CHAPTER I

INTRODUCTION

Recent advances in the field of biomaterials and their medical applications have been substantially interested. Cellulose has been used as biomaterial for many medical applications (Hoenich, 2006). Basically, cellulose is the most widely distributed skeletal polysaccharide and represents about 50% of cell wall material of plants (Pretre *et al.*, 1999). However, plant cellulose is unpurified associated with other kinds of natural fiber like lignin and hemicelluloses. Moreover, the production of plant cellulose products is facing some environmental problems (Saied *et al.*, 2004). Therefore, the production of cellulose products by using a new process that minimize environmental impacts by using *Acetobacter xylinum* has been developed (Jonas and Farah, 1998; Saied *et al*, 2004). In general, BC was extracellularly synthesized into nano-sized fibrils by the bacteria *Acetobacter xylinum*, with coconut water being used as liquid medium. Plant-derived cellulose and BC have the same chemical structure, however, BC displays advantages superior to the counterpart from plants with its physical and chemical properties. It has the unique properties such as high mechanical strength, high crystallinity, high hydrophilicity and ultra-fine network structure.

There have been several applications of BC in medical fields such as artificial skin for humans with extensive burns (Fontana *et al.*, 1990), artificial blood vessels for microsurgery (Klemm *et al.*, 2001), scaffold for tissue engineering of cartilage (Svensson *et al.*, 2005) and wound-dressing (Czaja *et al.*, 2006). Especially in the wound dressing application, BC is eligible because of its outstanding properties.

The excellent dressing should be maintained the wound in a wet condition, inexpensive, lightweight, and flexible. In addition, it is necessary to prevent infection, dehydration and protein loss with good permeability and non-toxicity. On the other hand, it should accelerate wound healing, decrease scars and so on (Czaja *et al.*, 2006; Deng *et al.*, 2007). BC shows high water content, good sorption of liquids, non-allergenic and can be safely sterilized without much change of its characteristics. Sanchavanakit *et al.* (2006) reported that at 48h, the relative living cell number of human normal skin fibroblast on BC film (~180%) was less than that on the polystyrene culture plate (~220%). However, Lee *et al.* (2003) found that at 48h, the relative living fibroblast cell number of 100% w/w gelatin sponge was comparable to that on the polystyrene culture plate (~283%).

Gelatin, natural polymer, is obtained by a controlled hydrolysis of the fibrous insoluble collagen, which is a protein widely found in nature. Collagen is the major constituent of skin, bones and connective tissue and constitutes the major part of extracellular matrix in animals. Collagen has antigenicity due to its animal origin, in contrast, gelatin has relatively low antigenicity compared to its precursor, yet it still retains some of the information signals which may promote cell adhesion, differentiation and proliferation. Ratanavaraporn *et al.* (2006) prepared the gelatin scaffolds for fibroblast cell culture compared to the collagen scaffolds. They found that, the number fibroblast cell attached and proliferated on type A and type B gelatin scaffolds comparing to the collagen scaffolds were not significantly different. In addition, gelatin is less expensive than collagen. Recently, gelatin based biomaterials have been applied to artificial skin, wound dressing, bone grafts, plasma expander, scaffolds for tissue engineering, adhesive and absorbent pad

because of its excellent biodegradability and biocompatibility (Neumann *et al.*, 1981; Tabata and Ikada, 1998; Dong *et al.*, 2006; Lien *et al.*, 2009).

Based on the advantageous properties of BC and gelatin, this study aims to prepare BC-gelatin film from microbial synthesis under static conditions by *Acetobacter xylinum* in coconut-water, which was a waste from coconut milk production plants. Microstructure and mechanical properties of the BC-gelatin films are then characterized. Furthermore, the growth of Vero cell on the modified BC films is examined. The present study will provide indications for gelatin supplement in culture medium during BC-gelatin biosynthesis and BC hydrogel impregnated in gelatin solution for the uses as biocompatible films in therapy of skin wounds.

Objectives

1. To develop BC-gelatin composite film by using two different methods,
   (I) Supplementation of gelatin into the culture medium during biosynthesis (Biosyn)
   (II) Impregnation of BC gels with gelatin solution then cross-link with tannic acid (Impreg).
2. To investigate the effects of difference gelatin content on BC-gelatin film characteristics.

Research Scopes

1. Prepare BC film from biosynthesis under static conditions by *Acetobacter xylinum*.
2. Examine effects of gelatin concentrations in the culture medium and the impregnated solution on the film development. Gelatin (type A) was used for the experimental study.
3. Characterizing the developed BC-gelatin film by
    a. Scanning electron micrographs (SEM) for preliminarily investigating morphology.
    b. Fourier transform infrared (FT-IR) spectrometer for identifying the chemical structure.
    c. Instron testing machine for determining tensile strength and elongation at break.
    d. X-ray diffraction (XRD) for finding crystallinity index.
    e. Oxygen permeation tester for measuring oxygen transmission rate (OTR)
    f. Water vapor permeation tester for measuring water vapor transmission rate (WVTR)
    g. Water absorption capacity (WAC)
    h. Antibacterial ability
    i. Antifungal ability
4. *In vitro* study of Vero cells (Monkey kidney cells) on the developed film in 24-well culture plates.