Carbohydrate Polymers 85 (2011) 548–553

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Characterization and biocompatibility of bacterial cellulose/alginate composite sponges with human keratinocytes and gingival fibroblasts

Nadda Chiaoprakobkij[a], Neeracha Sanchavanakit[b,c], Keskanya Subbalekha[d], Prasit Pavasant[b,c], Muenduen Phisalaphong[a,*]

[a] Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok 10330, Thailand
[b] Research Unit of Mineralized Tissues, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand
[c] Department of Anatomy, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand
[d] Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand

ARTICLE INFO

Article history:
Received 15 January 2011
Received in revised form 1 March 2011
Accepted 4 March 2011
Available online 15 March 2011

Keywords:
Bacterial cellulose
Alginate
Human keratinocytes
Gingival fibroblasts

ABSTRACT

The novel composite sponge of bacterial cellulose/alginate (BCA) has been developed for the use as mucosal flaps in oral tissue regeneration. The porous sponge was fabricated by a freeze drying process. The sponge matrix appeared organized in a three-dimensional network of nanofibrils. The FTIR spectra indicated the intermolecular interaction between bacterial cellulose (BC) and alginate. The in vitro studies with human keratinocytes (HaCat) and gingival fibroblasts (GF) demonstrated that the pure BC and the BCA sponges supported proliferations of the cells. However, in the wet state, only the BCA sponge with 30% alginate had a good tear resistance for sewing. The BCA composite sponge is a promising material for use as a non-adherent hydrogel dressing due to many advantages in terms of skin tissue compatibility, excellent water uptake ability, and high mechanical strength and stability in both water and PBS buffer.

1. Introduction

For the past ten years, a number of natural polymers have been studied for use as new alternative biomaterials. Bacterial cellulose (BC), which is synthesized by *Acetobacter xylinum* using glucose as a substrate, has been considered highly biocompatible with suitable properties allowing application in the tissue engineering and biomedical fields (Andrade, Pertile, Dourado, & Gama, 2010; Czaja, Young, Kawecki, & Brown, 2007). With the fine web-like network structure and high purity, BC provides significant benefits over plant cellulose. BC has advantages in terms of biocompatibility, non-toxicity, high mechanical strength, high swelling ability and high stability to pH variations. Compared to collagen or gelatin, BC does not contain any components of animal origin; additionally, it does not appear to cause allergic reactions. BC has been applied as artificial skin for patients with burns and ulcers (Fontana et al., 1990), temporary skin substitute for animals (Jonas & Farah, 1998), artificial blood vessels for microsurgery (Klemm, Schumann, Udhardt, & Marsch, 2001), material for a meniscal implant (Bodin et al., 2007) and as a potential scaffold for tissue engineering (Gao et al., 2011; Svesson et al., 2005).

Alginate, a linear polysaccharide (copolymer of (1-4)-linked β-mannuronic acid (M) and α-guluronic acid (G) monomers), is a natural polysaccharide derived primarily from brown seaweed. Due to its hydrogel properties and biocompatibility, alginate has also been widely used in biomedical applications, including wound management. The major function of alginate in tissue engineering applications is to provide mechanical integrity (Drury, Dennis, & Mooney, 2004). Alginate gel can form a gel on absorption of wound exudates, prevent the wound surface from drying out, minimize discomfort during removal (Thomas, 2000) and enhance the rate of healing of skin wounds (Jarvis, Galvin, Blair, & McCollum, 1987). Several therapeutic agents, including antibiotics, enzymes, growth factors and DNA, have already been successfully incorporated in alginate gels in order to maintain high biological activity (Smidsrod & Draget, 1996). Furthermore, alginate hydrogels have been applied as scaffolds for cartilage and bone regeneration (Alsberg, Anderson, Albeiruti, Franceshi, & Mooney, 2001).

To gain the beneficial properties of both BC and alginate, a novel porous sponge from a blend of BC and alginate was developed in this study. The structure, morphology, mechanical strength and biodegradability were characterized. In addition, for the further application as a mucosal flap in oral tissue regeneration, the biocompatibility of the alginate modified BC sponge was determined by examining its effect on HaCat and GF cells.

