CHAPTER III

LITERATURE REVIEW

3.1 Medical Application of BC

Various polymeric materials recently have been investigated for wound dressing application, but the search for an ideal skin substitute with properties and functionality similar to human skin is still continuing. Being similar to human skin, bacterial cellulose can be applied as skin substitute in treating extensive burns (Czaja *et al.*, 2006) including the care of surgical wounds, bedsores and ulcers (Cienchanska, 2004). With special characteristics, such as high mechanical strength in the wet state, substantial permeability for liquids and gases, low irritation of skin (Saied *et al.*, 2004), low toxicity, chemical stability (Kurosumi *et al.*, 2009), purity and uniformity (Mayall *et al.*, 1990), the gelatinous membrane of the bacterial cellulose can be used for the manufacture of artificial skin for temporary covering of wounds. The advantage of the BC includes its transparency, which allows for continuous clinical observation of the healing progress. What is important for the production of bacterial cellulose and in what way the resulting materials could be used by industry or possible fields of application such as health applications of bacterial cellulose has been reviewed (Jonas and Farah, 1998; Saied *et al.*, 2004).

Fontana *et al.* (1990) first reported the application of BC as temporary skin substitutes. The product, called Biofill®, can provide non-woven, shaped objects in medicine such as artificial arteries, vessels, skin, and etc. It has been still utilized for several skin injury treatments such as basal cell carcinoma/skin graft, severe body burns, facial peeling, sutures, dermabrasions, skin lesions, chronic ulcers, and both donor and receptor sites in skin grafts (Czaja *et al.*, 2006).

Mayall *et al.* (1990) used a Biofill® skin substitute in the treatment of trophic ulcerations of the limbs and showed that this material was very effective by shortening the cicatrisation time, reducing the contamination, and saving the cost of treatment.

Kucharzewski *et al.* (2003) showed two methods of treating non-healing venous leg ulcers. The experimental group of patients was treated with BC wound dressing (Bioprocess®), whereas the control group was treated with Unna's boot hydrocolloid dressing that is widely used in the therapy of these types of wounds. The authors inferred BC wound dressing was more effective in the treatment of the chronic venous leg ulcers than Unna's boot.

Alvarez *et al.* (2004) demonstrated the use of BC in the form of a hydrated membrane in the treatment of chronic venous ulcers. BC was more effective than a standard protocol (non-adherent cellulose acetate gauze) in the process of autolytic debridement.

Another very important advantage of bacterial cellulose is its mouldability *in situ*. By using special technologies, it is possible to prepare hollow fibers [bacterial synthesized cellulose (BASYC®)-tubes], used as artificial blood vessels and ureters. Artificial vessels are used to replace arteries or veins damaged as a result of tumors or accidents. High mechanical strength in wet state, enormous water retention values, low roughness of the inner surface and a complete vitalization of BASYC® microvessel- interpositions in rat experiments demonstrate the high potential of BASYC® as an artificial blood vessel in microsurgery (Saied *et al.*, 2004).

Artificial skin's BC for burn and skin injuries treatment displays dramatic clinical results such as immediate pain relief, diminished post-surgery discomfort, faster healing, reduced infection rate and reduced treatment time and cost (Fontana *et al.*, 1990). In addition, the wound healing effects of never-dried BC are fully biocompatible and also successfully protected burn wounds from excessive external fluid loss, thus accelerating the entire process of healing (Czaja *et al.*, 2006).

BC have potential application as an orthopedic biomaterial. Shi *et al.* (2009) developed nanocomposites consisting of calcium-deficient carbonate-containing hydroxyapatite (CaDHCAp) in the three-dimensional (3D) network of BC nanofibers. The experimental results showed that the obtained nanocomposites had outstanding osteoconductivity and bioactivity.

In addition, BC was applied as an additional membrane to protect immobilized glucose analyzers in biosensors used for assays of glucose levels. This bacterial cellulose membrane introduced the electrode stability. In human blood, the biosensor-coated commercial protecting membranes, as cuprophan (AKZO, England), was stable for 3-4 h, whereas bacterial cellulose membrane prolonged its stability to 24 h. Cellulose gels containing immobilized animal cell were used for their culture to produce interferon, interleukin-1, cytostatic and monoclonal antibodies (Saied *et al.*, 2004).

