Characteristics of Curcumin-Loaded Bacterial Cellulose Films and Anticancer Properties against Malignant Melanoma Skin Cancer Cells

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Received: 6 June 2018; Accepted: 11 July 2018; Published: 20 July 2018

Abstract: Curcumin-loaded bacterial cellulose films were developed in this study. Curcumin was absorbed into never-dried bacterial cellulose pellets by 24-h immersion in solutions of curcumin in the range of 0.2–1.0 mg/mL. The curcumin-loaded bacterial cellulose pellets were then air-dried and characterized. The mechanical properties of curcumin-loaded bacterial cellulose films, particularly the stretching properties, appeared to be lower than those of bacterial cellulose film. This was especially evident when the loading concentration of curcumin was higher than 0.4 mg/mL. Fourier-transform infrared spectroscopy analysis indicated an interaction between bacterial cellulose microfibrils and curcumin. Controlled release of curcumin was achieved in buffer solutions containing Tween 80 and methanol additives, at pH 5.5 and 7.4. Curcumin-loaded bacterial cellulose films prepared with curcumin solutions at concentrations of 0.5 and 1.0 mg/mL displayed antifungal activities against Aspergillus niger. They also exhibited anticancer activity against A375 malignant melanoma cells. No significant cytotoxic effect was observed against normal dermal cells, specifically, human keratinocytes and human dermal fibroblasts.

Keywords: bacterial cellulose; curcumin; absorption; release; skin cancer; malignant melanoma

1. Introduction

Cellulose is the most abundant natural polymer in the world and has been used in various biomedical applications throughout the past decades. Bacterial cellulose (BC) is obtained from cellulose-producing bacterial strains such as Gluconacetobacter xylinus (previously named Acetobacter xylinus, and originally Acetobacter xylinum) [1]. BC is a source of highly pure cellulose, which possesses superior properties to plant cellulose, including high mechanical strength, high crystallinity, ultra-fine network structure, high hydrophilicity, and biocompatibility [2,3]. The potential of never-dried BC has been studied in biomedical applications such as cartilage tissue engineering [4], replacement of blood vessels in rats [5], artificial skin for humans with extensive burns [6], wound dressings [7], and controlled release [8]. Many medical treatments require the constant and slow release of a particular drug at a controlled concentration [9]. The rapid release of active components from never-dried BC gel has been reported [8], and so, modification of BC gel to achieve slower release has been studied,
using techniques such as irradiation. However, these techniques are energy-consuming [10,11], and lower energy alternatives are required to make the use of BC gels for drug release economically viable. Due to the high shrinkage that occurs during drying, it has been demonstrated that drugs or active components can be entrapped in BC film, and slowly released as the BC film is rehydrated [12]. Thus, dried BC could be a candidate polymer matrix film for the local slow release of active components. This is a promising method to overcome the problems of degradation of active components by enzymes or acids that commonly occur following oral administration or injection.

Skin cancer is a major, global public health problem [13]. Malignant melanoma cases have quadrupled over the 30-year period between 1982 and 2012 [14]. It represents around 4% of all cases of skin cancer, but is responsible for 65% of all deaths caused by skin cancer [15]. The most common risk factors for malignant melanoma are ultraviolet radiation, prolonged childhood sun exposure, aging, family history, and immunosuppression [15]. Current treatment for skin cancer is typically surgery [14].

Curcumin (diferuloylmethane) is one of the bioactive compounds found in turmeric (*Curcuma longa* Linn). The crude extract of this plant is rich in polyphenols, mostly in the keto or enol forms, such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin [16–18]. Extracts of turmeric are used extensively for coloring and flavoring South Asian cuisine, and are also used in alternative medicine. Among the compounds contained in crude turmeric extract, curcumin exhibits outstanding pharmacological activities. For example, the compound has been shown to have anti-inflammatory, antioxidant, antihapatotoxic, antimicrobial, antidepressant, and anticancer properties. It has been reported to kill tumor cells and inhibit the proliferation of cancer cells, primarily through induction of apoptosis. This activity has been observed against malignant cells including: breast [19,20], thyroid [21,22], colon [23], leukemia [24,25], prostate [26], lung [27], ovarian [28] and pancreatic cancer cells [29], as well as malignant melanoma cells [30]. Curcumin has also been shown to inhibit vital processes of cancerous cells, including adhesion, migration, invasion, and spreading. Therefore, curcumin is cytotoxic to cancer cells but not to normal cells. The effects of curcumin entrapped in BC film have not yet been investigated. However, most curcumin is degraded within 30 min at physiological pH [18]. Furthermore, in vivo, low oral bioavailability has been reported in rodents and humans due to degradation by acidic bile and rapid intestinal metabolism [31].

