CHAPTER V

RESULTS AND DISCUSSIONS

As previously stated in Chapter 4, the bacterial cellulose–gelatin (BCG) composite films were developed by two different preparation methods, (i) supplementation of gelatin into the culture medium during biosynthesis (Biosyn) and (ii) impregnation of BC gels with gelatin solution, and subsequently cross-linking them with tannic acid (Impreg). The films obtained by both techniques were then characterized for surface and cross sectional morphologies, chemical structure, tensile properties, crystallinity index, porous structure, water absorption capacity, water vapor permeability and oxygen permeability. Furthermore, the antibacterial and antifungal properties of Impreg-BCG30 were investigated compared with uncross-linked Impreg-BCG30. Finally, Cytotoxic activity of BCG films against Vero cells were evaluated.

5.1 Characterization of Biosynthesized BC-Gelatin Films

In this section, the characteristics of BC-gelatin composite films prepared via *in situ* fermentation (as described in chapter IV) were analyzed. Herein, samples prepared by this method were referred to as Biosyn-BCG*n*, where *n* is the concentration of gelatin solution added directly into the culture medium.

5.1.1 Morphology

Medically, transparency of the film used as wound dressing is of considerable significance as transparent wound dressing would permit clinical observation of the wound area and of the healing process without removal of the wound dressing (Wang *et al.*, 2004). It is therefore interesting to investigate whether the incorporation of gelatin into BC matrix affects the optical transparency of BC-gelatin films.

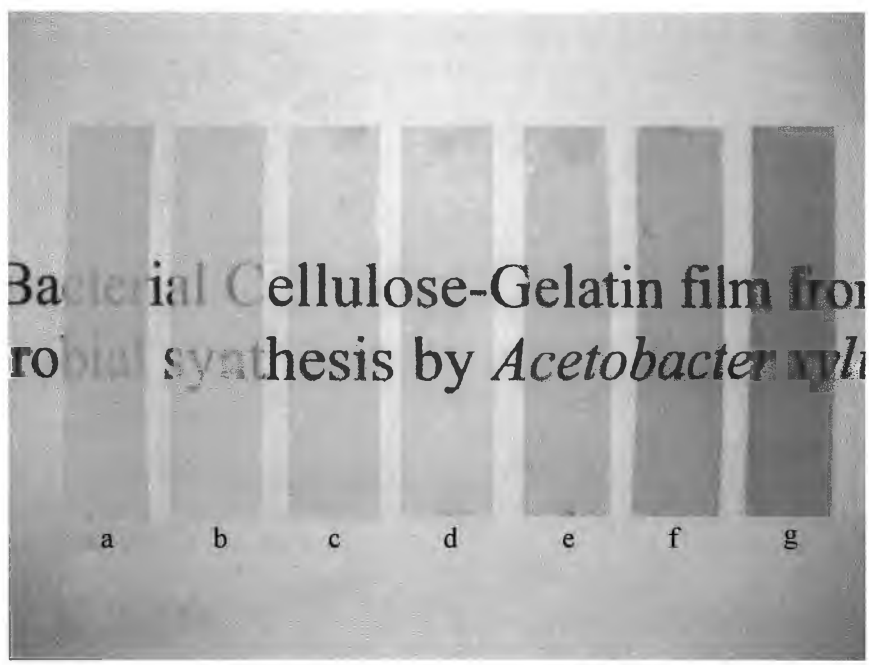


Figure 5.1 Optical photographs of BC-based films: BC (a), Biosyn-BCG1 (b), Biosyn-BCG3 (c), Biosyn-BCG5 (d), Biosyn-BCG7 (e), Biosyn-BCG10 (f) and Gelatin (g)

Figure 5.1 illustrates the effect of the presence of gelatin in the BC film on the transparency of BC-based composite film. From the observation, it was found that the more gelatins incorporated in BC, the greater the optical transparency was. This suggested that incorporation of gelatin into BC resulted in an improvement in the optical transparency of the BC films. According to previous studies (Yano *et al.*, 2005; Cai and Kim, 2010; Gea *et al.*, 2010), certain polymers can easily fill in empty space between BC fibrils while retaining the optical transparency of each polymer. Not only did molecules of such polymers coat on the BC fibril surface but also penetrated into BC fiber networks.

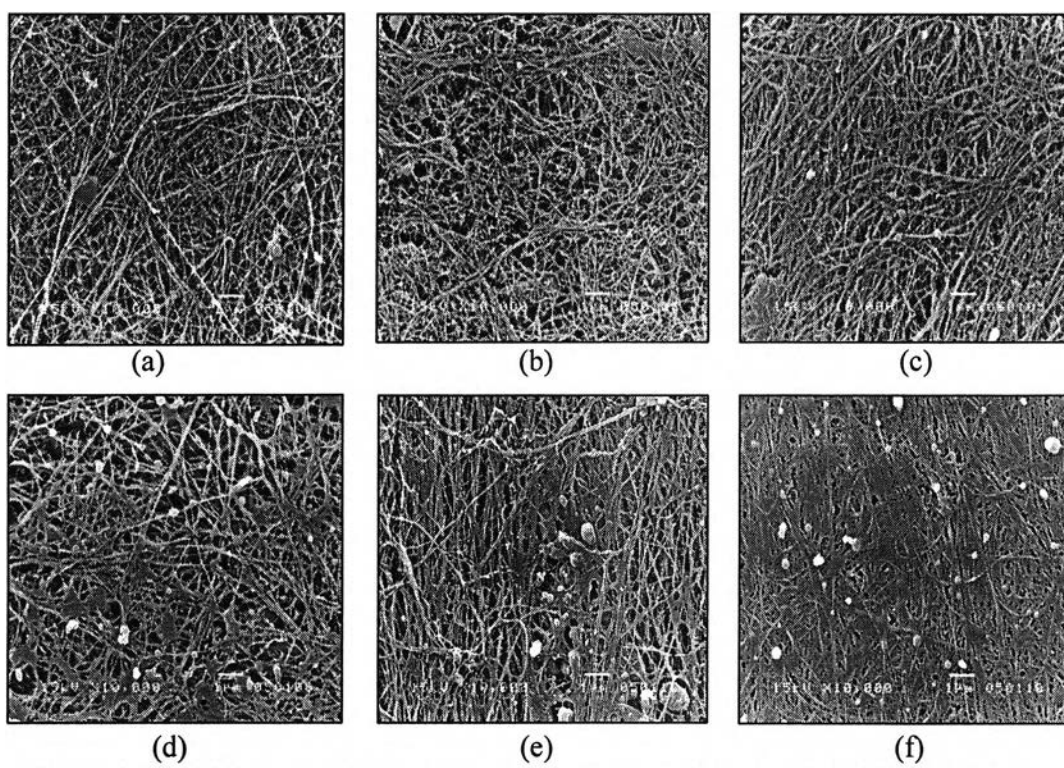


Figure 5.2 SEM images of surface morphology at magnification 10,000X of BC-based films: BC (a), Biosyn-BCG1 (b), Biosyn-BCG3 (c), Biosyn-BCG5 (d), Biosyn-BCG7 (e) and Biosyn-BCG10 (f)

Figure 5.2 represented the micrographs of surface for a pure BC film and BC-gelatin composite films. By adding gelatin into the culture medium of *Acetobacter xylinum*, finely dispersed bacterial cellulose BC/gelatin nanocomposites were produced in a wide range of morphologies. As information on SEM, BC ribbon-shaped fibrils can be observed on the surface and the mean diameter of pure BC fibrils was approximately 50-100 nm (Figure 5.2(a)). Considering Figure 5.2 (b)-(d), by adding gelatin at concentration of 1-5% w/v to culture medium, the mean fiber diameter of the BC fibrils did not change significantly, indicating that gelatin mixed with the cellulose on the nanometer scale. However, the film structure became denser and fibril diameter was slightly enlarged when 7-10% w/v gelatin was supplemented into the culture medium.