3.2 Gelatin for Wound Dressing

The literatures concerning the wound healing process and physical and physiologic factors that can affect the rate of this process, including the wound dressing product and the importance of the wound dressing in maintaining an optimal environment for wound healing had been reviewed by Hanna *et al.* (1997)

The wound dressing must be preserving moisture, soft, good permeability, non-toxicity and no pyrogen, as well as have no bad reaction and stimulation to flesh, and could promote the skin regeneration, accelerate wound healing by protecting wounds from infection and by keeping an environment of wound, decrease scars and so on (Deng *et al.*, 2007). The denatured type collagen, gelatin, has been used in medical industry as wound dressing because of its excellent biodegradability, biocompatibility (Dong *et al.*, 2006), bioabsorptivity, low antigenecity (Nagahama *et al.*, 2008) and high hemostatic effect (Tomihata *et al.*, 1994; Lee *et al.*, 2003).

Lee *et al.* (2003) investigated the cross-linked gelatin/β-glucan scaffolds for use as a support for the growth of fibroblasts and keratinocytes (normal human skin cell). This study was initiated to evaluate the attachment and proliferation of fibroblasts into a scaffold composed of gelatin and β -glucan mixed in various ratios. The result showed that the porous scaffolds composed of gelatin and β-glucan had the inter-connected pore structure and the sufficient pore size for use as a support for the growth of fibroblasts and keratinocytes. Moreover, the cell attachment and proliferation improved as the ratio of gelatin increased in the mixture, whereas water uptake increased with β-glucan contents. Therefore, the bio-artificial skin composed of gelatin and β -glucan will be useful to promote wound healing. Furthermore the cross-linked gelatin sponges using a salt-leaching method have been also investigated for their potential application as a component of artificial skin or tissue transplants to promote epithelialization and granulation tissue formation in wounds. The fibroblasts after 1 week of *in vitro* culturing showed a good affinity to the scaffold (Lee *et al.*, 2005).

Acceleration of wound healing by dressing with growth factors has been studied. Gelatin was found to be a suitable material for production of dressing with growth factor. Tanaka *et al.* (2005) demonstrated the biological activity of epidermal growth factor (EGF) included in the gelatin film by cell proliferation assay using NIH3T3 fibroblasts and PAM212 keratinocytes. EGF containing gelatin sheet might have therapeutic potentialities for dressing of various skin wounds (burns surgical removal of skin). The results showed that the developed gelatin film was very thin and preservable, which the high activity of EGF remained stable even after dried in gelatin film. Moreover the gelatin sheet containing EGF was effective to accelerate the rate of wound closure. It induced early reepithelialization and highly regulated repair of extracellular matrix in dermis.

Deng *et al.* (2007) developed the chitosan-gelatin sponge wound dressing (CGSWD), evaluated the toxicoligical according to the national sanitation ministry criterion WS5-1-87, ISO and ASTM and tested the antibacterial properties compared with medicine (used medicine-sensing test paper). They found that the use of the CGSWD in wound healing made wound healing speed quicker than using Vaseline sterile gauze and the formed scar was not bulge distinctly.

The ideal systems for tissue repair have to permit an effective release of therapeutic agents and flow of nutrients to proliferating cells. The mass transfer of keratinocyte growth factor (KGF) to wound healing through Semi-interpenetrating networks (sIPNs) that prepared to mimic extracellular matrix from unmodified gelatin (aid in wound healing) and poly(ethylene glycol) diacrylate (PEGdA) was characterized by Bader *et al.* (2009). The sIPNs were shown to be effective matrices for the delivery of drugs and growth factors, as well as for the transport of nutrients to proliferate cells during wound healing.

3.3 Gelatin-Carbohydrate Blends

Blending of polymers is one of the simplest methods to obtain a variety of physical and chemical properties from the constituent polymer at a molecular level. The gain in newer properties depends on the degree of compatibility or miscibility of the polymer (Nagahama *et al.*, 2008). Gelatin, because of its low intensity and high brittleness, is rarely used alone, being often used after modification through several methods, such as blending (Dong *et al.*, 2006). There were several literatures showed the efficacy of cross-linked gelatin-based sponges composed of gelatin and polysaccharides for wound-dressing materials (Choi *et al.*; 1999 (a,b); Choi *et al.*, 2001; Hong *et al.*, 2001).