In this study, the potential of BC as a matrix for controlled release of curcumin was evaluated. Curcumin was absorbed into BC pellicles, after which the pellicles were air-dried to produce curcumin-loaded BC films (BCC). The absorption and release characteristics of curcumin from BCC were assessed, and various properties including morphological, chemical, physical, and mechanical properties were characterized. The antibacterial properties of the BCC films were assessed against *Escherichia coli* and *Staphylococcus aureus*, as well as the antifungal properties against *Aspergillus niger*, and the cytotoxic effects and anticancer properties against A375 malignant melanoma skin cancer cells.

2. Materials and Methods

2.1. Materials

Curcumin (>95% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sucrose and sodium acetate were purchased from Ajax Finechem (NSW, Australia). Ammonium sulfate, sodium hydroxide and acetic acid were purchased from Carlo Erba (Cornaredo, Italy). Absolute ethanol was purchased from Merck (Darmstadt, Germany). Acetone and dimethylacetamide were purchased from RCI Labscan (Bangkok, Thailand). Dulbecco’s Modified Eagle Medium (DMEM), Roswell Park Memorial Institute culture medium (RPMI), Fetal Bovine Serum (FBS), and cell culture antibiotics (penicillin and streptomycin solution) were purchased from Gibco (Carlsbad, CA, USA). Other chemicals unless otherwise stated were purchased from Sigma-Aldrich (Buchs, Switzerland).
2.2. Cells, Microbial, and Fungal Strains

Gluconacetobacter xylinus (G. xylinus) strain AGR60 used for BC synthesis was kindly supplied by Pramote Thammarad, the institute of Research and Development of Food Product, Kasetsart University, Bangkok, Thailand. Escherichia coli (E. coli; ATCC 25922), Staphylococcus aureus (S. aureus; ATCC 12600), Aspergillus niger (A. niger; ATCC 16404) were used for the study of antimicrobial activities. Human dermal fibroblast (HDF), human keratinocyte cells (HaCat) and human malignant melanoma cells (A375) were used for the study of cytotoxicity and anticancer properties [32].

2.3. Preparation of Curcumin-Loaded BC Film

The culture medium was coconut water supplemented with 5.0% sucrose, 0.5% ammonium sulfate, and its pH was adjusted at 4.5 by 30.0% (v/v) acetic acid. The medium was sterilized at 110 °C for 5 min. Pre-cultures were prepared by transferring 10 mL of a stock culture of G. xylinus AGR60 to 200 mL of medium in 500 mL bottles, followed by static incubation at 33 °C for 7 days. After the surface pellicle was removed, the 5% (v/v) pre-culture broth was added to the main culture medium. The 75 mL of activated medium was inoculated in a 14.5 cm diameter sterile Petri-dish under ambient conditions (33 °C, 70% relative humidity and 1 atm). The BC pellicle was harvested, washed with running tap water for 2 h, treated with 1% w/v NaOH for 24 h, boiled, and washed several times with DI water until pH became neutral.

Curcumin solutions at the concentrations of 0.2 to 1.0 mg/mL were prepared by dissolving curcumin powder in absolute ethanol. Purified BC pellicles were immersed into 150 mL of the curcumin solutions under ambient conditions for 24 h. Afterwards, the BC-curcumin films (BCC) were air-dried at room temperature (30 °C) and stored in the plastic film. The film of BCCx refers to the BCC film prepared by immersion into the curcumin solution at x mg/mL.