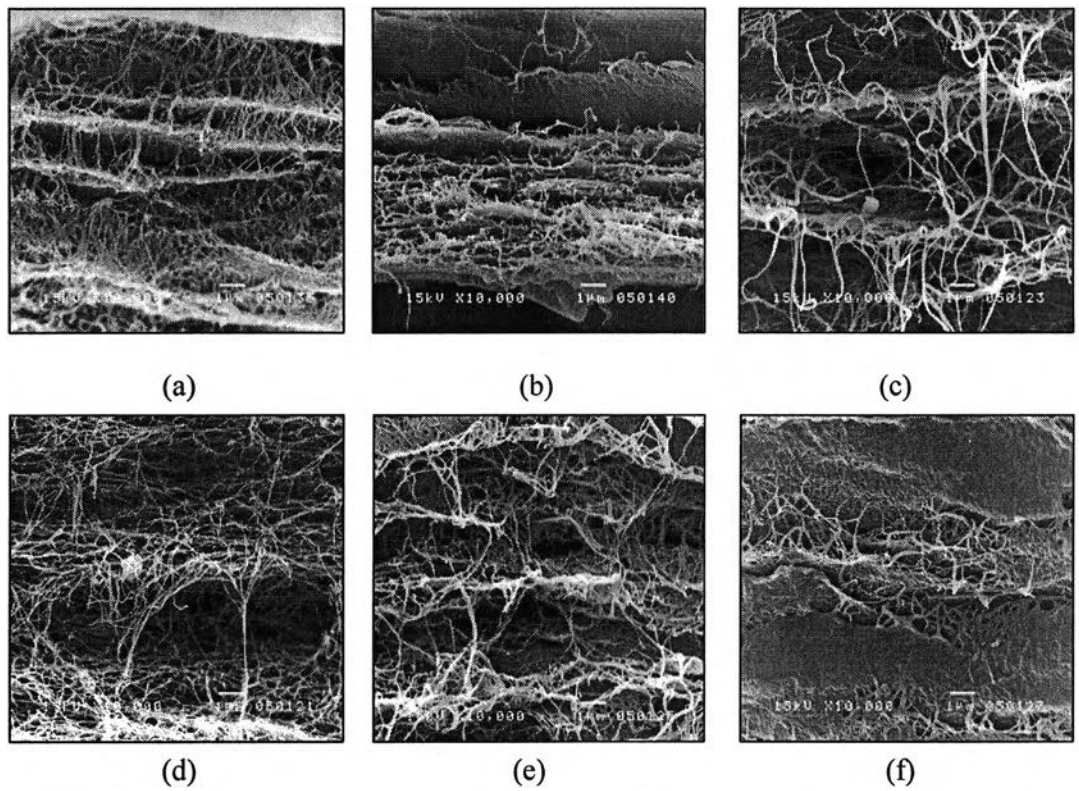


Figure 5.3 SEM images of cross section at 10,000X: BC (a), Biosyn- BCG1 (b), Biosyn- BCG 3(c), Biosyn- BCG5 (d), Biosyn- BCG7 (e) and Biosyn- BCG10 (f)

As seen in Figure 5.3, all films possessed numerous sheets composed of a network of nano-fibrils assembling together forming porous structure. These films had well interconnection between layers. By adding gelatin into culture medium, the cellulose layers were formed differently, depending on the concentration of gelatin. In case of adding gelatin (1-7% w/v), the more gelatin was directly added to the culture medium, the more space between cellulose layer was observed (Figure 5.3 (b)-(e)). Nevertheless, in the presence of 10% w/v gelatin in the culture medium,

the excessive gelatin gel could fill in the empty space between fibrils of BC-gelatin matrix (as showed in Figure 5.3 (f))

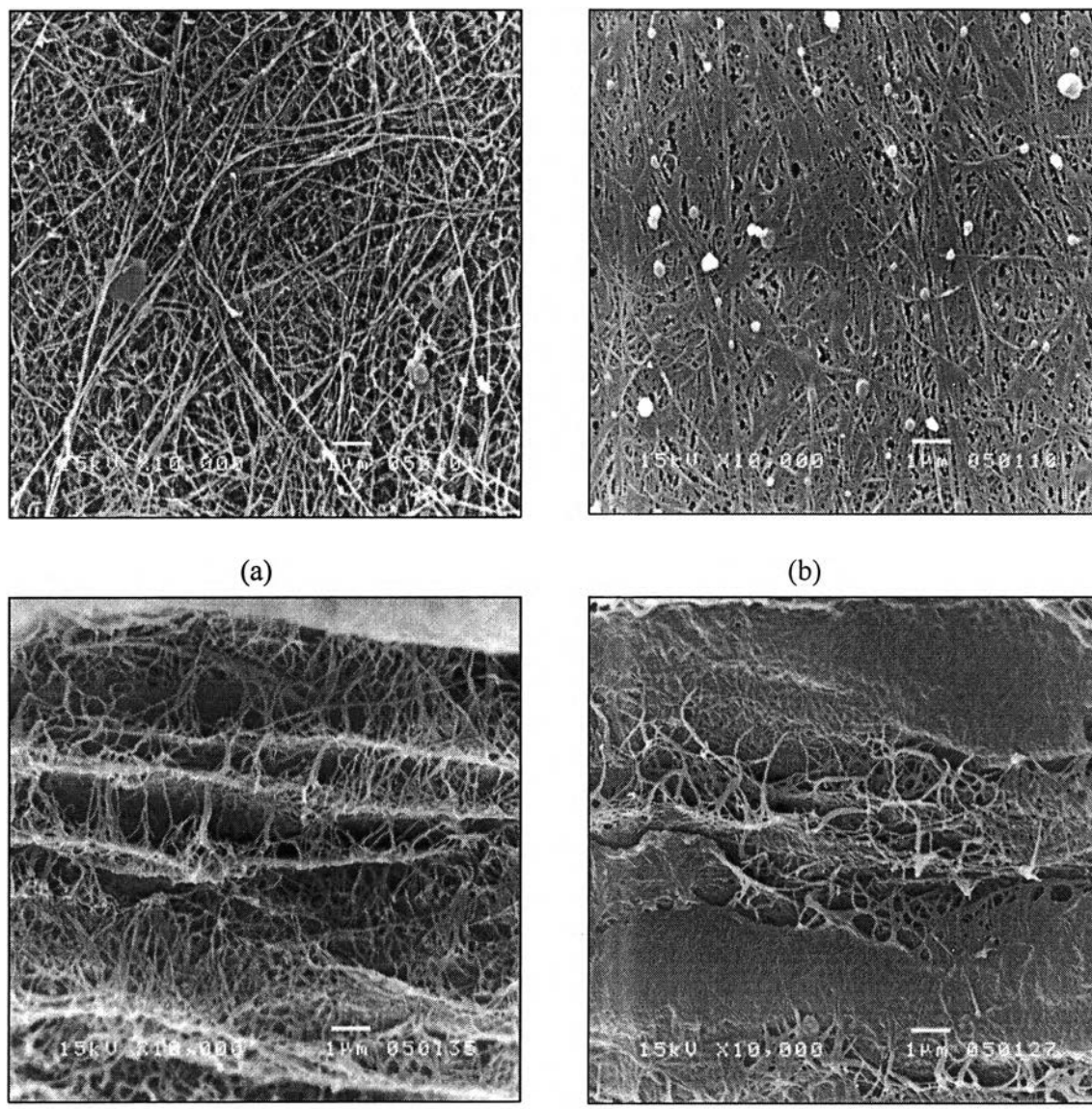


Figure 5.4 SEM images of surface morphology of: BC (a) and Biosyn-BCG10 (b) and SEM images of cross section of: BC (c) and Biosyn-BCG10 (d)

Figure 5.4 presented typical SEM images of BC film and BC/gelatin composite film prepared by *in situ* fermentation (biosynthesis) with a solution of 10% w/v gelatin directly added to the culture medium. It was found that the fibrils of Biosyn-BCG10 became larger owing to covering of gelatin on the fibril surface (Figure 5.4(b)). From cross sectional image of Biosyn-BCG10 (Figure 5.4(d)), gelatin gel could penetrate into the fiber networks and partially filling in empty space between BC fibrils.

Overall, it could be concluded that adding gelatin during the biosynthesis altered the high order structure of cellulose assemblies. The possible reason to explain this phenomenon was that the increased viscosity of culture medium from gelatin supplement might reduce the speed of *Acetobacter* motion during the cellulose synthesis, leading to the alteration of the BC structure. It was previously reported that growing bacterium in a viscous medium (Shibazaki *et al.*, 1998) or at low temperature (Hirai *et al.*, 2002) affected the bacterial motion, which may be connected to the growth rate of cell division (Hesse and Kondo *et al.*, 2005). Therefore, the supplement of agents during microbial synthesis could affect the assembly and crystallization of glucan chains as the motion of bacterium was changed (Hong *et al.*, 2008).

5.1.2 FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy has often been utilized as the useful tool in determining specific functional groups or chemical bonds that exist in a material. Therefore, in this work, the sample of BC and Biosyn-BCG films were analyzed by FTIR. The FTIR spectra of all samples were detected at wave number ranging from 4000 to 450 cm^{-1}. The BC film showed a band at 3375.16 cm^{-1} and 1646.81 cm^{-1} which were attributed to -OH group and -COO group of cellulose, respectively (Phisalapong and Jatupaiboon, 2008; Kanjanamosit *et al.*, 2009; Cai and Kim, 2010). The characteristic absorptions of gelatin film were the bands at 3299.60, 1631.10 and 1529.82 cm^{-1}, which were assigned to O-H stretching vibration, Amide I (C=O and C-N stretching vibration) and Amide II (N-H bending vibration), respectively (Dong *et al.*, 2006; Kim *et al.*, 2010). The FTIR results demonstrate that the characteristic absorption bands of all the Biosyn-BCG films, which supplemented of 1-10% w/v gelatin in the culture medium, are slightly shifted from a wave number at 1646.81 cm^{-1} to a wave number at 1650.06, 1649.18, 1649.92, 1646.42 and 1647.88 cm^{-1}, respectively as showed in Figure 5.5(b-f). At the same time, those modified films also show the new band of Amide II at 1552.25, 1542.62, 1541.30, 1535.61 and 1541.23 cm^{-1}, respectively, due to the presence of N-H bending vibration of gelatin (Dong *et al.*, 2006). All those changes could imply some specified interactions between the -COO group of BC and the N-H groups of gelatin. From Figure 5.5 the first peak of the modified BC films was attributed to O-H stretching vibrations at a wave number of 3350.97, 3382.96, 3400.35, 3412.05 and 3411.70 cm^{-1} which are comparatively different from the band of BC film and gelatin.

Liang *et al.* (1995, 2008) suggested that the frequency difference could be considered as a measure of the average strength of the intermolecular hydrogen bond. Therefore, the absorption band difference between the stretching bonds of BC and Biosyn-BCG films indicated the intermolecular interaction of hydrogen bonds occurred between -OH group of bacterial cellulose and gelatin, which could lead to a good molecular compatibility between BC and gelatin (Dong *et al.*, 2006, Cai and Kim., 2010).

