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Biocompatibility and antibacterial activity of chitosan and collagen immobilized poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid)

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Abstract

Acrylic acid (AA) was grafted to ozone treated poly (3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV) membrane. The resulting membranes were further grafted with chitosan (ChS) via esterification. Afterward ChS-grafted membranes were immobilized with collagen (COL). The anti-bacterial activity of chitosan against *Staphylococus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* was reserved after COL-immobilization. In addition, after COL-immobilization, the L929 fibroblasts attachment and proliferation to membranes were improved, and the protein adsorption was decreased. The results indicate that by grafting with ChS and immobilizing with COL, PHBV can not only exhibit antibacterial activity, but also improve the cell attachment as well proliferation for fibroblast.

Keywords: Poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid); Chitosan; Antibacterial activity; Protein adhesion; Collagen; Cell attachment; Cell proliferation

1. Introduction

Poly (3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV) is a natural polyester polymerized by bacteria. This polyester is biodegradable and biocompatible, thus PHBV has been used as recyclable packing materials, kitchen films, diaper, and sanitary napkins. For the same reason, PHBV can also be used for biomedical applications. The biomedical applications mentioned in the literature include surgical suture, surgical swabs, wound dressings, vascular graft, blood vessel, scaffold for new tissue in growth, body parts, syringe (Hocking & Marchessault, 1994). In general, the content of hydroxyvaleric acid (HV) in PHBV is about 0–25%. The addition of HV can improve the flexibility and impact strength of the material, especially for making chiral building blocks. Because the mechanical and thermal properties of PHBV are similar to those of polypropylene (PP), it can be processed using common equipment. In addition, the degradation rate of PHBV is slower than polylactide (PLA) and poly(latide-co-glycolide) (PLGA), thus it can be made into implant of long-term purpose (Hocking & Marchessault, 1994).

The surface modification of polymer becomes important when polymeric material is contacting with the physiological component such as blood and living tissues. In order to covalently immobilized biomacromolecules onto polymer surfaces, they are required to have functional groups such as amine, imine, carboxyl hydroxyl, isocyanate, expoxy etc. Since most conventional polymers except for polysaccharides have no such functional groups on the surfaces, they should be modified so as to have reactive groups for covalent immobilization of biomacromolecules. Indeed, several papers have reported that the introduction of functional group to the surfaces of PHBV can be achieved by plasma (Flösch, Geckeler, Schue, & Göpel, 1992), γ-ray radiation grafting of acrylic acid (AA) (Mitomo & Enôjji, 1995), and oxidizing chemical grafting (Haene & Remsen, 1999).

Chitosan (ChS) is the deacetylated derivative of chitin which is one of the most abundant nature polysaccharides containing nitrogen. Chitosan is a widely used natural, abundant biopolymer, and produced commercially from

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crab and shrimp waste shells. Chitosan (ChS) and its derivatives have been identified as hydrophilic, non-toxic, biodegradable, antibacterial and biocompatible, should be extraordinarily suitable for tissue engineering as the scaffold (Muzzarelli et al., 1990; Olsen, Schwartzmiller, Weppner, & Winandy, 1988; Seo, Mistuhashi, & Tanibe, 1992). It has been used as a wound healing accelerator, a health food to reduce the blood cholesterol level, and an immune system stimulant. When sticking to the bacterial cell wall, ChS can suppress the metabolism of bacteria (Iida et al., 1987; Koneman, Allen, Janda, Sohreckenberger, & Winn, 1997). The antibacterial study of poly(ethylene terephthalate) (PET) grafted with chitosan and its derivatives has been performed to *Staphylococus aureus* and *Escherichia coli* (Huh et al., 2001).

The degree of acetylation plays a key role in cell adhesion and proliferation, but does not change the cytocompatibility of chitosan (Chatelet, Damour, & Domard, 2001). Therefore, chitosan cannot be considered as a suitable biomaterial for in vitro fibroblast cultivation. However, its cytocompatibility towards fibroblasts allows its use in combination with other materials, such as collagen (Shahabeddin et al., 1991).