2.4. Curcumin Content Analysis

The amount of curcumin absorbed in BC was determined. Each of the BCC specimens (2.5 × 2.5 cm²) were immersed in 4 mL of 2:1 v/v acetone/dimethylacetamide (DMAc) solution at room temperature (30 °C). A 0.5 mL of the solution was mixed with 8 mL of the acetate buffer solution, and the actual amount of curcumin content was measured by a UV-vis spectrophotometer (Shimadzu UV-2550, Tokyo, Japan) at the wavelength of 420 nm.

2.5. Characterization of Curcumin-Loaded BC Films

Surface and cross-sectional morphologies of BCC films were characterized by Scanning electron microscopy (SEM) (JEOL JSM-5410LV, Tokyo, Japan). The BCC films were sputtered with a thin layer of gold in Balzers-SCD 040 sputter coater (Balzers, Liechtenstein). The specimens were examined at 10,000× magnification and 15 kV. The rehydrated BC was dehydrated in ascending grade of ethanol and dried in a Tousimis Samdri-780 critical point dryer (Rockville, MD, USA) prior to sputtering with gold and scanning.

Water vapor transmission rates (WVTR) of BC and BCCs with an area of 50.00 cm² were investigated by means of desiccant method according to ASTM E-96 under 38 °C and a relative humidity of 98%.

Oxygen transmission rates (OTR) of BC and BCCs were measured using an oxygen permeation analyzer 8000 (USA) conforming to ASTM D3985. The test condition was carried out at 23 °C and relative humidity of 0%.

Chemical interactions between BC and curcumin were identified by Fourier transform infrared (FTIR) spectroscopy (Spectrum One, MA, USA) in the region of 2000–1000 cm⁻¹.

Mechanical properties of dried BC and BCC films were measured by Instron Testing Machine 5567 (Copiague, NY, USA). The test conditions followed ASTM D882. The film samples were cut into 1 × 10 cm². Two ends of the specimens were placed between the upper and lower jaws of the instrument, leaving the testing area of 1 × 6 cm². Extension speed of the instrument was 10 mm/min. Tensile strength, Young’s modulus, and elongation at break were the average values determined from at least five specimens.
2.6. In Vitro Release Assay

The release characteristic of curcumin from BC was investigated by placing BC/curcumin film specimen in modified Franz diffusion cells [12]. Physiological PBS (pH 7.4) and acetate buffer (pH 5.5), along with buffers with 0.5% v/v Tween 80 and 3% v/v methanol were used as release medium. Each water-swollen film specimen (35 mm in diameter) was placed on a receptor compartment of the diffusion cell before covering with a glass lid. The opening of the donor cell was sealed with a plastic film. The receptor chamber was fully filled with 11.5 mL of acetate buffer (pH 5.5) or phosphate buffer (pH 7.4). The diffusion cells were water jacketed and the temperature was maintained at 37 °C throughout the experiment via a circulating water bath. The receptor fluid was kept at 37 °C with constant stirring using a Teflon-coated magnetic stir bead. During the release testing (0–48 h), 0.3 mL sample was withdrawn from the receptor through a sampling port and an equal amount of the fresh buffer was immediately refilled. The concentration of released curcumin was then analyzed by measuring the absorbance of collected samples using the UV-vis spectrophotometer at the wavelength of 420 nm.

2.7. Antimicrobial Property Assessment

The antibacterial property against E. coli and S. aureus was assessed by using the agar diffusion test, according to AATCC Test Method 147-1998. The BCC films were cut into 25 × 50 mm² and sterilized by UV irradiation for 20 min on each side. The film was put on bacteria cultured on agar for 24 h at 37 °C. The antifungal property against A. niger was evaluated according to AATCC Test Method 39-1989. The films were punched into a round shape with 3.8 cm diameter and then sterilized. The test for A. niger was performed on an agar plate at 30 °C for 7 days.