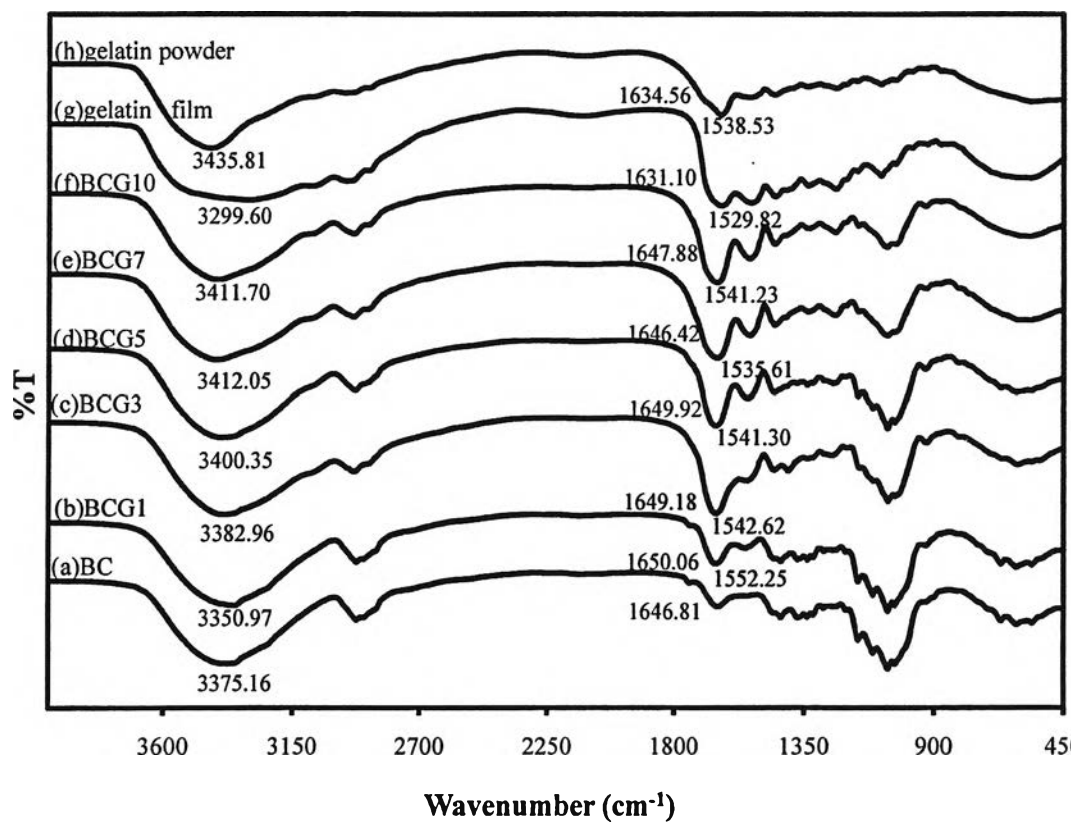


Figure 5.5 The FTIR spectra of BC (a), Biosynthesized gelatin-BC films which were supplemented of 1%, 3%, 5%, 7% and 10%w/v of gelatin powder in culture medium (b-f), gelatin film (g) and gelatin powder (h)

5.1.3 XRD Analysis

X-ray diffraction (XRD) is a rapid analytical technique primarily used for phase identification of crystalline materials. In this study, the XRD was used to identify the crystallinity index of the films. The estimated degree of crystallinity index was calculated according to the literature method (Segal *et al.*, 1959). The XRD patterns of BC and Biosyn-BCG films are shown in Figure 5.6. For BC (Figure 5.6(a)), three main peaks were at 14.37, 16.86 and 22.58 deg for the $(1\bar{1}0)$, (110) and (200) plane, respectively, which can be identified in reflexion planes of cellulose I (Kim *et al.*, 2010; Cai and Kim *et al.*, 2010). Besides, those observed peaks were attributed to the BC cultured in static circumstance (Phisalaphong *et al.*, 2008). Generally, gelatin has a broad diffraction peak in the 2θ range 20–22 deg (Kim *et al.*, 2010) and 1200 count in intensity (Pal *et al.*, 2007). For the Biosyn-BCG films (Figure 5.6(b-f)), there was no observation of gelatin diffraction peak. From this, we could infer that the crystallinity of the films was mainly due to BC. However, the percentages of gelatin supplemented in the range of 1 – 10% w/v in the culture medium affect on crystallinity indices (C.I.) of the modified films compared with typical BC film. The C.I. of the films gradually decreased from 82.48% of BC film to 71.57, 62.67, 55.08, 54.20 and 48.81%, respectively.

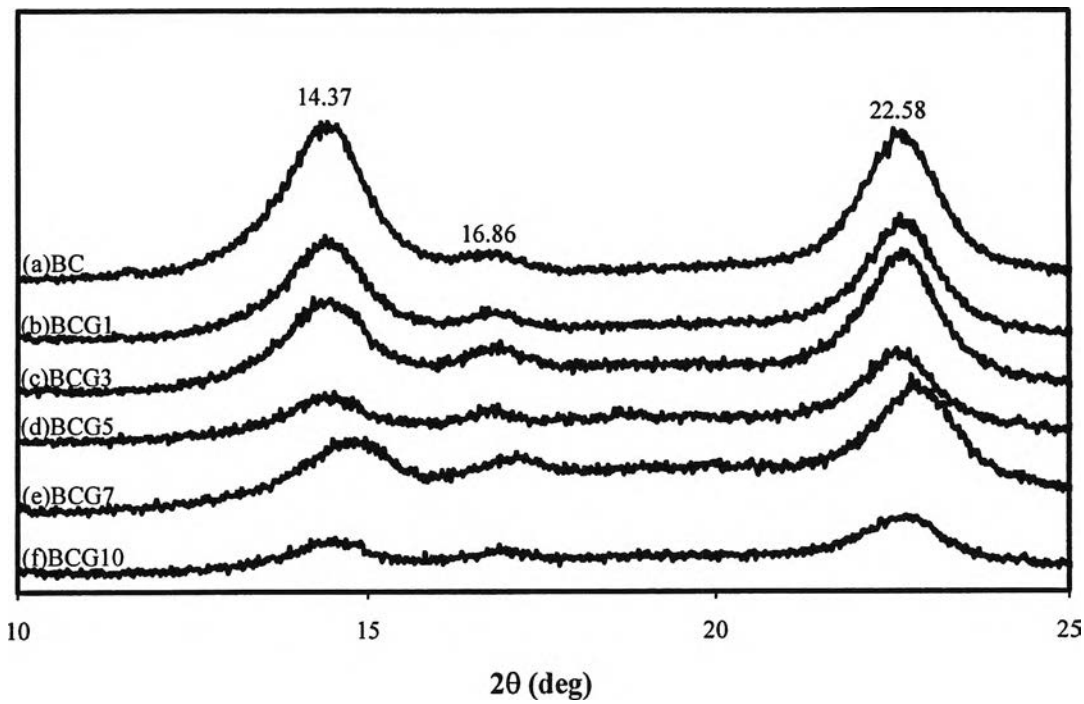


Figure 5.6 The XRD patterns of BC (a) and Biosynthesized BC-gelatin films (b-f)

5.1.4 Mechanical Property

In general, biomaterials used as wound dressing have to be handled by physician while applying them onto wound surfaces. So, the mechanical properties are often one of the most important properties because virtually all service condition and the majority of end-use applications involve some degree of mechanical loading. Therefore, in this study, the mechanical properties of the films were examined in terms of the tensile strength, which is the maximum stress applied to a point at the films specimen breaks (Vemuri, 2000) and elongation at break. As shown in Figure 5.7 and 5.8, the average tensile strength and the average elongation at break of dried BC film with the average thickness of 0.12 mm were 63.02 MPa and 1.39%, respectively. After the film was immersed in DI water until equilibrium swelling, these values became 8.32 MPa and 20.70%, respectively. In other words, the strength of the BC film in reswollen form was lower but it exhibited more elastic behavior than that of the BC film in dried form.

The results of tensile strength of the Biosyn-BCG films are shown in Figure 5.7. The tensile strength gradually reduced related to the amount of gelatin supplementation. The trend line illustrates a gradual decrease in tensile strength until minimum values at 29.33 and 0.03 MPa for dry and reswollen films, respectively. The reduction of mechanical properties of these films could confirm that the intermolecular interaction between cellulose and gelatin might present (see FT-TR section). The dried films yielded about 7-9 times of the tensile strength higher than that of the reswollen films. The dense fiber structure of the dried films and also

the stiffness of gelatin in dry state (Keenan, 2003; Phisalaphong and Jatupaiboon, 2008) could result in exceedingly high resistance to impact.

Moreover, Cai and Kim (2010) described about the reduction in tensile strength phenomenon for modifying BC with biopolymer such as PEG that the incorporation of biopolymer could affect the preferential orientation of the $(1\bar{1}0)$ plane during drying process. Consequently, this incomplete orientation of the $(1\bar{1}0)$ plane decreased in crystallinity of BC and which in turn leaded to a decrease in strength.

