CHAPTER II
LITERATURE REVIEW

2.1 Bone Therapy: The Current Situation

Bone composes of collagen and hydroxyapatite (HA). Two of the specific bone cells are osteoclasts and osteoblasts. Osteoclasts are involved in the destruction of bone and osteoblasts are involved in the rebuilding of bone. While osteoclasts secrete acids to dissolve HA and enzymes to break down the collagen, osteoblasts construct new bone, both collagen and hydroxyapatite (Ovaginian, 2001).

At present, the medical treatments for reconstructing bone are autograft, allograft and transplanting artificial materials. The procedure of autograft is the harvesting of a bone from a healthy part of the patient's body and transplanting it into the damaged part of the same patient. The procedure of allograft is similar to autograft except that the bone comes from a cadaver. However, these two methods show the limitation in terms of expensive cost, and possibilities in infections including painful operations. For transplanting artificial materials, traditionally, these materials are made from metals, plastics, and ceramics that have different mechanical properties from bone and are subject to fatigue, fracture, toxicity and lack of biocompatibility (Scheme I).

Scheme I

Methods of Bone Therapy

Autograft

Disadvantages

- Surgical cost
- Infection
- Pain

Allograft

Disadvantages

- Surgical cost
- Infection
- Pain

Transplanting Artificial Materials

- Metals
- Plastics
- Ceramics

Disadvantages

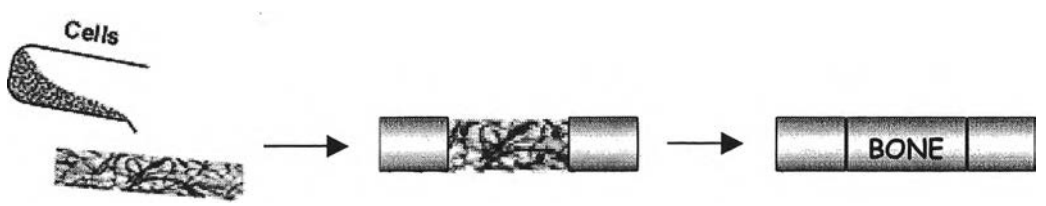
- Fatigue
- Fracture
- Toxicity
- Lack of biocompatibility

An alternative way to develop artificial material is to use a natural polymer with the properties of cell-compatibility, non-toxicity, biodegradability as a carrier for the cell bone growth. The biomaterial would serve to fill the void among the damaged bone while the surrounding bone grows into the gap. Since the material is biodegradable, the material degrades as the new bone generated (Laurencin *et al.*, 2000).

2.2 Tissue Engineering of Bone

Tissue engineering has been defined by Laurencin as the application of biological, chemical, and engineering principles toward the repair, restoration, or regeneration of living tissues by using biomaterials and cells. In recent years, tissue engineering has emerged as a potentially effective approach to the repair and replacement of damaged or diseased bone. A common and practical method is to use biodegradable and scaffold porous materials. The important point of biodegradable materials is the non-toxicity and biodegradability during the bone tissue growth. In addition, the porous structures increase the surface area allowing a great number of osteoblasts to attach on the matrix. When using osteoconductive polymers such as chitin-chitosan, poly(lactic acid) or poly(glycolic acid), the porous structure of the polymers provides a favorable surface for cell adhesion and growth. This enhances the regeneration of the implant by allowing the growth of bone tissue directly on the inner surface of the matrix (Scheme II).

Scheme II

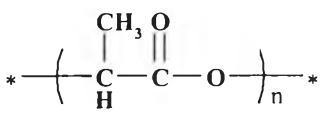


Porous biomaterial Implant Healthy bone

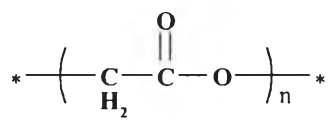
2.3 Biomaterials

There are numerous research projects focusing on novel biomaterials for bone fixation. These projects include synthetic polymers, ceramics, and composites. For synthetic polymers, poly(lactic acid) and poly(glycolic acid) (Scheme III) which are biodegradable polymers have tremendous potential in biomedical application, such as bone fracture fixation (Leeslag *et al.*, 1987), drug delivery system (Schakenraad *et al.*, 1998), surgical suture (Postema *et al.*, 1989) or prosthetic devices (Gilding *et al.*, 1978). However, these polymers exhibit some undesirable properties for bone regeneration. Both poly(lactic acid) and poly(glycolic acid) degrade by bulk hydrolyzation of the polymer backbone. The nature of this type of degradation results in a release of a significant amount of acidic monomers and oligomers at the end of degradation. This can lead to tissue necrosis and other undesirable side effects (Laurencin *et al.*, 2000). Other biodegradable polymers being developed currently are polycaprolactone (Marra et al., 1999), polyanhydrides (Langer, 1995), polyphosphazenes (Laurencin *et al.*, 2000), and polycarbonates (Ertel *et al.*, 1994).