2.8. Measurement of Anticancer Properties

The BCC0.5 and BCC1.0 films were tested for cytotoxic effects against A375 cells. HDF and HaCat cells were used as controls. BCC films were cut into circular sheets with diameters of 15 mm. They were then sterilized by autoclaving at 121 °C for 15 min, and dried at 70 °C overnight. HDF and HaCat cells were cultured in Dulbecco modified eagle medium (DMEM), while A375 cells were cultured in Roswell Park Memorial Institute medium. Both types of media were supplemented with 10% fetal bovine serum (FBS), and 1% antibiotics. The cells were cultured and maintained in 5% CO₂ at 37 °C.

For the cell adhesion assay, sterilized samples were placed over the glass coverslips in a 24-well plate. For control wells, plain glass coverslips were used. Samples were incubated in 5% CO₂, at 37 °C for 1 h. Cells were seeded into each well to give a final number of 1 × 10⁵ cells per well, and incubated for 24 and 48 h. The samples were fixed using 2.5% glutaraldehyde in 0.1 M phosphate buffered saline (PBS) for at least 1–2 h, and then rinsed twice with PBS and once with deionized water. Samples were dehydrated by immersion in a series of ethanol solutions with increasing concentrations, and dried in a critical point dryer (Leica EM-CPD300, Vienna, Austria). The samples were mounted in a sputter coater (Balzers SCD-040, Wetzlar, Germany) and coated with gold. Cell adhesion measurements were carried out with a scanning electron microscope (SEM; JEOL JSM-IT300, Tokyo, Japan) operated at 5000× and 15 kV.

For cytotoxicity measurements, the samples were soaked in the appropriate culture medium and incubated at 37 °C for 24 h. The medium containing the sample extract was then filtered through a 0.45-µm syringe filter, and the concentration adjusted to 1000 µg/mL. Cells were seeded into each of the wells of a 96-well plate to give 1 × 10⁴ cells per well. The plate was incubated at 37 °C, 5% CO₂ for 24 h, and then 90 µL of the filtered culture medium were added into each well. The plate was incubated again for 24 and 48 h, and then 10 µL of PrestoBlue™ solution (Life Technologies, Carlsbad, CA, USA) added. The plate was incubated for 2 h, after which the absorbance at 570 and 600 nm was measured by UV-visible spectrophotometry (Thermo Scientific, Waltham, MA, USA). The absorbance values were then processed, and the cytotoxicity values were calculated.
3. Results and Discussion

3.1. Absorption of Curcumin into BC

Due to the unique properties of BC, studying the absorption and release of curcumin from BC revealed interesting information about the characteristics of the material. Our results suggest that BC has potential as a carrier for the controlled release and transdermal delivery of drugs or active components. Immersion in curcumin solution was carried out for 24 h because absorption occurs rapidly in the first 12 h, then gradually increases, reaching a plateau within 24 h. The actual amount of curcumin absorbed into BCC0.25, BCC0.5, and BCC1.0 films was found to be 0.19 ± 0.03, 0.36 ± 0.03, and 0.47 ± 0.03 mg/cm² of film (with a thickness of ≈20 µm), respectively. During immersion, the curcumin solution enters the loose matrix of the never-dried BC film. Entrapment of curcumin is a result of the interaction between BC microfibrils and curcumin that occurs during air drying [33]. As the concentration of curcumin solution increased, more curcumin molecules were observed to diffuse and absorb into the BC matrix.

3.2. Characterization of BC-Curcumin Membrane

3.2.1. Surface and Cross-Sectional Morphologies

Figure 1a illustrates the surface morphology of the air-dried BC film, revealing a well-organized network structure of microfibrils with diameters in the range of 0.05–0.1 µm. The spaces between fibrils indicate a pore size of less than 0.1 µm. The formation of H-bonds between the cellulose chains occurs during air drying and the resulting densely packed matrix was especially apparent when the films were observed in cross section [33] (Figure 2). Due to the hydrophilic nature of BC, the pore diameter of the BC film increased to 0.2–1.0 µm after rehydration (Figure 1b). Water penetration caused relaxation of the polymer chain, which resulted in a loosening of the packed structure of the BC matrix. These results indicate that BC could be suitable for use as a releasing matrix of drugs or active components. After absorption of curcumin into BC at concentrations of 0, 0.2%, 0.4%, 0.6%, 0.8%, and 1% (w/v), uniform distribution of curcumin aggregates was obvious on the surface of binary composites of BCC films. The formation of aggregates was not apparent between the fibril layers; except at the maximum curcumin concentration (BCC1.0), when some aggregates were seen between layered fibrils. Previously, we have reported similar observations from the preparation of films containing ethanolic extract of mangosteen peel [12].