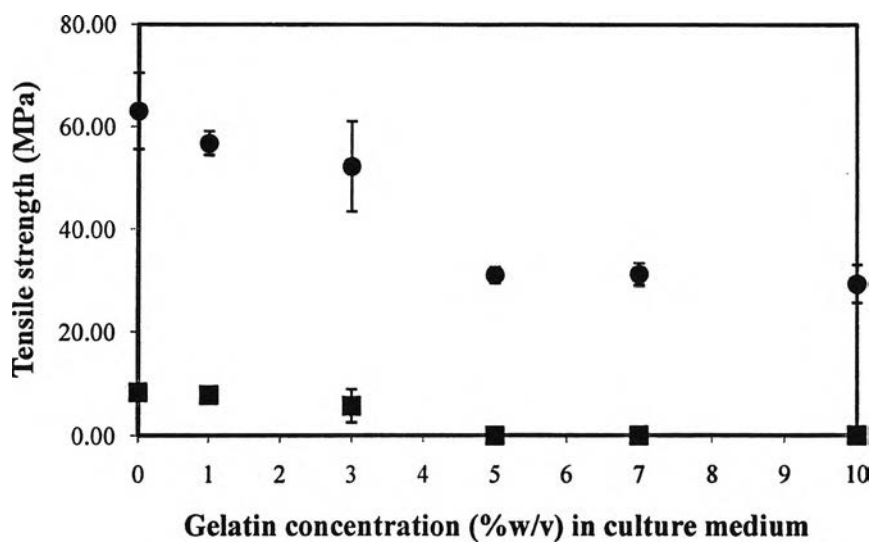


Figure 5.7 The tensile strength of the Biosynthesized BC-gelatin dried films (●) and reswollen films (■) as a function of gelatin concentration in culture medium.

Together, with increasing concentration of gelatin, the elongation at break of both reswollen and dry film continuously decreased to 7.34 and 1.04 %, respectively with the adding of 10% w/v gelatin as shown in Figure 5.8. Opposite to the results of the tensile strength, the dried films yielded about 0.07-0.14 times of the elongation at break lower than that of the reswollen films. These results suggested that the supplement of gelatin resulting in a harder and more brittleness films, which was due to the brittle characteristic of gelatin in a dry state (Keenan, 2003). After these Biosyn-BCG films were immersed in DI water until equilibrium swelling, they behave more elastic due to the swelling of cellulose fiber and gelatin in water (Phisalaphong and Jatupaiboon, 2008).

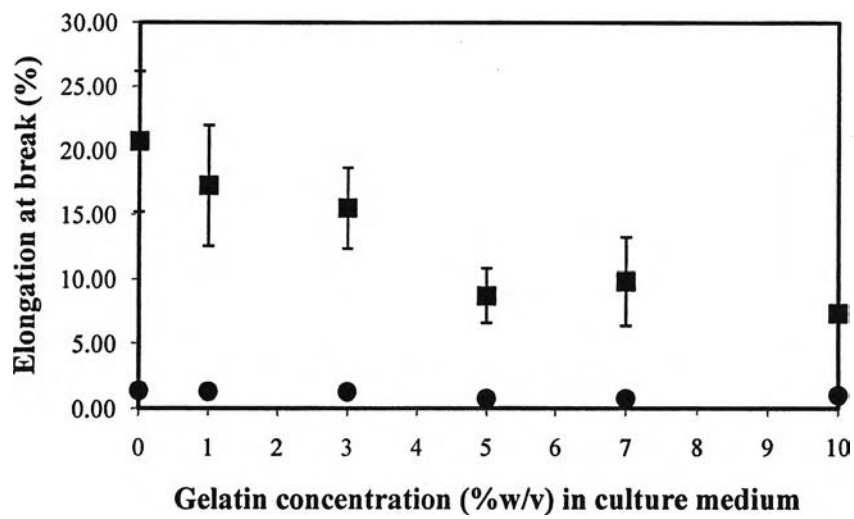


Figure 5.8 The elongation at break of the Biosynthesized BC-gelatin dried films (●) and reswollen films (■) as a function of gelatin concentration in culture medium.

5.1.5 Water Absorption Capacity (WAC)

Liquids sorption analysis of biomaterials is very important. Especially in the wound dressing application, the abilities to absorb the wound exudates and keep the wound dry and prevent airborne infection are essential. Consequently, the water absorption capacity analysis of biosynthesized BC-gelatin films was done by immersing the preweighted of dried film in distilled water at room temperature until equilibration.

From Figure 5.9, the water absorption capacity of BC was 613.84%. BC is extremely hydrophilic, absorbing 60 to 700 times its weight in water (Suwanmajo, 2006). In addition, the WACs of all modified BC films were higher than that of the BC. The Biosyn-BCG3 showed the maximum value of WAC at 762.53% or 1.24 times the value of the BC. Because of hydrophilic characters of gelatin (Keenan, 2003), the Biosyn-BCG films were more hydrophilic and enable to absorb more water than that of the BC film. However, with the gelatin supplementation from 3% to 5% and 7% w/v (Biosyn-BCG5 and -BCG7), the WACs of the films gradually decreased from 762.53% to 721.27% and 676.32%, respectively. It could be due to the loose fibrils network of these films. The decline in WAC with the gelatin supplementation from 3%-10% w/v was found analogous to the XRD result.

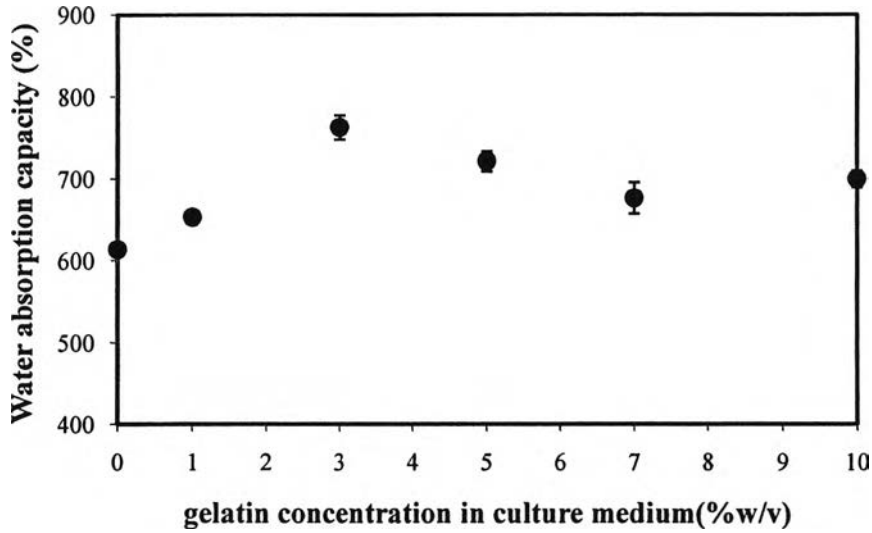


Figure 5.9 The water absorption capacity of Biosynthesized gelatin-BC films as a function of gelatin concentration in culture medium

5.1.6 Oxygen Permeability Test

Oxygen is essential to life. In wound healing, it plays a key role by supporting tissue regeneration and repair, by inhibiting anaerobic bacteria and supporting the body's natural defense mechanisms. In this study, all films were analyzed for oxygen transmission rate by following the ASTM D3985.

Table 5.1 shows the results of the oxygen transmission rate (OTR) of the BC and Biosyn-BCG film. As gelatin content increased to 5% w/v in culture medium, the OTR was slightly increased because of the relatively increased pore diameter in nanometers of these films (see SEM images), whereas the approximate diameter of oxygen molecule is 0.36 nm (Kanjanamosit *et al.*, 2009). In spite of this, gelatin filling in empty space between BC fibrils was observed in the Biosyn-BCG10 sample

leading to a reduction in the pore diameter of the Biosyn-BCG10 and caused the reduction in oxygen permeability.

Compared with the commercial wound dressing such as Bioprocess® (Cardona *et al.*, 1996) and gauze (Lowe, 2008) as shown in Table 5.1, the BC and Biosyn-BCG films in dry state exhibited significant lower oxygen transmission.

Table 5.1 The oxygen transmission rate of Biosynthesized gelatin-BC films (Mean value form duplicate test) and commercial wound dressings (Cardona *et al.*, 1996; Lowe, 2008).

Material	OTR±S.D. ($cc/m^2/day$)
BC	1.59±0.05
Biosyn-BCG3	1.60±0.03
Biosyn-BCG5	2.65±0.00
Biosyn-BCG10	1.74±0.07
Bioprocess®	277.34±54.45
Gauze	14275.00

5.1.7 Water Vapor Permeability Test

The good wound dressings not only absorb wound exudates to keep the wound dry, but also have to control evaporative fluid loss from wounded skin which was a necessary property to accelerate the wound healing process. Generally, the evaporative water loss for normal skin is 204.0±115.2 $g/m^2/day$, while that for the burn skin is 5138±202 $g/m^2/day$. Thus, water losses from burn skin can be up to 20 times greater than from normal skin (Cardona *et al*, 1996; Wu *et al.*, 1996).

All films were analyzed for water vapor transmission property by following the ASTM E-96. From Figure 5.10, the water vapor transmission rate (WVTR) of BC films was 1026 $g/m^2/day$, which was higher than that of all Biosyn-BCG films. The Biosyn-BCG3 showed the minimum value of WVTR at 592 $g/m^2/day$. Despite the lower WAC value of the BC, according to the more uniform porous structure of the BC, the water vapor should be easier to diffuse though the film. However, in dry state, these films showed relatively lower WVTR compared with other commercial wound dressings such as Bioprocess®, Biobrane II® (Cardona *et al*, 1996; Lowe, 2008).

