CHAPTER I
INTRODUCTION

One of the essential extracellular matrices synthesized by fibroblast is collagen. It is widely known that type I collagen plays an important role in wound healing mechanism. Because of its many advantages, collagen has been used in various biomedical products such as hemostatic agents, sponges for burn wounds and tissue engineering. Type I collagen can be found in skin, tendon and ligament as well as bone. Although nowadays many researchers who have studied collagen from various sources including the skin of porcine, bovine, shark (Lin and Liu, 2006), frog (Li et al., 2004) and fish (Kimura et al., 1987a,b; Montero et al., 1990; Muyonga et al., 2004; Kittiphattanabawon et al., 2005 and Jongjareonrak et al., 2005), fish scale (Ikoma et al., 2003; Kimura et al., 1991), fish muscle (Montero et al., 1990), bird feet (Lin and Liu., 2006) and many marine organisms (Nagai and Suzuki., 2002 and Nagai et al., 2002). The collagen used for industrial purpose has only been extracted from skin and bone of cattles and pigs. Unfortunately, these animals can carry dangerous transmitting diseases such as bovine spongiform encephalopathy (BSE) and foot and mouth disease (FMD). Moreover, collagen extracted from pig is against Islamic belief (Senaratne et al., 2006). Therefore, in order to avoid these risks and unpleasant effect, scientists have paid more attention to other alternative collagen sources such as jellyfish (Nagai et al., 2002), paper nautilus (Nagai and Suzuki., 2002) sea cucumber (Cui et al., 2007), and fishes (Kimura et al., 1987, Matsui et al., 1991, Nagai and Suzuki, 2000a,b, Mizuta et al., 2003, Muyonga et al., 2004, Kittiphattanabawon et al., 2005, and Jongjareonrak et al., 2005). Generally, the raw material for collagen extraction is derived from the fishery processing wastes (Nagai and Suzuki, 2000a), for instance, brown blacked toadfish skin was extracted with an excellent yield (54.3% on the basis of lyophilized dry weight) (Senaratne et al., 2006). However, these alternative sources still have some problem concerning low thermal resistance.

Chemical and physical properties differences between collagens are associated with the extracting method or source of raw material. Collagen can be basically extracted by two methods. First of which is the conventional method using only weak acids and the other is the method combining the weak acids and proteolytic enzyme, pepsin. The product extracted from the first method is called "acid solubilized collagen" (ASC) while the second one is called "pepsin solubilized

collagen" (PSC). Even though the cost of enzymatic extracting method is higher, more yield and better product qualities such as less allergic reaction can be obtained due to the crosslink and antigenic portions of collagen is enzymatically removed (Friess, 1998).

Source of raw material is the other factor influencing its physicochemical properties. In general, the collagen structure is heterotrimer usually consisting of two chains of α1 and one α2 chain [$(\alpha 1)_2\alpha_2$] (Yamauchi, 2002) but merely in aquatic animal, α3- the distinct α chain, can be observed (Kimura et al., 1987, Nagai and Suzuki, 2001, Nagai et al., 2002). Besides the type of α chain, the thermal stability is also different. Ogawa and co-workers (2004) found that denaturation temperature of fish collagens were lower than vertebrate counterpart owing to their low imino acid content. Therefore, it may be assumed that collagen thermal stability might relate to the α3 component existence and imino acid composition. Additionally, the thermal stability is also related to the upper limit of physiological temperature of extracted animals (Nagai et al., 2002). According to the previous studies, collagen extracted from subtropical and tropical fish contained higher thermal stability than that of cold water fish (Muyonga et al., 2004 and Ogawa et al., 2004). Moreover, it is interesting to observe that α3 have not been found in almost all subtropical and tropical fish in their collagen molecules. As mentioned above, we may conclude that fish living in different environment may have genomic distinct resulting in diverse expression of physicochemical properties. Therefore, fish living in high temperature environment should have collagen with desirable quality.

Figure 1.1 Giant gouramy (Osphronemus guramy)

Giant gouramy (Osphronemus guramy) is a tropical fish which is classified in the family Osphronemidae. The Asian gouramy differs from common gouramies by its bulky appearance with a short spine in each pelvic fin, a long filamentous second pelvic fin ray and large scales (Figure 1.1) with approximately 1 kg weight. This fish has been widely cultivated in many parts of Thailand and appeared to be a popular food fish. The large amount of skin and scale of the fish left over as by-product can be used for collagen extraction. We hypothesized that collagen extracted from skin and scale of giant gouramy was type I collagen with higher thermal stability than that of the cold water fishes and could be used as a resource to build scaffold for synthetic wound dressing.

Therefore, the objectives of present study are, as follows:

1. To determine the percent yield of collagen extracted from skin and scale of giant gouramy.
2. To characterize the physicochemical properties of collagen extracted from skin and scale of giant gouramy.
3. To examine the proliferative responses of human dermal fibroblast culture to collagen extracted from skin and scale of giant gouramy.