![Figure 1. SEM images of surface morphology of a dried BC film (a), a rehydrated BC film (b), and dried films of BCC0.2 (c), BCC0.4 (d), BCC0.6 (e), BCC0.8 (f), and BCC1.0 (g) at the magnification of 10,000×.](image-url)
3.2.2. Gas Permeability Test

From Table 1, the water vapor transmission rate (WVTR) of BC film can be seen to have slightly reduced from about 597 to 434–506 g/m²/day by the absorption of curcumin at concentrations of 0.2–1.0% (w/v). The WVTR values of the BCC films were found to be similar to those of commercial films such as Ulteco® and Bioclusiose® [34]. This investigation revealed that the WVTR of BCC films falls within the acceptable range for a skin dressing. Generally, the evaporative water loss rate for normal skin is 204.0 ± 115.2 g/m²/day, while that for injured skin is 5138 ± 202 g/m²/day. The WVTR value for dressings has been recommended to be within the range of 70–9400 g/m²/day to control loss of fluid from the injured skin, and maintain the condition of the skin [35,36].

The oxygen transmission rates (OTR) of BC and BCC films are shown in Table 1. As the curcumin content increases, the OTR decreases. Furthermore, the OTR of BCC film was found to be significantly lower than the WVTR. This discrepancy is likely due to the incorporation of curcumin into the spaces between the BC microfibrils, resulting in a denser structure. Because oxygen molecules are larger than water molecules, the denser structure could cause a significant reduction in the diffusivity of oxygen through the film. The low OTR values could be due to the measurement at 0% RH. However, for clinical use, the OTR of the moist film might be significantly enhanced. A high relative humidity will cause the film to swell, resulting in an increase of OTR.

| Film   | WVTR (g/m²/day) | OTR (cm³/m²/day) |
|--------|----------------|-----------------|
| BC     | 597.3 ± 90.5   | 2.24 ± 0.15     |
| BCC0.2 | 481.6 ± 35.1   | 1.26 ± 0.01     |
| BCC0.8 | 434.8 ± 185.6  | 1.13 ± 0.16     |
| BCC1.0 | 506.2 ± 89.7   | 0.70 ± 0.05     |

Values were expressed as mean ± SD (n = 3).

3.2.3. FTIR Analysis

Chemical interactions between BC and curcumin were investigated by FTIR analysis. As shown in Figure 3, FTIR spectra of the BC and BCC films were recorded between 2000 and 1000 cm⁻¹. The BC spectrum showed a peak at 1639.38 cm⁻¹ (Figure 3), which was attributed to the carbonyl group of cellulose [12]. This peak was also observed in BCC spectra, but was shifted from 1639.38 cm⁻¹
to 1634.87, 1634.31, 1628.48, 1628.35, and 1627.49 cm$^{-1}$, depending on the curcumin concentration. This shift could be attributed to the interaction between BC and curcumin. After absorption of curcumin, the BCC film spectra exhibited an additional peak at around 1515–1510 cm$^{-1}$ due to the aromatic skeletal vibrations of the benzene ring. This confirms the presence of curcumin in the matrix [37].