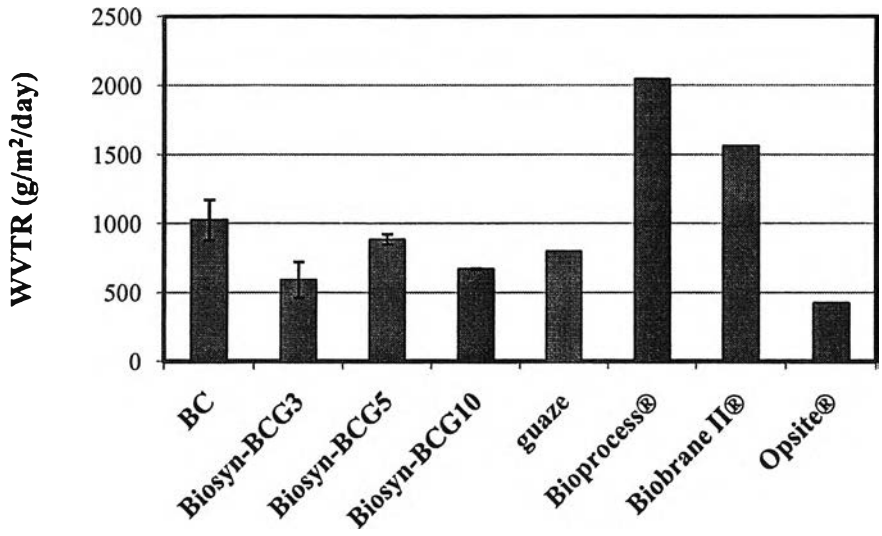


Figure 5.10 The water vapor transmission rate of Biosynthesized BC- gelatin films compared with commercial dressing (Cardona *et al.*, 1996; Lowe, 2008).

5.2 Characterization of Gelatin-Impregnated BC Films

The characteristics of BC-gelatin composite films prepared by impregnation method (as previously described in chapter IV) were analyzed in this section. Herein, all BC-based composite films prepared by this technique were referred to as Impreg-BCG*n*, where *n* is the concentration of gelatin aqueous solution used for creating gelatin-impregnated BC films.

5.2.1 Morphology

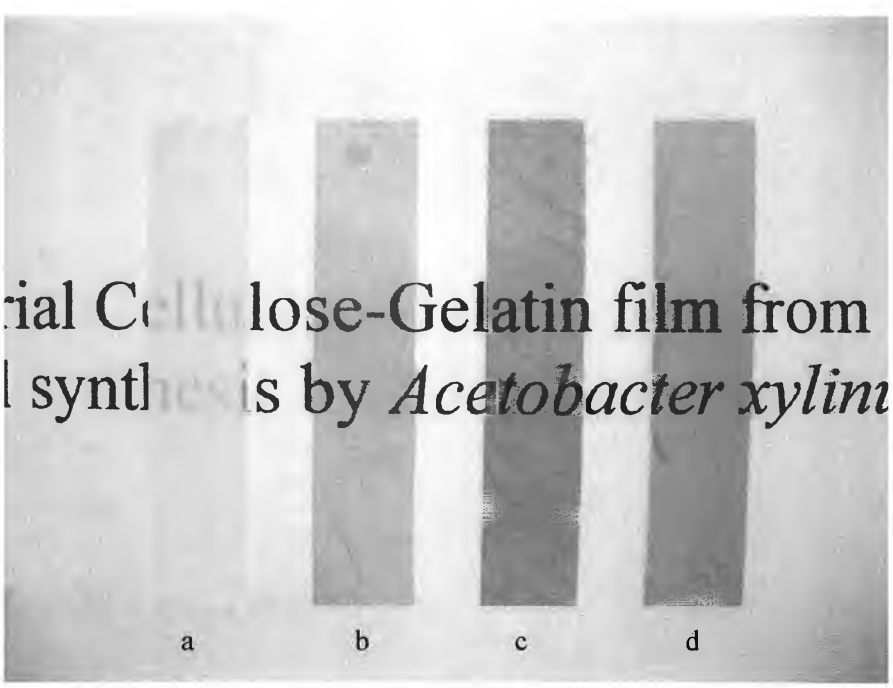


Figure 5.11 Optical photographs of BC-based films: BC (a), Impreg-BCG15 (b), Impreg-BCG30 (c) and gelatin films (d).

Figure 5.11 illustrated the effect of the presence of gelatin in the BC film on the transparency of BC-based composite film. Despite being prepared from different approaches, the effect of gelatin on the optical transparency of nanocomposite films developed by impregnation method was similar to those of the Biosyn-BCG films. The incorporation of gelatin into BC leads to an improvement in the optical transparency of BC films. BC is therefore a promising material for creating transparent nanocomposite films owing to its high porosity.

Figure 5.12 shows the surface morphology of the reswollen Impreg-BCG films. From observation, the incorporation of gelatin gel in the BC matrix of the Impreg-BCG films was different from that of the Biosyn-BCG films. Due to the ability of tannic acid to precipitate protein (Yan and Bennick, 1995), gelatin in form of small particles was observed in the Impreg-BCG films. In Figure 5.12 (b) and (c), the amount of gelatin particles related to the gelatin concentration in the impregnated solution and the gelatin particles appeared to be randomly distributed on the Impreg-BCG surface.

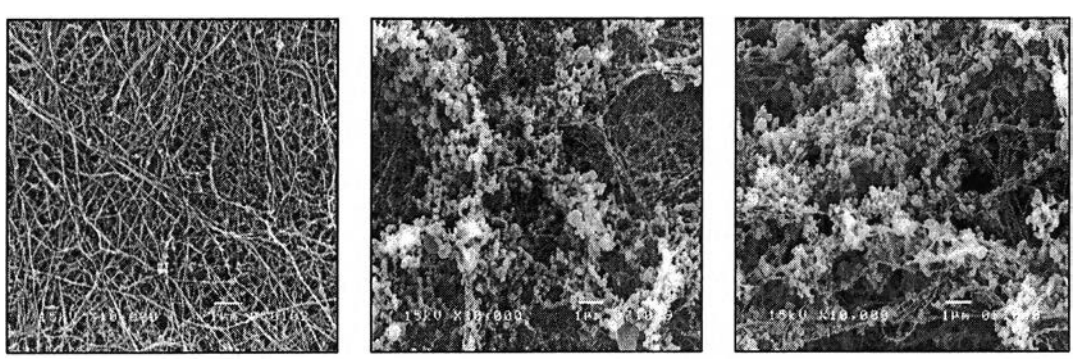


Figure 5.12 SEM images of surface morphology of BC (a), Impreg-BCG15 (b) and Impreg-BCG30 (c) film in reswollen form from Gelatin Impregnation method

From cross sectional images (Figure 5.13), the gelatin particles can penetrate into the BC fibrils and form a thin layer. As a result, significantly changes occurred in the arrangement of BC fibrils and the film thickness. The mean thickness of the Impreg-BCG15 and Impreg-BCG30 films were 300 and 355 μm, respectively, which was about 83-85 percent higher than that of the unmodified BC film (50 μm).

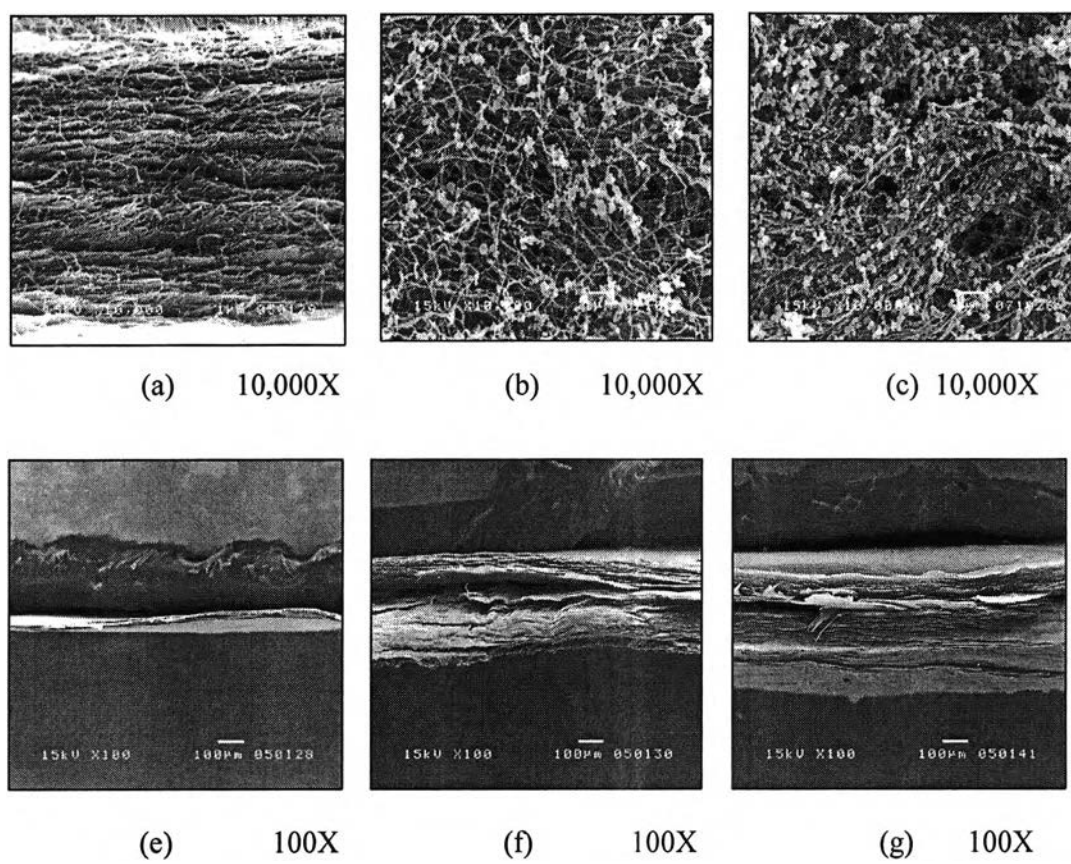


Figure 5.13 SEM images of cross section of BC (a, e), Impreg-BCG15 (b, f) and Impreg-BCG30 (c, g) film in reswollen form from Gelatin Impregnation method