* Corresponding author. Tel.: +66 2 218 6875; fax: +66 2 218 6877.
E-mail address: muenduen.p@chula.ac.th (M. Phisalaphong).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.03.011

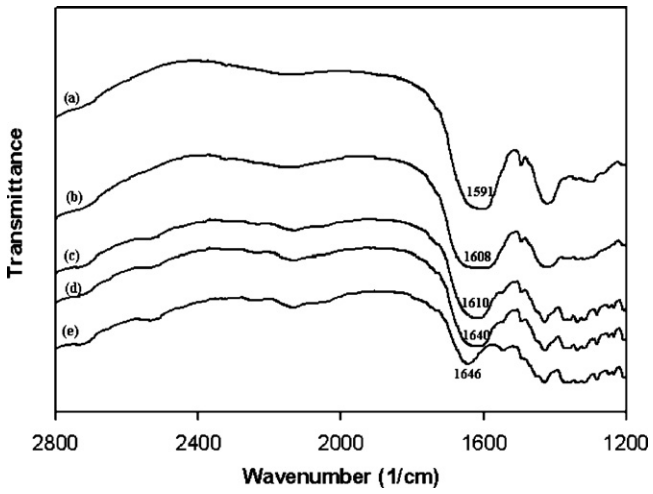

Fig. 1. FTIR spectra of (a) alginate, (b–d) BCA and (e) BC sponges in wave numbers ranging from 2800 to 1200 cm^{-1}. The weight ratios of BC/alginate are: (b) 30/70, (c) 50/50 and (d) 70/30.

2. Materials and methods

2.1. Preparation of the BCA composite sponge

The BC used in this study was the gel-like cellulose pellicle formed by *A. xylinum* AGR 60, kindly supplied by the laboratory of Pramote Tammarate (the Institute of Food Research and Product Development, Kasetsart University, Bangkok). The medium for the inoculums was coconut-water containing 5.0% sucrose, 0.5% ammonium sulfate $(NH_4)_2SO_4$ and 1.0% acetic acid. Cell cultivation for BC biosynthesis was performed under static conditions as previously described (Sanchavanakit et al., 2006). The developed gel-like cellulose pellicle was then purified by washing with deionized (DI) water, treated with 1.0% (w/v) NaOH at 35 °C for 24 h to remove bacterial cells and rinsed with DI water until the pH was 7.0. The never dried BC was stored in DI water at 4 °C before use.

The BC pellicles (size 1 cm × 1 cm × 1 cm) were crushed to form BC slurry by using a homogenizer at room temperature. Sodium alginate of 1.5% (w/v) was dissolved in distilled water at room temperature to form a gel-like solution. Then, the BC slurry was thoroughly mixed with the alginate solution at weight ratios of BC/alginate at 100/0, 70/30, 50/50, 30/70, or 0/100 until homogeneous slurries were formed. Next, 50 g of the slurry mixtures were placed in Petri dish plates, cross-linked by an aqueous solution of 1.5 (w/v) $CaCl_2$ and rinsed with distilled water to remove the excess chlorides. Then, the mixtures were pre-frozen at −40 °C for 24 h prior to drying under vacuum pressure (∼90 mTorr) at −40 °C for 24 h.

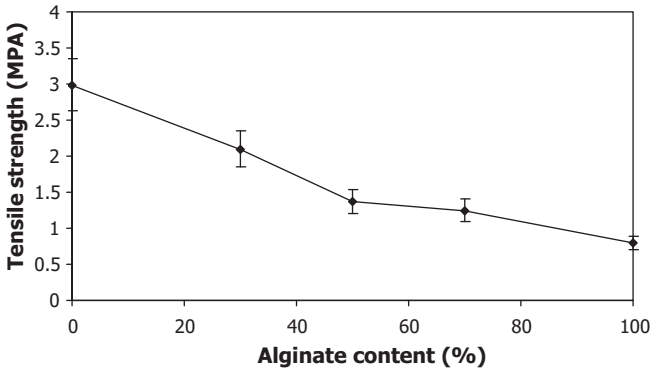

Fig. 2. The tensile strength of the BCA sponges as a function of alginate content (wt%).

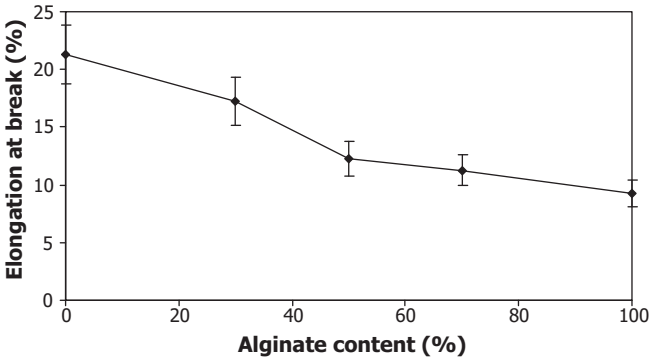

Fig. 3. The elongation at break of the BCA sponges as a function of alginate content (wt%).