![FTIR spectra of BC, BCC0.2, BCC0.4, BCC0.6, BCC0.8 and BCC1.0.](image)

**Figure 3.** FTIR spectra of BC, BCC0.2, BCC0.4, BCC0.6, BCC0.8 and BCC1.0.

### 3.2.4. Mechanical Properties

Figure 4 summarizes the mechanical properties of typical BC and BCC films, highlighting that the mechanical properties of the films were affected by curcumin content. Tensile strength, Young’s modulus, and elongation at break all tended to decrease as a function of increasing curcumin content (Figure 4a–c). The tensile strength of BC film with an average thickness of 20 µm was 126.41 MPa. This was reduced to 47.91 MPa in the case of BCC 1.0. With increasing curcumin incorporation, BCC films showed reduced toleration toward the applied stress during the tension test. When compared with that of BC film, the Young’s modulus and elongation at the break of BCC1.0 were found to be approximately 2.6 and 2 times lower, respectively. According to the previous report [33], hydrogen bonding between BC fibrils could be disrupted by the addition of interfering compound, which might result in the decrease in mechanical properties of the films.
Figure 4. Tensile strength (a), Young’s modulus (b), and % elongation at break (c) of BC and curcumin-loaded BC films. Values were expressed as mean ± SD (n = 5).

3.3. Release Assay

The release of curcumin was evaluated by placing rehydrated BCC film on the surface of a buffer solution in modified Franz diffusion cells. The release profiles under non-transdermal conditions are shown in terms of concentration of released curcumin (Figure 5a), and percentage of curcumin relative to the actual amount of curcumin loaded into the BC pellicles (Figure 5b). The hydrophobicity of curcumin means that it is poorly soluble in water, and its release into aqueous solutions without the addition of surfactant was very low (data not shown). The release rate was enhanced by the addition of surfactant to increase the solubility of hydrophobic molecules. On previous work, Li et al. (2015) also reported that the release of curcumin from silk hydrogel films was significantly enhanced when the release assay was performed in PBS with Tween80 and methanol supplement compared to that of PBS without supplement [38]. The pH of the solution was found to have had a minor effect on the dissolution of curcumin, but the addition of surfactants to the solution was observed to significantly increase the molecule’s solubility. Burst release was observed in the first 8 h, as the packed matrix of air-dried BCC became loose when immersed in water [12]. The release rate then slowed in a controlled manner. The total amount of curcumin released from BCC films was also found to increase in line with the initial amount of curcumin that was absorbed into the films. Pure curcumin is highly unstable
in alkaline aqueous solutions (pH ≥ 7.0). Therefore, the concentrations of curcumin released from BCC films into the acetate buffer were higher than those in the PBS buffer. Degradation should occur very slowly at pH 1–6, whereas degradation could occur at a significantly higher rate in PBS at pH 7.4 due to the instability of curcumin at this pH [18,39]. This degradation could cause the decrease in the concentration of curcumin in the solution that was seen in the release assay after 10 h. This result demonstrates that BC is a good candidate matrix for the controlled release of curcumin. For the use of the film as a topical patch on skin, the control release of curcumin into the skin might also be adjusted by the addition of surfactant on the target area.

![Figure 5. Release profiles of curcumin from BCC0.5 (gray) and BCC1.0 (black) into buffer solutions (acetate buffer at pH 5.5 (▲,▲); PBS at pH 7.4 (♦,♦)) supplemented with Tween80/methanol: (a) concentration of released curcumin and (b) percentage of release based on actual amount of curcumin loaded in BCC films.](image)

3.4. Antibacterial and Antifungal Properties

The antibacterial effects of BC, BCC0.5, and BCC1.0 against *E. coli* and *S. aureus* were studied (Figure 6, rows a and b). Because curcumin may be unable to diffuse through the film into the agar media, BC, BCC0.5, and BCC1.0 did not show a clear zone of inhibition [40]. However, inhibition of bacterial growth under these samples was observed with optical microscopy.

The antifungal effects of BCC against *A. niger* were also evaluated (Figure 6, rows c and d). BCC0.5 and BCC1.0 inhibited the growth of *A. niger*, whereas significant growth (more than 60% of area) was observed on BC film. Growth of *A. niger* was reduced to less than 10% on BCC0.5 and BCC1.0 films. The fungal cells appeared unchanged throughout the 7 days, and occupied the same areas on day 7 as they did when the suspensions were applied to the films. This result demonstrates that the antifungal property of BC film is improved by the absorption of curcumin into it.
Curcumin has been previously reported to have antimicrobial effects against common foodborne pathogens such as *E. coli* and *S. aureus* [41]. The antimicrobial effects of curcumin against Methicillin-resistant *S. aureus* have also been demonstrated; as a required concentration of antibiotics was reduced when curcumin was also present [42]. The polymers in BC films have been reported to be susceptible to colonization by bacteria, leading to the formation of a bacterial biofilm [43]. Therefore, BC alone does not have antimicrobial activity, but the incorporation of curcumin into the BC films could prevent bacterial adhesion.