5.2.2 FTIR Analysis

The sample of BC and Impreg-BCG films were also analyzed by Fourier Transform Infrared (FTIR) spectroscopy. The FTIR spectra of all samples were detected at wave number ranging from 4000 to 450 cm^{-1} as shown in Figure 5.14. The BC film showed a band at 3427.77 and 1643.38 cm^{-1} (Figure 5.14(a)) which was attributed to –OH group and -COO group of cellulose, respectively (Phisalaphong and Jatupaiboon, 2008; Kanjanamosit *et al.*, 2009; Cai and Kim, 2010). From Figure 5.14 (d), the characteristic absorptions of gelatin film were the bands at 3299.60, 1631.10 and 1529.82 cm^{-1}, which were assigned to O-H stretching vibration, Amide I (C=O and C-N stretching vibration) and Amide II (N-H bending vibration), respectively (Dong *et al.*, 2006; Kim *et al.*, 2010). The FTIR results demonstrated that the characteristic absorption bands of all the Impreg-BCG films were only slightly shifted from a wave number at 1643.38 cm^{-1} to a wave number at 1643.49 and 1643.10 cm^{-1}, respectively as showed in Figure 5.14 (a-c). At the same time, those films also showed the new band of Amide II at 1534.92 and 1530.69 cm^{-1}, respectively which were due to the presence of N-H bending vibration of gelatin (Dong *et al.*, 2006). In addition, the peaks attributed to O-H stretching vibrations at wave numbers of 3413.63 and 3415.80 cm^{-1} were slightly different from the band of the BC film and gelatin. All those changes could imply weak interactions between the OH group of BC and gelatin (Dong *et al.*, 2006).

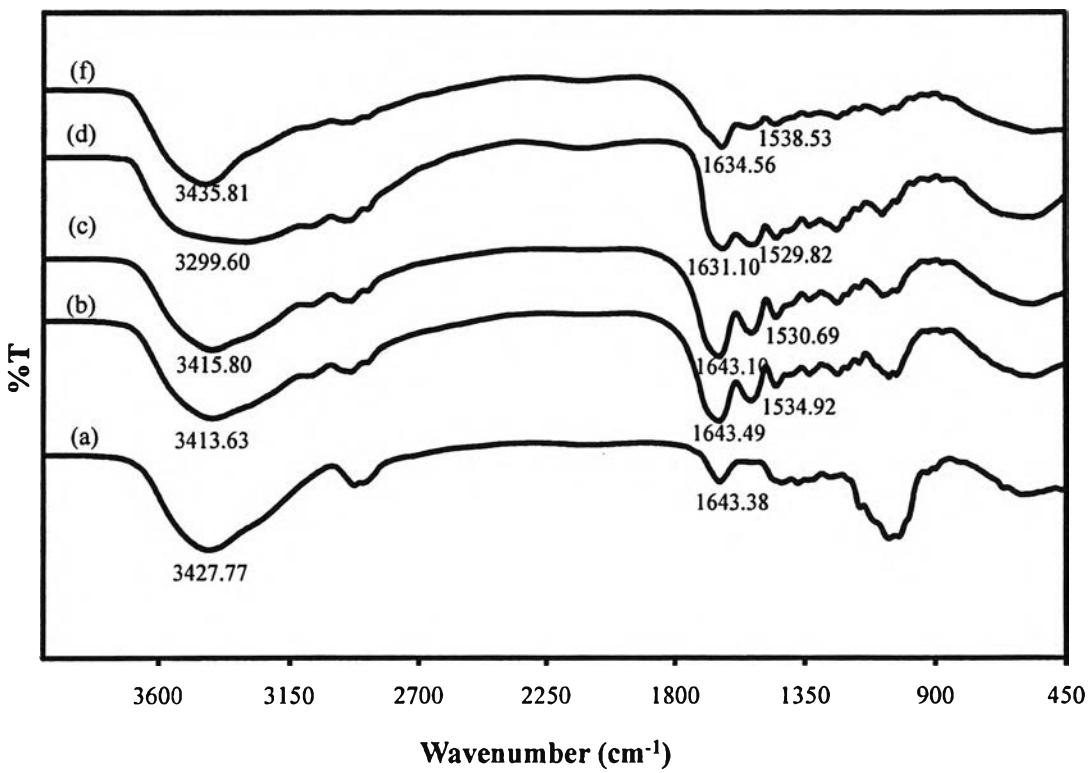


Figure 5.14 The FTIR spectra of BC (a), gelatin impregnated BC films which were immersed in 15% (b) and 30% w/w aqueous gelatin solution (c), gelatin film (d) and gelatin powder (f).

5.2.3 XRD Analysis

In this study the crystallinity index of dried films were simply calculated from X-ray diffraction data. The XRD data of BC and Impreg-BCG films are shown in Figure 5.15. For BC, three main peaks was at 14.64 deg, 16.97 deg and 22.70 deg for the $(1\bar{1}0)$, (110) and (200) planes, respectively, which can be identified in reflexion planes of cellulose I (Kim *et al.*, 2010; Cai and Kim *et al.*, 2010). For the Impreg-BCG films, there were no observations of gelatin diffraction peak, which gelatin has a broad diffraction peak in the 2θ range 20–22 deg (Kim *et al.*, 2010) and 1200 count in intensity (Pal *et al.*, 2007). The diffraction peaks of Impreg- BCG films had no significantly changes in peaks comparing with BC. For BC, the C.I. was 55.67% and for Impreg-BCG15 and Impreg-BCG30 films, it was 55.94% and 56.67%, respectively. These results indicated that there were no significant changes in the crystalline structure of the Impreg-BCG films during the incorporation of gelatin.

The similar results were described by Kim *et al.*, 2010. Their BC/gelatin composites were prepare by immersing the wet BC pellicle in 1% gelatin aqueous solution for 2 h in room temperature and dried by a freeze-dryer at −40°C for 3 days. They found that the BC/gelatin composite had no significant changes in the crystalline structure.

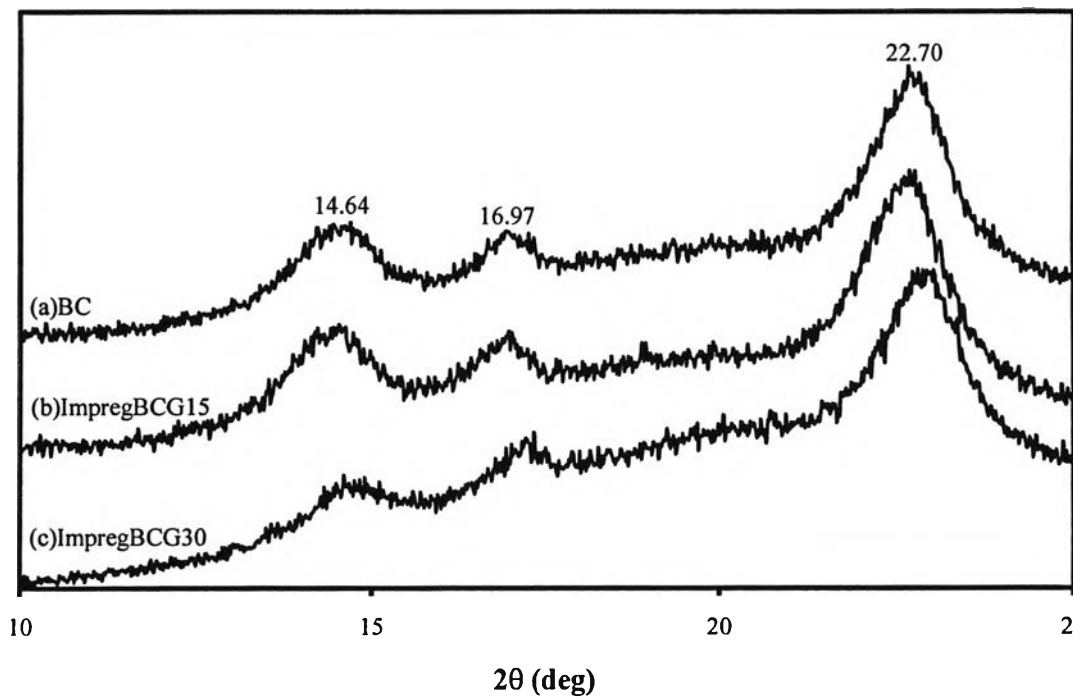


Figure 5.15 The XRD patterns of BC (a) and Gelatin impregnated BC films (b-c)

5.2.4 Mechanical Property

In this study, the mechanical properties such as the tensile strength and elongation at break of the films were examined. In general, the biomaterial used as wound dressing have to be handled by physician while applying them onto the wound surfaces. So, the mechanical properties are often one of the most important properties because virtually all service condition and the majority of end-use applications involve some degree of mechanical loading.