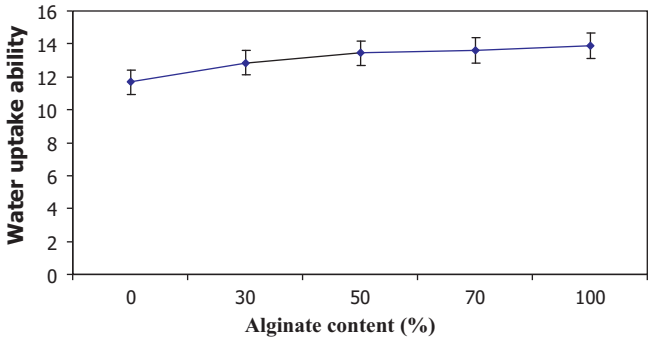

Fig. 4. The water uptake ability of the BCA sponges as a function of alginate content (wt%).

2.2. Characterization of the sponges

2.2.1. Elemental analysis

The calcium and sodium content were determined with an X-ray fluorescence (XRF) spectrometer (model ED2000, Oxford, United Kingdom).

2.2.2. Fourier transform infrared (FT-IR) spectroscopy

The structural information was collected using a Perkin Elmer Spectrum GX FT-IR Microscope (Waltham, MA). The FT-IR spectra of the samples were measured from 2800 cm^{-1} to 1200 cm^{-1} at a resolution of 4 cm^{-1}.

2.2.3. Mechanical testing

The samples were cut into strip-shaped specimens that were 10 mm in width and 10 cm in length (50 mm between the grips). A universal testing machine (INSTRON 5567, Rochester, NY) was used to determine tensile strength of the samples at the stretching speed of 2 mm/min. The ten-

Table 1
The sponge elemental content analyzed by X-ray fluorescence spectroscopy (XRF).

Blending composition (BC/alginate)	Elemental content (%) by XRF	
	Na	Ca
100/0	–	–
70/30	–	0.88
50/50	–	2.49
30/70	–	2.96
0/100	–	5.57

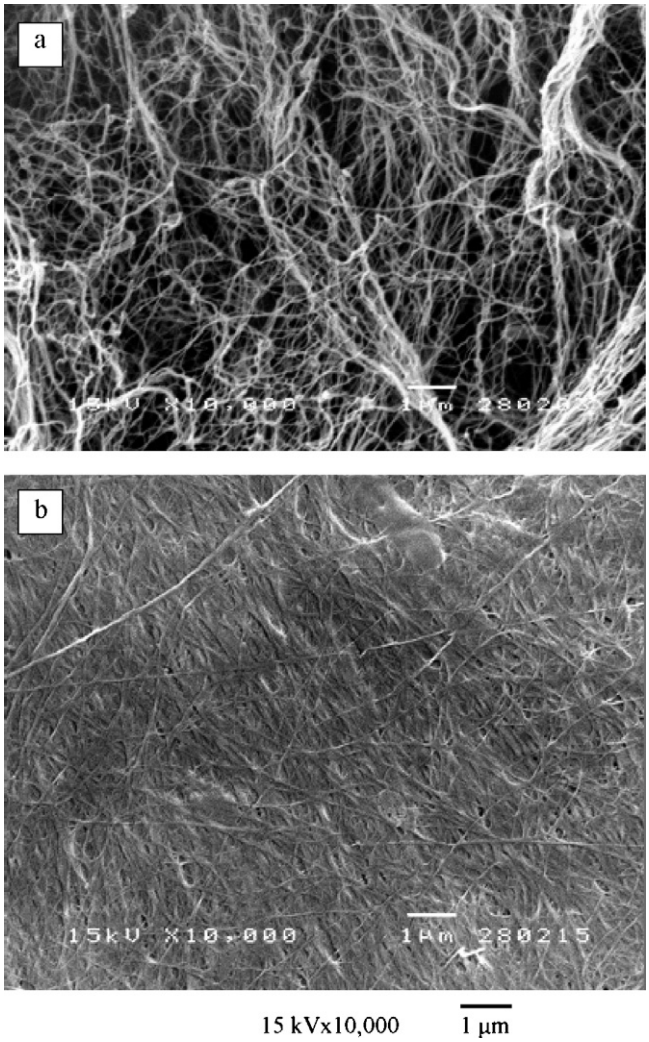

Fig. 5. SEM images of cellulosic fibers after homogenization (a) and surface morphology of the BCA sponge (b).

sile strength was the average value determined from five specimens.

2.2.4. Water uptake ability

To measure the water uptake ability (the equilibrium water content) of the sponge, pre-weighed dry sample was immersed in distilled water for 1 min. After the bulk water was removed by placing the wet sponge on the Petri dish for 1 min, the weight of wet sample was measured. All testing was run in triplicate, and the equilibrium water content was determined using the following equation:

$$\text{Water uptake ability} = \left(\frac{W_w - W_d}{W_d}\right)$$

where W_w and W_d represent the weight of wet and dry samples, respectively.

2.2.5. Scanning electron microscopy (SEM)

The morphology of BC, BCA and alginate sponges was analyzed by scanning electron microscope (SEM); model JSM-5410LV, JEOL (Tokyo, Japan). The freeze-dried samples were sputter coated with gold (JEOL JFC 1100 E ion sputtering device) and photographed at an accelerating voltage of 15 kV and magnifications of 35–10,000.