![Figure 6. Antimicrobial activities of BC (column 1), BCC-0.5 (column 2), and BCC-1.0 (column 3) against *E. coli* (row “a”), *S. aureus* (row “b”), *A. niger* after 1 day-incubation (row “c”) and *A. niger* after 7-day incubation (row “d”).](image)

3.5. Cytotoxicity and Anticancer Properties

The morphologies of HaCat, HDF, and A375 cells on BCC0.5 and BCC1.0 films were examined by SEM (5000× magnification), at 24 h and 48 h after seeding (Figures 7 and 8, respectively). After 24 h of incubation, both BCC0.5 and BCC1.0 were observed to inhibit the growth of dermal cancer cells. The presence of curcumin released from BCC films into the culture medium caused a decrease in the viability of the cancer cells. At 24 h, A375 cells appeared to shrink and exhibited a rounded shape on BCC-0.5 and BCC-1.0 (Figure 7, Column 3). Moreover, cell membrane damage was observed when A375 cells were cultured with BCC1.0 film. More significant damages and breakage of cells on BCC-0.5 and BCC1.0 were observed when the cells were cultured for 48 h (Figure 8, Column 3). The results indicated that the release of curcumin from BCC films significantly affected the morphologies of A375 and caused cell death. Measurement of HaCat and HDF cells revealed that cell shrinkage occurred in the first 24 h of incubation with BCC1.0 (Figure 7, Columns 1 & 2). However, when these cells were cultured with BCC0.5, progressive cell adhesion onto the film was observed. After prolonged incubation (48 h), adhesion of HaCat and HDF cells onto BCC0.5 and BCC1.0 was observed (Figure 8, Columns 1 & 2). Therefore, we can conclude that curcumin is not cytotoxic against normal skin cells, but is selectively cytotoxic against skin cancer cells. Our observation under SEM (5000×) agrees with the previous observation under a microscope (200×) by Y. Zhang et al. (2015) on the study of the effect of curcumin on the apoptosis of A375 cells, that the morphology of A375 cells appeared contracted,
shrunk, turned into irregularly round shapes, and detached from adherence after treatment with curcumin for 48 h [30]. They also confirmed that the A375 cells were killed by apoptosis by flow cytometry [30].

Figure 7. SEM images (5000 × magnification) showing cytotoxic effects of BCC0.5 (Row B) and BCC1.0 (Row C) against plain cover class (Row A, control) on HaCat (Column 1), HDF (Column 2), and A375 (Column 3) cells after 24-h treatment.