As shown in Figure 5.16, the tensile strength of the BC, Impreg-BCG15 and Impreg-BCG30 dried film significantly decreased from 54.32 MPa to 33.78 and 11.53 MPa, respectively. In the similar way, the tensile strength of the BC, Impreg-BCG15 and Impreg-BCG30 reswollen film decreased from 10.26 MPa to 0.43 and 0.38 MPa, respectively. The dried films had higher tensile strength than that of the reswollen films.

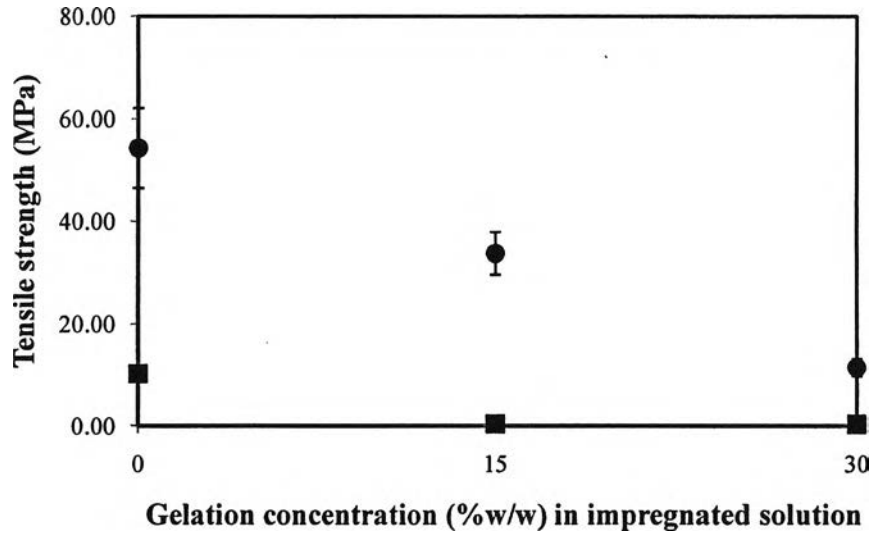


Figure 5.16 The tensile strength of the Biosynthesized BC-gelatin dried films (●) and reswollen film (■) as a function of gelatin concentration in impregnated solution.

In a similar way to the previous observation, the dried films yielded about 0.026 - 0.071 times the elongation at break lower than of the reswollen films (Figure 5.17). The elongation at break of the dried films impregnated in 15 and 30% w/w of gelatin aqueous solution relatively decreased from 1.73% of typical BC to 1.38% and 0.46%, respectively. The elongation at break of the reswollen films impregnated in 15% and 30% w/v of gelatin aqueous solution relatively decreased from 24.15% of typical BC to 21.52% and 17.24%, respectively. Owing to the brittle characteristic of gelatin in a dry state (Keenan, 2003), it was found that the supplement of gelatin caused harder and more brittle films.

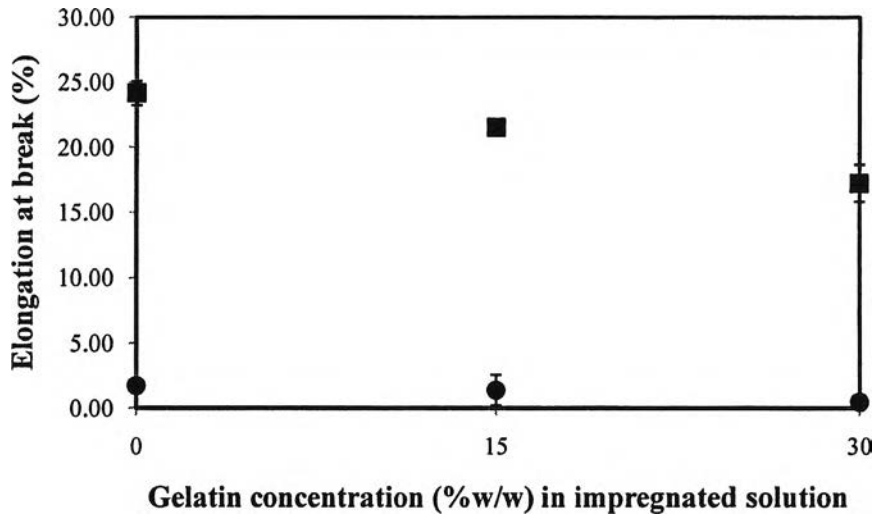


Figure 5.17 The elongation at break of the Biosynthesized BC-gelatin dried films (●) and reswollen film (■) as a function of gelatin concentration in impregnated solution.

5.2.5 Water Absorption Capacity (WAC)

The modified film with gelatin exhibited the higher water absorption capacity. From Figure 5.18 the water absorption capacities of BC, Impreg-BCG15 and Impreg-BCG30 were 605.94%, 664.18% and 677.94%, respectively. The WAC increased related to the increase of gelatin content. Because of hydrophilic characters of gelatin which has hydroxyl group, amine group and carboxyl group on its backbone (Keenan, 2003), the Impreg-BCG films were more hydrophilic and enable to absorb more water than that of the BC film. In comparison to the Biosynthesis method, a larger amount of gelatin could be incorporated into the BC film by the impregnation method. However, it was found that the water absorption capacity of the Impreg-BCG films

was less than that of the Biosyn-BCG films. Because of binding between tannic acid cross-linking agent and gelatin, amine group of gelatin was largely bound via hydrogen bond with the phenolic hydroxyl groups of the tannic acid as well as the hydrophobic interactions in tannic acid – gelatin interaction took place (Buren *et al.*, 1969; Yan *et al.*, 1995). Consequently, the WAC was limited.

This result was similar to the report by Natarajan *et al.* in 2005. The WAC of Fibrin-Chitosan-Gelatin composite film, prepared by Fibrin paste, chitosan and gelatin solutions mixed in different stoichiometric ratios, decreased with the cross-linking reaction by glutaraldehyde. It was explained that the two –CHO groups of glutaraldehyde might have cross-linked with the $-NH_2$ groups of both fibrin/chitosan and gelatin, thereby forming a bridge (Schiff bases) between the two backbones resulting in the decrease of WAC.

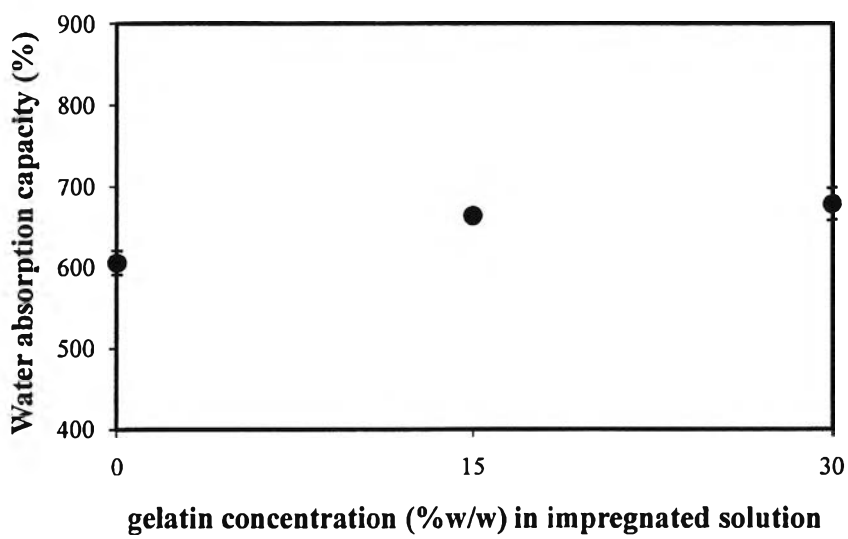


Figure 5.18 The water absorption capacity of Gelatin impregnated BC films as a function of gelatin concentration in impregnated solution

5.2.6 Oxygen Permeability Test

In this study, water vapor transmission property of all films was analyzed following the ASTM D3985. From table 5.2 shows the results of the oxygen transmission rate (OTR) of the BC and Biosyn-BCG films comparing with the commercial wound dressing such as Bioprocess® (Cardona *et al.*, 1996) and gauze (Lowe, 2008). Due to the small pore diameter of the BC and BCG films in dry state, the corresponding values of OTR were considerably low. The increase in gelatin concentration in impregnated solution (0-30% w/w) had no considerably effect on the OTR of these films.

Table 5.2 The oxygen transmission rate of Biosynthesized gelatin-BC films (Mean value form duplicate test) and commercial wound dressings (Cardona *et al.*, 1996; Lowe, 2008).

Material	OTR±S.D. ($cc/m^2/day$)
BC	2.67±0.32
Impreg-BCG15	1.56±0.17
Impreg-BCG30	1.65±0.01
Bioprocess®	277.34±54.45
Gauze	14275.00

5.2.7 Water Vapor Permeability Test

From the previous result, the gelatin concentration in impregnated solution (0-30% w/w) did not significantly affect the degree of swelling of the films. However, the water vapor transmission rate (WVTR) of the Impreg-BCG films was relatively enhanced in comparison to the BC film or the Biosyn-BCG films (Figure 5.19). The more hydrophobic property from cross-linking with tannic acid was supposed to cause the less water vapor absorption and thus improve water vapor permeability. The Impreg-BCG films had a WVTR comparable to the WVTR of the commercial dressing such as Biobrane II® (composite of polydimethylsiloxane on nylon frabic).