2.2.6. In vitro studies

Cells were cultured in a 24-well polystyrene plate using growth medium composed of Dulbecco's modified eagle medium (DMEM) supplemented with 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin-B. The cells were incubated in a humidified incubator in an atmosphere of 5% CO_2 and 95% air. At confluence, HaCat and GF cells were harvested using a suspension of 0.25% trypsin and subcultivated in the same medium with 12 and 4 dilutions, respectively.

HaCat and GF cells were used to evaluate adhesion and proliferation as a direct contact test. The sponges (13 mm in diameter and 2 mm in thickness) were immersed in 70% ethanol for 5 min for sterilization, followed with four successive solvent exchanges with deionized water. The sponges were then placed on a 24-well polystyrene plate (NUNC, Roskilde, Denmark), and 350 μl of culture medium (Sanchavanakit et al., 2006) was added to each well before cell seeding (6×10^4 cells/well). Cells were allowed to initially attach for 5 h. For the proliferation test, cells were seeded onto each of the matrices, and the cultures were harvested after 6, 24 or 48 h. The attached or proliferated cells were then quantified using the 3-(4,5-dimethythiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay.

Three hundred and fifty microliters of MTT solution (0.5 mg/ml in DMEM without phenol red, filter-sterilized) was added to each culture well. After incubation for 5 h, the MTT reaction medium was removed, and 900 μl of dimethylsulfoxide and 100 μl of glycine buffer (pH 10.5) were added. Optical densities were determined by spectrophotometer (Genesis 10 UV scanning, Rochester, NY) at 570 nm.

3. Results and discussion

3.1. Elemental analysis

The X-ray fluorescence (XRF) spectroscopy results (Table 1) showed no sodium (Na) peak. Therefore, NaOH was completely removed from the freeze-dried sponges. The XRF spectra indicated a calcium content of 0.88–2.96 wt% in the BCA sponges, depending on the alginate content. The calcium accumulation in the BCA and alginate sponges should be due to the formation of a calcium/alginate gel network by cross-linking with Ca^{2+} ions, as previously described (Phisalaphong, Suwanmajo, & Tammarate, 2008).

3.2. FT-IR spectrophotometric analysis

The FT-IR spectra of the BCA sponges at various blending compositions were measured from 2800 to 1200 cm^{-1}, as shown in Fig. 1. The BC spectra showed a band at 1646 cm^{-1}, which was attributed to the glucose carbonyl of cellulose. The BCA sponges exhibited the characteristic absorption bands with the appearance of no new peaks or the disappearance of any peaks associated with the parent molecules. The interaction between BC and alginate could be identified by the carbonyl and carboxyl group bands present in the range of 1900–1500 cm^{-1}. The carboxyl group bands for the BCA sponges of 70, 50 and 30% alginate were shifted from 1591 cm^{-1} (100% alginate) to 1608, 1610 and 1640 cm^{-1}, respectively. The shifts could be attributed to intermolecular interactions between the hydroxyl group of cellulose and the carboxyl group of alginate, which might disrupt the hydrogen bonding between cellulose fibers. Similar observations were previously reported in cotton cellulose/alginate blend membrane (Yang, Zhang, Peng, & Zhong, 2000) and BC/alginate blend membrane (Phisalaphong et al., 2008).