Figure 8. SEM images (5000 × magnification) showing cytotoxic effects of BCC0.5 (Row B) and BCC1.0 (Row C) against plain cover class (Row A, control) on HaCat (Column 1), HDF (Column 2), and A375 (Column 3) cells after 48-h treatment.
The viabilities of HaCat, HDF, and A375 cells treated with extracts of BCC0.5 and BCC1.0 (Figure 9) were correlated with the SEM micrographs of adhered cells. HaCat cells initially showed shrinkage in the first 24 h after seeding onto BCC1.0 film, but adhered to the film when the incubation time was increased to 48 h. The effects of curcumin on the adhesion of HDF cells were insignificant because the cells were adhering to the films throughout the incubation period. After incubation with BCC0.5 extract, the viability of A375 cells was significantly reduced to 88.04 ± 1.36% after 24 h, and further reduced to 72.78 ± 1.80% after 48 h. When A375 cells were treated with BCC1.0 extract, the viability was 82.42 ± 6.80% after 24 h and 70.89 ± 0.69% after 48 h. These values suggest that curcumin has cytotoxic effects against A375 cells and that BC contributes to the controlled release of curcumin. Zhang et al. (2015) suggested that curcumin affects the cell invasion and proliferation of A375 cells by increasing the rate of apoptosis [30]. This effect has also been seen in other cancer cells treated with curcumin, including prostate [26], papillary thyroid [22], pancreatic [29], ovarian [28], and colon cancer cells [23]. Other effects on cancer cells have been observed, such as nuclear shrinkage and chromatin condensation in cutaneous T-cell lymphoma cells [25], and induction of cell cycle arrest in prostate cancer cells [26]. Recent studies with colon cancer cells revealed that curcumin induced reactive oxygen species production, which ultimately caused DNA fragmentation (the hallmark of apoptosis), cell cycle arrest, and induction of apoptosis through the mitochondrial pathway [44]. Another study by Shahar Lev-Ari et al. (2014) revealed that curcumin suppressed NF-κB (NFκB-dependent inflammation mediator) activation, and downregulated all of its target genes (involved in the regulation of apoptosis and proliferation) [27]. However, in vitro studies on lymphocytes, hepatocytes, HDF, and rat dermal fibroblasts have demonstrated that curcumin has no effect on cell proliferation of non-cancerous cells [45]. In healthy cells, p53 induces G1 arrest to allow the cells to repair before progressing the cell cycle [46]. This occurs in association with cyclin-dependent pathways. Curcumin causes cell cycle arrest at the G0 by downregulating cyclin D1, while simultaneously upregulating Cip1. This results in cell cycle arrest at the G0 phase without apoptosis. However, in cancer cells, levels of cyclin D1 level are so high that despite curcumin-induced upregulation of Cip1; it is insufficient to cause cell cycle arrest. This allows normal cells to be immune to curcumin-induced apoptosis at G2 phase, in which curcumin makes a significant induction of p53, accelerating apoptotic pathway [46,47].

![Figure 9](image_url)

**Figure 9.** Cell viabilities of HaCat, HDF, and A375 cells after seeding on BCC0.5 and BCC1.0 films for 24 h and 48 h. The values were expressed as mean ± SD (n = 3). Statistically significant differences in cell viabilities of HaCat, HDF and A375 were compared with the same treatment:  a p < 0.05 versus HaCat, b p < 0.05 versus HDF and  c p < 0.05 versus A375.
4. Conclusions

This study presents a new methodology for entrapment of curcumin in BC film, and the ensuing controlled release of the compound. Curcumin was absorbed into BC pellicles and then the pellicles were air dried. Interactions between BC microfibrils and curcumin molecules were revealed by FTIR analysis. High curcumin concentrations (above 0.4 mg/mL) led to a reduction in the BCC film’s stretching properties. BCC films showed antifungal activities against *A. niger*, and inhibited the growth of *E. coli* and *S. aureus* under the films. The non-transdermal controlled release of curcumin from BCC films was achieved in buffer solutions containing Tween 80 and methanol additives, at pH 5.5 and 7.4. Assessments of cytotoxicity and anticancer properties of BCC films revealed that the curcumin released from BCC films was cytotoxic towards A375 cells, but not towards normal cells. The result from our study shows potential use of curcumin-loaded BC film as a topical patch for the treatment of malignant melanoma skin cancer cells. However, the preliminary study on animal skin should be performed prior to clinical trials.

**Author Contributions:** Conceptualization, M.P.; Methodology, C.S., S.T. and N.C.; Validation, M.P. and A.S.; Formal Analysis, C.S. and W.W.; Investigation, M.P., C.S. and W.W.; Resources, M.P. and A.S.; Writing—Original Draft Preparation, C.S. and W.W.; Writing—Review & Editing, M.P.; Supervision, M.P.; Project Administration, M.P.; Funding Acquisition, M.P. and A.S.

**Acknowledgments:** This work was supported by the National Research University Program (Health Cluster), Chulalongkorn University, contract Grant No. NRU59-016-HR.

**Conflicts of Interest:** Authors declare no conflict of interest.

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