CHAPTER I

INTRODUCTION

Bone is an organ that serves to sustain and protect internal organs as well as supports the muscle movement. Although the progress of pharmaceutical and medical areas has been rapidly developed to a level that treatments are almost satisfied, the bone therapy is still in the need of more development.

There are many ways to treat bone fracture, depending on position of breakage or severity. Traditional techniques of bone therapy: splints, braces, plaster cast, and metal implantation are usually applied to immobilize bones and allow new bone tissue to regenerate. However, all of these methods require very long time (1-3 months) depending on patient conditions. In order to minimize the time of healing and to prevent dwarf muscle from inactivity and increase the efficiency of immobilized bone, alternative tissue engineering techniques such as ultrasound (Kaufman *et al.*, 1997), magnetic (Guillen *et al.*, 1985), autografts (Hulstyn *et al.*, 1993), allografts (Jager *et al.*, 2010), and xenografts (Poumarat *et al.*, 1993) are proposed. As these techniques have been developed recently, there are limitations to be solved. For example, ultrasound and magnetic treatments need the machine in treatment process, autografts have the problem of donor shortage and donor site morbidity whereas allograft and xenograft show a risk in disease transmission and immune response. Recently, a three-dimensional (3D) scaffold for bone regeneration has received much attention. Porous polymers with high mechanical properties as well as bio-related properties, especially bone cell compatibility are expected to be used as a replace material (Zhang, *et al.*, 1999).

Chitosan is a good candidate material for producing the 3D porous scaffolds. Up to now, many studies have shown that chitosan hydrogel has a potential to use as a tissue scaffold due to its biocompatibility (Richardson, *et al.*, 1999), biodegradability (Yamamoto and Amaike, 1997), bioactivity (Guibal *et al.*, 2004)., and non-toxicity (Rao and Sharma, 1992). Furthermore, chitosan is reported about its providing of biological primers for cell-tissue proliferation and reconstruction (Ilium, 1998). For tissue engineering, a porous chitosan has been reported as a matrix for bone

regeneration (Manjubala *et al.*, 2006). Moreover, chitosan is also well-known to have the same glucosamine unit as the lubricant in human joint (Freesia and Warren, 1972).

The fact that chitosan has rigid crystalline structures is formed by both inter- and intra-molecular hydrogen bonding, so chitosan is difficult to dissolve in typical polymer solvents (Mihammad *et al.*, 2010). The use of chitosan for bone therapy is not easy and simple. Chemical modification of chitosan to obtain desired properties is a good way to solve this limitation. Derivatizing of chitosan to water soluble carboxymethyl chitosan (Zhou *et al.*, 2010) is an alternative choice for producing hydrogel for bio-system.

For the past few years, our group focused on chitin whiskers and succeeded in preparing chitosan nanoscaffold from the chitin whiskers (Phongying *et al.*, 2007). The morphology was found to be under a three dimensional nano-fiber network with high surface area and pore volume. As the reaction was carried out only in water and mineral acid and base, biocompatibility for guiding cell growth is expected.

Based on the requirements of bone therapy in terms of bone breaking and bone fracture, injectable bone glue is an ideal case since the material can penetrate to the bone breaking area by injecting to that specific position. Therefore, it is an ideal to consider a material that (i) is in an appropriate viscous solution which is possible to inject the material into the bone area for connecting the bone fracture, (ii) is ready to crosslink and becomes a gel for bone connection, (iii) provides the network for bone cell and/ or hydroxyapatite, non-toxicity and biodegradability. In this work, we propose a novel chitosan nanoscaffold injectable hydrogel. Here, the key points to bring in those requirements into chitosan are (i) changing chitosan to water-based chitosan with reactive functional group for crosslinking, (ii) pre-crosslinking water-based chitosan as an injectable gel, (iii) further crosslinking injectable chitosan gel with chitosan nanoscaffold. To see the overall performance of the material, the present work also focuses on the gel properties in terms of gel formation conditions, gel strength, including other bio-related properties such as, cell viability, bone cell growth promotion and efficiency, etc.