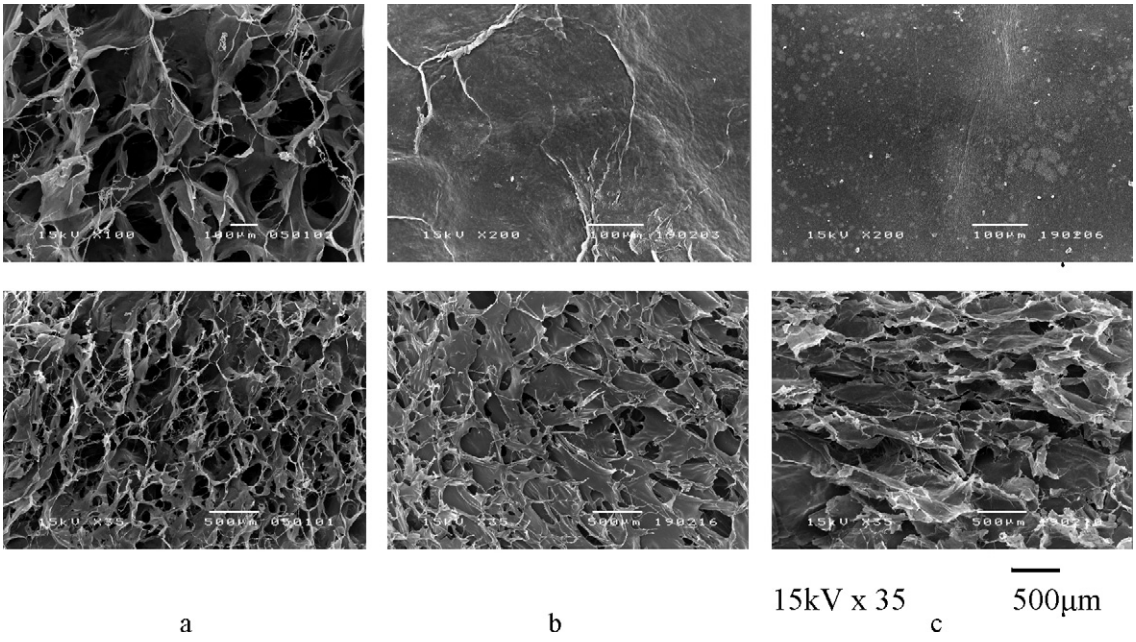

Fig. 6. SEM micrographs of surface (top) and cross-section (bottom) of (a) BC, (b) BCA and (c) alginate sponges.

3.3. Mechanical strength

Tensile strengths of freeze-dried sponges are shown in Fig. 2. The tensile strength of the BC sponge (0% alginate) was greater than that of the alginate sponge (100% alginate). For those of BCA sponges, the tensile strength decreased with an increase of alginate content. Similarly, the elongations at break of the BCA sponges also decreased with an increase of alginate content as shown in Fig. 3. It was previously reported that the presence of alginate decreases the mechanical properties of BC film (Kanjanamosit, Muangnapoh, & Phisalaphong, 2010; Phisalaphong et al., 2008; Wu et al., 2004), suggesting that the intermolecular hydrogen bonding of cellulose might be broken down or disrupted to form cellulose-alginate hydrogen bonds. The intermolecular interactions might reduce the crystallinity and mechanical strength of the composite material.

3.4. Water uptake ability and structural stability

Water uptake ability and structural stability of sponges play an important role in their practical use in biomedical applications. The water uptake ability of BCA sponges with different alginate concentration is shown in Fig. 4. The structural stability of the freeze-dried sponges was investigated by immersion in water and in a phosphate buffer solution (PBS).

The water uptake ability of the BC sponge was approximately 11.7 times of its weight in water. The freeze-dried sponges of BC swelled rapidly after the immersion in water or in PBS. However, because the BC sponge was composed of homogenized BC fibrils without a strong linking network, its structural stability was poor in the wet state. During the immersions, the BC sponges gradually collapsed and disintegrated within 2 and 3 h in PBS and in water, respectively.

The water uptake ability of the alginate sponge was 13.9 times of its weight, which was higher than that of the BC sponge.

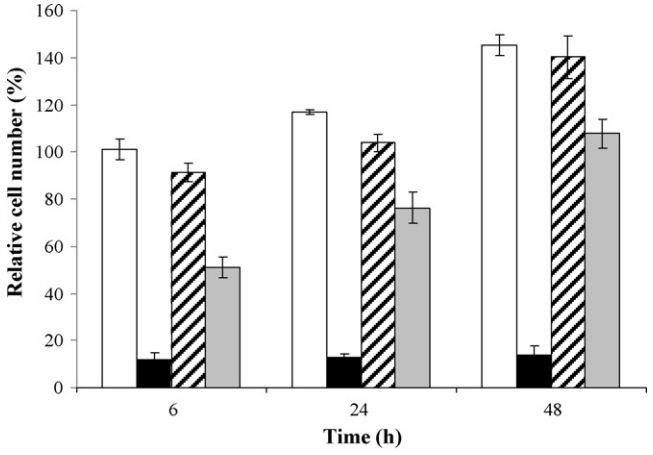

Fig. 7. Proliferations of HaCat on tissue culture plastic (□), the alginate sponge (■), the BC sponge (▨) and the BCA sponge (▩). Percentage of living cells was assessed at 6, 24, and 48 h of cultures by MTT assay.

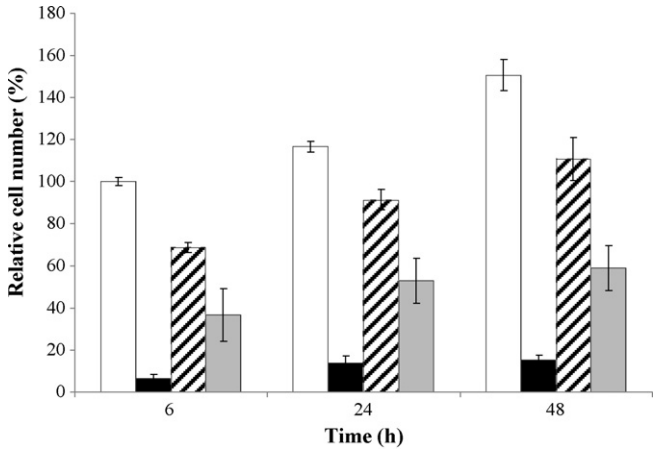

Fig. 8. Proliferations of GF on tissue culture plastic (□), the alginate sponge (■), the BC sponge (▨) and the BCA sponge (▩). Percentage of living cells was assessed at 6, 24, and 48 h of cultures by MTT assay.