Lii *et al.* (2002) demonstrated a suitable method for synthesis of carboxymethyl cellulose-gelatin complexes. In the complex, both components are bound with covalent bond by electrosynthesis method. The elastic modui of the microcrystalline cellulose-gelatin blends was studied by Kasapis (1999).

Lee *et al.* (2004) developed the composite films made from blending two different biopolymers, gellan and gelatin. First, they determined the effect of gellan/gelatin ratio and NaCl concentration on the mechanical properties of these films. The tensile strength of the composite films decreased linearly as the gelatin ratio increased, whereas the tensile elongation increased with increasing gelatin ratio. Then, they investigated the water solubility that ranged from 30 to 52%, which was lower than that of other biopolymer films such as cellulose (55-84%). The water solubility decreased with increasing gelatin ratio up to 40%. The swelling ratio of the composite films was also significantly decreased with increasing gelatin ratio up to 40%.

Dong *et al.* (2006) developed alginate/gelatin blend films to use in controlled release applications. The result from the FT-IR spectra showed a strong evidence of the intermolecular interactions and good molecular compatibility between alginate and gelatin. The X-ray diffraction patterns of AG film indicated the strong interaction between alginate and gelatin which has destroyed the close packing of the alginate molecules for the formation of regular crystallites. Moreover the maximum value of tensile strength and elongation at breaking were both observed when the content of gelatin was 50 % w/w. The results indicated that blending is effective in improving the mechanical properties of the drug loaded films.

Wound dressing material with dense surface is required for protecting the evaporation of exudates from wound. However, a dense surface layer was not formed in the gelatin-glucan, a natural complex carbohydrate, sponges (Lee *et al.*, 2003). This might be from the low concentration of gelatin (0.7 % w/w). In addition, the average pore size and porosity of the sponges decreased with decreasing gelatin content. Deng *et al.* (2007) developed the chitosan-gelatin sponge wound dressing (CGSWD) made from chitosan as main material and gelatin as assistant material with appropriate cross-linking agent and some other assistants. Many former experiments had proved that CGSWD was soft, permeable and preserving moisture.

3.4 Gelatin Cross-linked by Tannic Acid

The cross-linking methods can be classified as physical cross-linking and chemical cross-linking. Physical treatments such as UV- and γ- radiation could induce cross-linked gelatin gels with weak interaction, whereas chemical agents such as aldehydes could cause cross-linking between the amino acid chains of gelatin with strong interaction. However, these agents have high toxicity and might contaminate the product. Therefore, the natural cross-linking agents such as genipin, ferulic and tannic acid have been used as cross-linking agent for gelatin (Bigi *et al*, 2002; Chambi and Grosso, 2006; Cao *et al.*, 2007).

Tannins also referred to as tannic acid (TA) is a water-soluble polyphenol consisting of a central carbohydrate (glucose) and phenol carboxylic acids which have a molecular weight between 500 and 3000 Daltons. It is found in a variety of fruits and vegetables and acts as a natural antimicrobial agent and antioxidants (Chung *et al.*, 1998; Taitzoglou *et al.*, 2001; Cao *et al.*, 2007; Balange and Benjakul 2009; Kim *et al.*, 2010). Due to its ability to bind protein, Cao *et al.* (2007) studies influences of concentrations of TA on the properties of gelatin to be used in packing as edible films. The result showed that TA could act as a cross-linking agent for gelatin film and could decrease swelling ratio as well as had no obvious effect on water vapor permeability of the film. Moreover, the properties of the films treated by TA could become better after being stored for more than 90 days.

Furthermore, tannic acid was reported as a therapeutic agent to heal burned skins. The normal tanning process consists of an increase in the amount of cross-linking between collagen fibrils in a collagen matrix which is a similar process that takes place in the denatured protein mass of burned skin. Consequently, the burn wounds exhibited the positive effects such as pain reduction, lower infection risk, stimulation of epidermal regeneration, faster wound healing, better scar formation, reduced mortality and reduction of plasma loss (Hupkens *et al.*, 1995). In the same way, Heijimen *et al.* (1997) investigated cross-linking of dermal sheep collagen which has been used as a model of human skin by tannic acid. The results suggested that tannic acid could have a function *in vivo* in burn treatment by binding burn toxins and inhibiting degradation of the remaining dermal matrix.