Scheme III



Poly(lactic acid)



Poly(glycolic acid)

Ceramics are also widely used in bone tissue engineering applications. Hydroxyapatite (HA; $Ca_{10}(PO_4)_6(OH)_2$) is a calcium-phosphate based ceramic that is a major inorganic component of bone. At present, HA is commercially available and proven for biocompatibility, biodegradability and osteoconductivity. However, the material is difficult to shape in specific forms required for bone substitution due to its hardness and brittleness (Hollinger *et al.*, 1996).

Composites of ceramics and polymers are alternative ways to develop the material. For example, poly(D, L-lactic-co-glycolic acid) is reported in blending

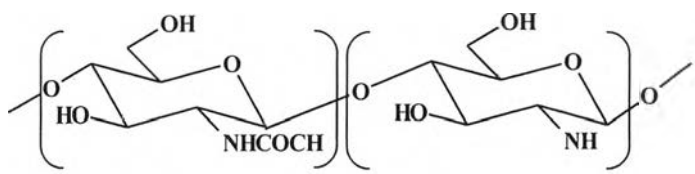
with HA to improve mechanical properties as well as increase the osteoconductive nature of the composite (Attawia *et al.*, 1995).

2.4 Chitin-chitosan: Specific Properties for Bone Therapy

Chitin is a linear polymer of 1,4 β-linked N-acetyl-D-glucosamine which is obtained from crustacean shell. Chitosan is a partially N-deacetylated chitin. Chitin-chitosan is proposed as biomedical material because it exhibits many unique properties such as biodegradability (Amano *et al.*, 1978), biocompatibility (Singh *et al.*, 1994), and non-toxicity (Chandy *et al.*, 1992). Chitin-chitosan was reported to assist in wound healing with a hemostatic property and the utility in propagation of human osteoblasts and chondrocytes (Lahiji *et al.*, 2000).

By considering the chemical structure of chitin-chitosan (Scheme IV), it consists of the reactive hydroxyl and amino groups, which are attractive in functionalization. In addition, the free amino groups of chitosan can be protonated to give cationic polymer, which is effective for binding with hydroxyapatite, the main inorganic component of bone (Tachaboonyakiat *et al.*, 2001).

Scheme IV



Chitin **Chitosan**

Many researchers studied on bone-like chitosan/HA composite. This material shows the unique properties in biodegradability, biocompatibility, and osteoconductivity. In the past, chitosan/HA composites obtained from the mixing of HA powder with the chitosan solution (Ito *et al.*, 1994) and the coating of HA particles onto a chitosan sheet (Varma *et al.*, 1999) were microscopically inhomogeneous and often caused inflammation when implanted. Yamaguchi *et al.* (2001), thus, prepared homogeneous HA/chitosan nanocomposites using a co-

precipitation method with H_3PO_4 and $Ca(OH)_2$. The composite obtained was found to be mechanically flexible and easily formed into any desired shape. In addition, the mechanical strength could be enhanced by heat treatment in a saturated steam, which may be used to produce a crosslinked chitosan/HA composite without the need for a crosslink reagent such as glutaraldehyde.

Tachaboonyakiat *et al.* (2001) reported the formation of hydroxyapatite in the chitosan network. An alternate soaking with $CaCl_2$ and Na_2HPO_4 (Tahuchi *et al.*, 1998) was applied to initiate small crystalline hydroxyapatite formation with the crystal structure similar to that of bone hydroxyapatite. However, the use of glutaraldehyde as a crosslinker limited its application in human body due to its toxicity. Another study is the preparation of chitosan/HA scaffold by using enzymatic degradation to create highly porous composite followed by the bone tissue formation.

2.5 Motivation of the Present Work

Even though many kinds of artificial bone have been investigated, each of them has its own advantages and disadvantages. The present work stands on a unique point to develop chitosan for a glue chitosan/hydroxyapatite composite, which will be a guildline to develop material for bone therapy. The material is attractive in terms of biodegradability, biocompatibility, and osteoconductivity including adhesiveness to fix up the fracture bone. It can also be expected that the generation of the bone tissue allows the repairing of bone naturally. The work proposes the effectiveness of epoxy ring opening reaction to form chitin-chitosan network. It is important to note that the work also aims to develop chitosan composite with hydroxyapatite by using alternate soaking process.