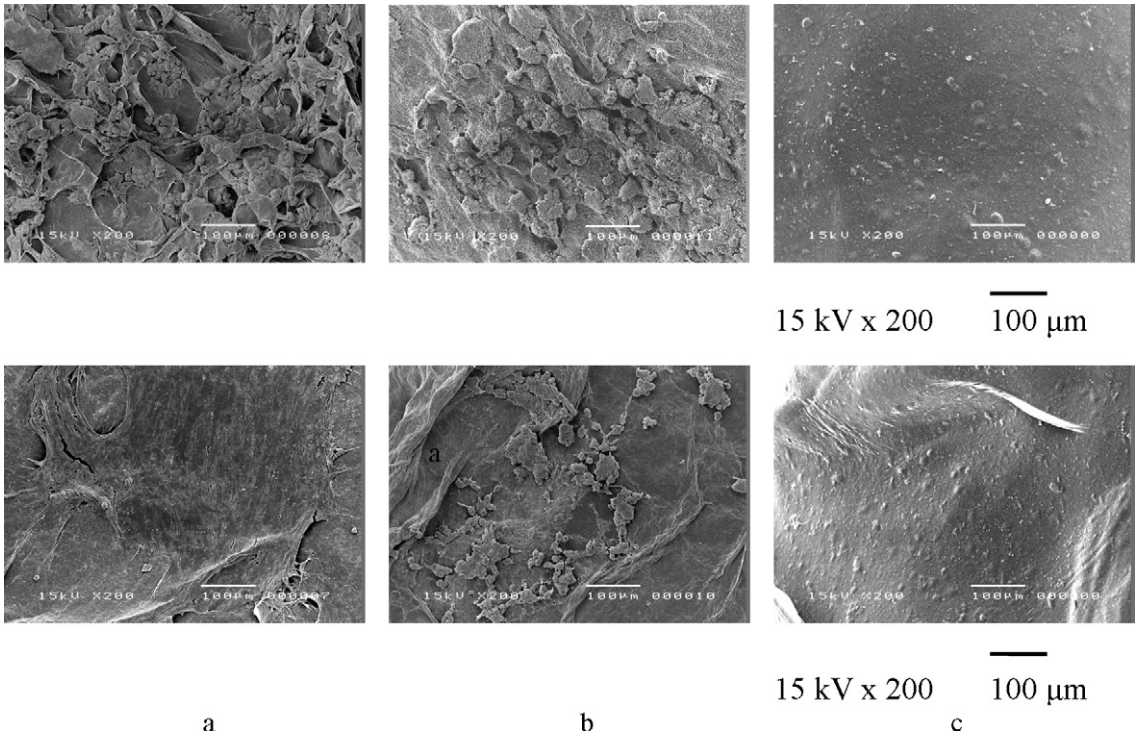

Fig. 9. SEM images of HaCat (top) and GF (bottom) on the (a) BC, (b) BCA and (c) alginate sponges at 48 h after seeding.

Water molecules are easily absorbed into alginate due to its highly hydrophilic property. The freeze-dried alginate sponge was stable in distilled water; however, it completely collapsed within 30 min in PBS. Drury et al., in 2004 reported that the dissolution of alginate hydrogel leads to the weakening of sponges based on alginate. Because the cross-linked calcium ions can be exchanged with other non-gel-inducing cations in PBS, the reduction in cross-linking is induced in the buffer solution causing erosion and destruction of alginate gel. Phosphate can sequester the calcium ions cross-linked with alginate, consequently destabilizing the calcium-alginate gel (Smidsrod & Skjak-Braek, 1990).

The supplement of alginate in the BCA sponges resulted in the increase in water uptake capacity. Blending with alginate could disrupt the hydrogen bonding between the cellulose fibers and result in the increase in the water uptake ability of the modified BC. The BCA sponges rapidly swelled and were stable in water. However, some structural disruption and disintegration of the sponges with an alginate content of more than 30% occurred after 2 h of immersion in PBS. The freeze-dried BCA sponge of 70% BC and 30% alginate was more stable and retained its overall size for the entire course of the examination (48 h). Because the structure of BCA sponge with 30% alginate could remain stable in water and PBS, it has better potential for use in a wide range of clinical settings. In addition, the excellent swelling ability of the sponge could facilitate adsorption of wound exudates.

