering. Natural polymers of chitosan and collagen were blended and underwent simple freeze-drying process resulting in 3-D porous scaffolds. Biocompatibility of the scaffolds was tested in the cell culture system. To elucidate optimal ratios of collagen in chitosan-collagen sponges in supporting growth and differentiation of osteoblasts, growth and differentiation of osteoblasts on the sponges with different ratios of collagen in chitosan-collagen sponges and the microstructrue of the sponges were investigated.

The 3-D cell culture of the osteoblast cell line is an effective tool in studying biocompatibility of the scaffolds and cellular response to biomaterials in tissue engineering. To study the growth of cells into an internal porous structure of scaffolds, the cell seeding technique must be optimized to homogenously distribute seeded cells into an inner structure of the scaffolds. When the size of the scaffolds, as in this study of a 3x5 mm, is not too small to achieve sufficient infiltration of nutrient and excretion of waste products, a conventional cell culture can be applied in the 3-D cell culture study model.

In this study, it was found that an incorporation of chitosan into collagen suspension, creating a chitosan-collagen composite suspension possibly with a molecular structure differing from chitosan or collagen suspension, as it could be observed that porous structure and osteoconductive properties of chitosan-collagen composite scaffolds were enhanced.

The scaffolds can be fabricated by using a simple freeze-drying technique. A constant cooling rate and drying temperature applied in this study ensured a homogenous porous structure of the scaffolds. A controlling of a formation of ice crystals in size and numbers is an another critical factor attributing to homogenous porous structure of the scaffolds.

Collagen in the composite helped to stabilized the internal porous structure of chitosan-collagen sponges compared to chitosan and collagen sponges. It was clearly demonstrated that collagen did not only improve the physical structure of scaffolds and

promote growth and differentiation of osteoblasts, but collagen also had a greater effect than chitosan in supporting the mineralization of ECM *in vitro*.

This study demonstrated that chitosan-collagen scaffolds are potential scaffolds in bone tissue engineering. Collagen in a chitosan-collagen composite improved the porous structure and osteoconductive property of chitosan scaffolds. The porous structures of chitosan-collagen scaffolds support growth and differentiation of osteoblasts.

Chitosan-collagen 1:2 sponges show promising results demonstrating a porous structure and a high level of osteoconductivity. In order to develop this scaffold to be applicable in human skeletal defects, physical and mechanical properties such as internal porous structure, strength, degradation rate and swelling pattern. It is essential that biocompatibility and tissue reaction of the scaffolds in the immunocompetent host must be elucidated.