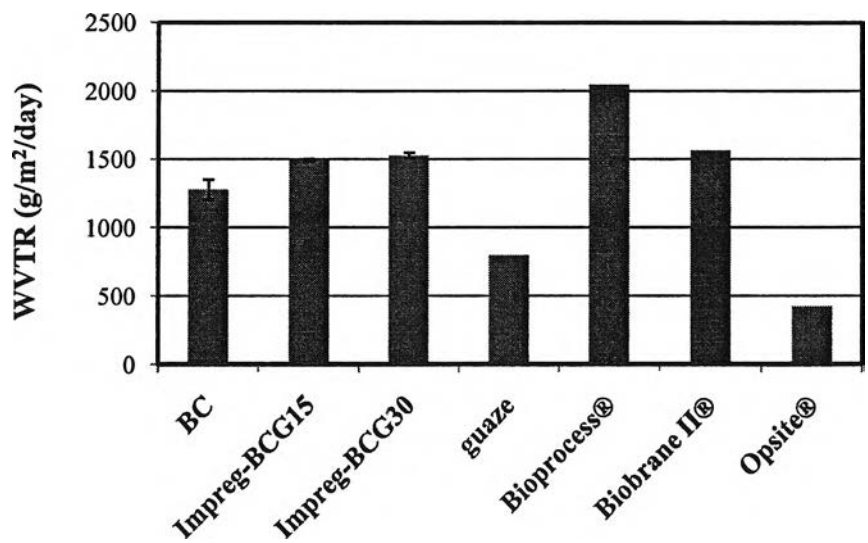


Figure 5.19 The water vapor transmission rate of Impregnated BC- gelatin films compared with commercial dressing (Cardona *et al.*, 1996; Lowe, 2008).

5.2.8 Antibacterial Ability

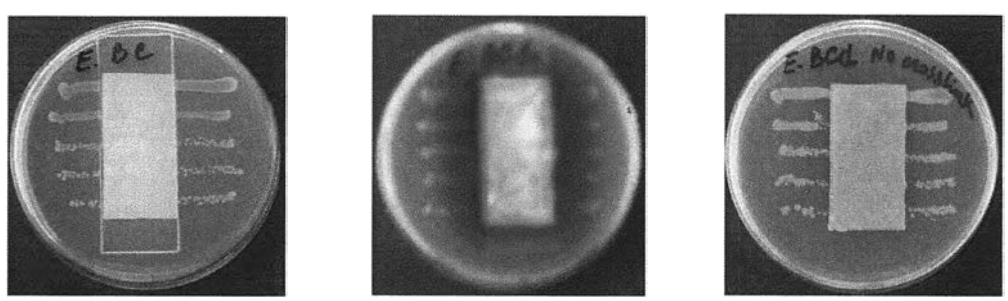
In this study, both *Escherichia coli* and *Staphylococcus aureus* which is representative Gram (-) and Gram (+) bacteria, respectively were used as the test bacteria to examine the antibacterial properties of BC and BCG films. The antimicrobial activities of the BC, Impreg-BCG30 and uncross-link Impreg-BCG30 films are shown in Table 5.3 and Figure 5.20. The presences of BC and uncross-linked ImpregBCG30 films inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* under the samples. The result indicated that the BC and uncross-linked Impreg-BCG films could be restrain proliferation of both bacterial cells. Besides, it could imply that the Biosyn-BCG films exhibited antimicrobial activity specifically under the films. The Impreg-BCG30 films inhibited the growth of *Escherichia coli* under the sample. Moreover, it inhibited the growth of *Staphylococcus aureus* under the sample and around the sample with the clear zone of 5.0 mm in width. It indicated that tannic acid, the cross-linking agent, had strongly effect on the growth of *Staphylococcus aureus*. Akiyama *et al* (2001) reported that 100 mg/l of tannic acid exhibited the antibacterial action against *Staphylococcus aureus* after incubation for 24 h; moreover, tannic acid could be use as adjuvant agent for treatment of *Staphylococcus aureus* skin infections in addition to antibiotics.

Table 5.3 The antibacterial activity of BC and BC-gelatin film on *Escherichia coli* and *Staphylococcus aureus* at the end of the incubation for 24 h.

Bacteria	Sample	Observed growth
		Result
Escherichia coli	BC	Inhibition of growth under the sample
	Impreg-BCG30 (No crosslink)	Inhibition of growth under the sample
	Impreg-BCG30	Inhibition of growth under the sample
Staphylococcus aureus	BC	Inhibition of growth under the sample
	Impreg-BCG30 (No crosslink)	Inhibition of growth under the sample
	Impreg-BCG30	Inhibition of growth 5.0 mm. in width of clear zone

BC | Impreg-BCG30 | Impreg-BCG30 (No crosslink)

On *Staphylococcus aureus*



BC | Impreg-BCG30 | Impreg-BCG30 (No crosslink)

On *Escherichia coli*

Figure 5.20 Inhibition tests of samples on bacteria for 24 h. incubated at 37°C

5.2.9 Antifungal Ability

In this study, *Aspergillus niger* was used as the test fungi to examine the antifungal activity of BC and BC-gelatin films. The antimicrobial effect of BC, Impreg-BCG30 and uncross-link Impreg-BCG30 are shown in Table 5.4 and Figure 5.21, *Aspergillus niger* could grow completely on all films. The result implied that tannic acid did not exhibit antifungal property.

Diepeningen *et al.* (2004) studied about degradation of tannic by *Aspergillus niger*. They reported that *Aspergillus niger* could grow in tannic acid solution (20% w/v) and utilized high concentration of tannic acid, which set them apart from all related fungi. On the other hand, tannic acid could act as an inducer in the production of tannase enzyme by using *Aspergillus niger* (Aissam *et al.*, 2005; Lokeswari and Jayaraju, 2007).

Table 5.4 The antifungal activity of BC and BC-gelatin film on *Aspergillus niger* activity at the end of the incubation for 7 days.

Fungi	Sample	Observed growth
		Result
Aspergillus niger	BC	Heavy growth (more than 60%)
	Impreg-BCG30 (No crosslink)	Heavy growth (more than 60%)
	Impreg-BCG30	Heavy growth (more than 60%)

BC | Impreg-BCG30 | Impreg-BCG30 (No crosslink)

Figure 5.21 The growth of *Aspergillus niger* on the specimens, at 30°C at the end of the incubation 7 days.

5.3 Cell Study

The MTT assay was used for the evaluation of cell growth. The data of this assay were showed in terms of absorbance which can be quantified by measuring at a certain wavelength by a spectrophotometer and proportional to the number of living cells cultured on the samples (Svensson *et al.*, 2005). In this study, the evaluation of Vero cells growth on the samples which has been recommended for cytotoxicity studies in biomaterial research (Santos *et al.*, 2009) was performed by using MTT assay at a wavelength of 550 nm. From Figure 5.22, the absorbance of living cells after seeding on Biosyn-BCG5 and Biosyn-BCG10 film for 0, 24 and 48 h was comparable to that of the cells cultured on BC (control). The cells were able to grow on all samples. After 48 h, the growth rate of cells on the Biosyn-BCG10 was

1.8 times higher than that on BC whereas, the growth rates of cells on Biosyn-BCG5 and the impreg-BCG15 were lower. From these results, it was possible to conclude that the modified BC film did not present toxicity against Vero cells.

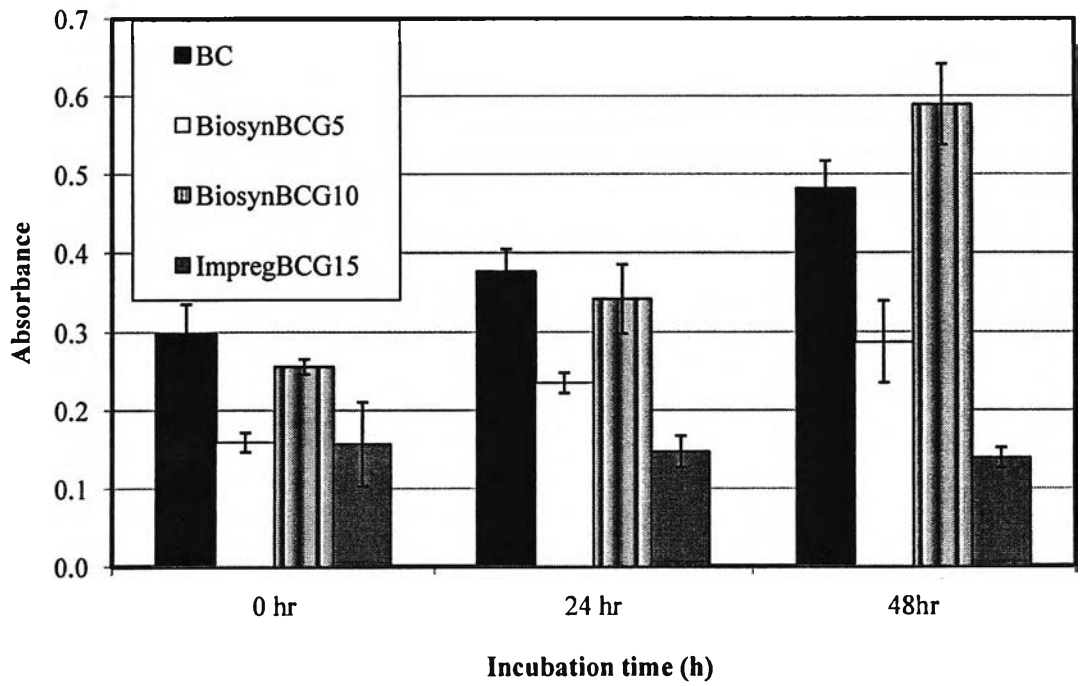


Figure 5.22 MTT results of viability of Vero cells on BC (control) and modified BC films