3.5. Morphology

After the homogenization treatment, defibrillated BC appeared in form of groups of cellulose nanofibrils dispersed in aqueous solution (Fig. 5(a)). In order to fabricate the BCA sponge, the homogeneous mixture composed 30% alginate and 70% defibrillated BC was cast in Petri dish plates and was cross-linked in an aqueous solution of 1.5 (w/v) $CaCl_2$ before freeze-drying. The SEM image for close look of BCA sponge surface was shown in Fig. 5(b). After the cross-linking and freeze-drying, the water-insoluble connected network of BC-alginate was obtained. The conditions used in the cross-linking and freeze-drying processes strongly affected the structure of the sponge.

SEM micrographs of the BC, BCA and alginate sponges (Fig. 6) show the porous structure with the three-dimensional interconnection throughout the sponges. The BCA and alginate sponges had an asymmetric structure consisted of a top skin layer and sponge-like porous layer, similar to a functional wound dressing of chitosan sponge proposed by Mi et al., in 2003. The dense outer layer helps to prevent bacterial invasion and to avoid wound dehydration, whereas the porous support layer provides for the drainage of wound exudates and mechanical strength. The pore size of the sponges fell in the range of 100–500 μm, which is suitable for usage in tissue engineering (Yang, Leong, Du, & Chua, 2001).

3.6. In vitro study

Cell culture experiments with HaCat and GF cells were carried out to evaluate the biocompatibility of the sponges. Proliferations at 6, 24 and 48 h after seeding of HaCat and GF cells on the BC, BCA and alginate sponges, in comparison to those on the tissue culture plastic (polystyrene), are shown in Figs. 7 and 8, respectively. The BC and BCA sponges supported proliferations of HaCat and GF cells, while few cells of both cell types were detected on the alginate sponge. The proliferative rates of HaCat and GF cells were in the following order: on tissue culture plastic > BC > BCA > alginate. In a previous report by Svensson et al., in 2005, the proliferation tests of bovine chondrocytes showed that the percentage of relative cell viability on BC scaffolds were greater compared to those on tissue culture plastic and calcium alginate.

Morphologies of HaCat and GF cells on the BC, BCA and alginate sponges at 48 h after seeding are revealed in Fig. 9. HaCat cells responded to the BC and BCA sponges by exhibiting good attachment and spreading. The growing GF cells on the BC sponge demonstrated normal cell morphology with typical spindle shape and spread covering the material surface. GF cells only attached

onto the BCA sponge, however, the cells were still in round shape indicating that this modified material selectively supported spreading of HaCat, not GF. Very few cells of both cell types were observed on the alginate sponge.

Preliminary study on tear resistance was performed in water saturated BCA sponge by using 24 mm surgical needle (18-8 stainless steel, spring eye, cutting edge, 3/8 circle, Mani Inc., Tochigi, Japan) and braided silk US 3/0 (Pearsalls Ltd., Somerset, England) to suture material only together. The result showed that BCA was able to be sutured, since it resisted to the surgical needle and silk including force during the procedure. However further study, especially in vivo experiment, is required for definite information.

The BCA sponge has the potential to be used as a wound dressing material due to its biocompatibility, structural stability and good tear resistance. The BC sponge supported growth and spreading of HaCat and GF cells; however, it could not retain its shapes throughout the cell culture study (48 h).

4. Conclusions

A novel biocompatible porous sponge from the blend of BC and sodium alginate has been developed. The blend was fabricated in the form of a porous sponge by cross-linking with a $CaCl_2$ solution followed by a freeze drying process. FTIR analysis indicated that an intermolecular interaction exists between BC and alginate. The BCA sponge with 30% alginate has advantages in terms of biocompatibility, high mechanical strength, high water uptake ability and structural stability. The BCA sponges supported the proliferation of HaCat and GF cells. The BCA composite sponge was conceived as a temporary dressing and should be removed everyday of contact. It had an asymmetric structure consisted of a top skin layer and sponge-like porous layer. The dense outer layer helps to prevent bacterial invasion and to avoid wound dehydration, whereas the porous support layer provides for the drainage of wound exudates and mechanical strength. The pore size of the sponges fell in the range of 100–500 μm, which is suitable for usage in tissue engineering. In addition, the sponge had good tear resistance during sewing procedures. Therefore, the BCA sponge has a good potential to be used in the oral cavity to cover the surgical wound.

Acknowledgements

The authors are grateful for the financial support from the Thailand Research Fund (TRF) – the Commission on Higher Education – Chulalongkorn University, contract grant number RMU 5380024, TRF (contact grant number DBG 5280015) and Chulalongkorn University Centenary Academic Development Project. N.C. also received support from The Royal Golden Jubilee PhD Program from TRF.

References

Alsberg, V., Anderson, K., Albeiruti, A., Franceshi, R. T., & Mooney, D. J. (2001). Cell interactive alginate hydrogels for bone tissue engineering. *Journal of Dental Research*, *80*, 2025–2029.

Andrade, F. K., Pertile, R. N., Dourado, F., & Gama, F. M. (2010). Bacterial cellulose: Properties production and applications. In A. Lejeune, & T. Deprez (Eds.), *Cellulose: Structure and properties* (pp. 427–458). New York: Nova Science Publishers, Inc.

Bodin, A., Backdahl, H., Fink, H., Gustafsson, L., Risberg, B., & Gatenholm, P. (2007). Influence of cultivation conditions on mechanical and morphological properties of bacterial cellulose tubes. *Biotechnology and Bioengineering*, *97*(*2*), 425–434.

Czaja, W. K., Young, D. J., Kawecki, M., & Brown, R. M. (2007). The future prospects of microbial cellulose in biomedical applications. *Biomacromolecules*, *8*(1), 1–12.

Drury, J. L., Dennis, R. G., & Mooney, D. J. (2004). The tensile properties of alginate hydrogels. *Biomaterials*, *25*, 3187–3199.

Fontana, J. D., de Souza, A. M., Fontana, C. K., Torriani, I. L., Moreschi, J. C., Gallotti, B. J., et al. (1990). Acetobacter cellulose pellicle as a temporary skin substitute. *Applied Biochemistry and Biotechnology*, *24/25*, 253–263.

Gao, C., Wan, Y., Yang, C., Dai, K., Tang, T., Luo, H., et al. (2011). Preparation and characterization of bacterial cellulose sponge with hierarchical pore structure as tissue engineering scaffold. *Journal of Porous Materials*, *18*, 139–145.

Jarvis, P. M., Galvin, D. A. J., Blair, S. D., & McCollum, C. N. (1987). How does calcium alginate achieve haemostasis in surgery? *Thrombosis and Haemostasis*, *58*(1), 80.

Jonas, R., & Farah, L. F. (1998). Production and application of microbial cellulose. *Polymer Degradation and Stability*, *59*, 101–106.

Kanjanamosit, N., Muangnapoh, C., & Phisalaphong, M. (2010). Biosynthesis and characterization of bacteria cellulose-alginate film. *Journal of Applied Polymer Science*, *115*, 1581–1588.

Klemm, D., Schumann, D., Udhardt, U., & Marsch, S. (2001). Bacterial synthesized cellulose artificial blood vessels for microsurgery. *Progress in Polymer Science*, *26*(9), 1561–1603.

Mi, F., Wu, Y., Shyu, S., Chao, A., Lai, J., & Su, C. (2003). Asymmetric chitosan membranes prepared by dry/wet phase separation: A new type of wound dressing for controlled antibacterial release. *Journal of Membrane Science*, *212*, 237–254.

Phisalaphong, M., Suwanmajo, T., & Tammarate, P. (2008). Synthesis and characterization of bacterial cellulose/alginate blend membranes. *Journal of Applied Polymer Science*, *107*, 3419–3424.

Sanchavanakit, N., Sangrungraungroj, W., Kaomongkolgit, R., Banaprasert, T., Pavasant, P., & Phisalaphong, M. (2006). Growth of human keratinocytes and fibroblasts on bacterial cellulose film. *Biotechnology Progress*, *22*(*4*), 1194–1199.

Smidsrod, O., & Draget, K. I. (1996). Chemistry and physical properties of alginates. *Carbohydrate in Europe*, *14*, 6–13.

Smidsrod, O., & Skjak-Braek, G. (1990). Alginate as immobilization matrix for cells. *Trends in Biotechnology*, *8*, 71–78.

Svesson, A., Nicklasson, E., Harrah, T., Panilaiis, B., Kaplan, D. L., Brittberg, M., et al. (2005). Bacterial cellulose as a potential scaffold for tissue engineering of cartilage. *Biomaterials*, *26*, 419–431.

Thomas, S. (2000). Alginate dressings in surgery and wound management. Part 1. *Journal of Wound Car*, *9*(2), 56–60.

Wu, Y., Yu, S., Mi, F., Wu, C., Shyu, S., Peng, S., et al. (2004). Preparation and characterization on mechanical and antibacterial properties of chitosan/cellulose blends. *Carbohydrate Polymers*, *57*, 435–440.

Yang, G., Zhang, L., Peng, T., & Zhong, W. (2000). Effect of Ca^{2+} bridge cross-linking on structure and pervaporation of cellulose/alginate blend membranes. *Journal of Membrane Science*, *175*, 53–60.

Yang, S., Leong, K. F., Du, Z., & Chua, C. K. (2001). The design of scaffold for use in tissue engineering. Part I. Traditional factor. *Tissue Engineering*, *7*(6), 679–689.