2. Experimental

2.1. Materials

Poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) with 5 wt% HV content was purchased from Aldrich Chemical Co., Inc. USA. Chitosan (molecular weight about 1.6×10^5 and a degree of deacetylation of 85.3%) was purchased from Shin Era Technology Co., Taiwan. Acrylic acid and 25% glutaraldehyde (GA) were purchased from Ferak Laborat Gmbh, Germany. 1-ethyl-3-(3-dimethy-laminopropyl) carbodiimide hydrochloride (EDC) was purchased from Acros Organics, New Jersey, USA. Type I collagen (purity: 98%) and human plasma fibrinogen (HPF) with clottable proteins >95% were purchased from Calbiochem, USA. Human serum albumin (HSA, type V) was purchased from Sigma, USA.

2.2. Preparation of membrane

The PHBV powder was dissolved at 60 °C in chloroform to a concentration of 50 mg/ml. The solutions were cast on glass plates and then dried in a hood. The thickness of the resulting membranes was about 0.3 mm. Afterwards, these membranes were treated by the Soxhlet extraction with methanol for 24 h to remove residual chloroform.

2.3. Ozone treatment

A piece of membrane with a dimension of 2×8 cm^2 was placed in a 250 ml Erlenmeyer flask and flushed with air containing 10.2 g/m^3 of ozone for a 20 min. The ozone was generated using an ozone generator (Kang I Ozone Enterprise Co., Ltd., Taiwan) and the ozone concentration was controlled by an Ozone UV Photometric Analyzer (SOZ-6000, SKEI Electronics Co., Ltd., Japan). Afterwards, the sample was evacuated for 2 min to remove unreacted ozone, and then soaked at 65 °C in an aqueous solution containing 10% of acrylic acid, 0.2 M H_2SO_4 and 1 mM $FeSO_4$. After 60 min, the sample was retrieved and rinsed with adequate double-distilled water for 3 times, followed by soaking in 150 ml of double-distilled water at 75 °C for 24 h. In the first 10 h, the water was replenished every 2 h, and after 10 h, the water was replenished every 4 h. These procedures were carried out to remove unreacted AA and the homopolymer of AA.

2.4. Chitosan grafting

Acrylic acid grafted membranes were cut into pieces of 1×1 cm^2, and placed in the reacting solution containing 1N HCl and 0.25 mg/ml of ChS (dissolved in 5 mM acetic acid) at 45 °C to proceed the esterification for 5 min. Afterwards, the membrane was rinsed with PBS and double-distilled water three times to remove unreacted ChS. The reaction is given as scheme (a) in Fig. 1.

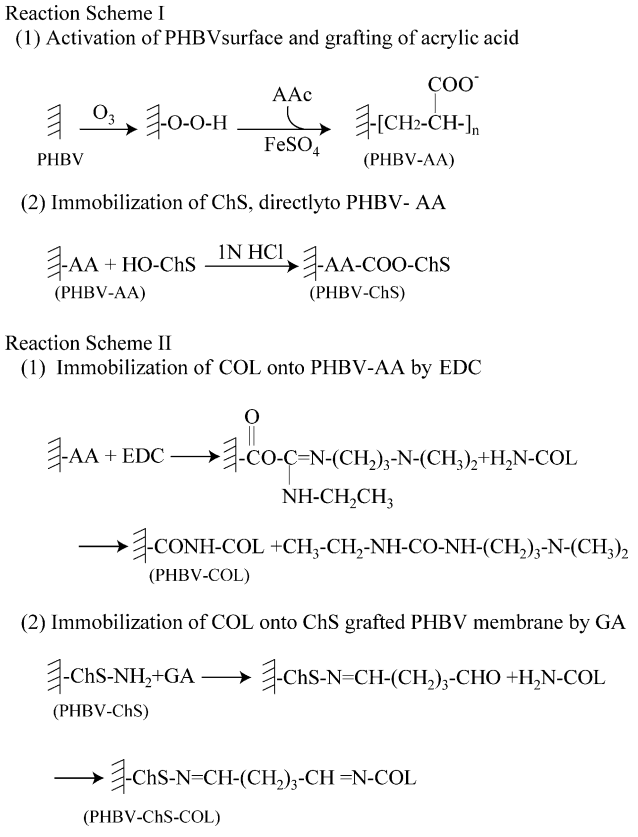

Fig. 1. The reaction schemes for grafting ChS and COL onto the membrane surfaces.

2.5. Collagen immobilization on modified PHBV membranes

Acrylic acid grafted membranes were cut into pieces of 1×1 cm^2, and placed in the reacting solution containing 0.2 mM EDC at pH 4.8 and 4 °C for 24 h, and were washed 3 times with phosphate buffer solution (PBS) and double-distilled water, then the membrane was dried in an oven at 65 °C. These EDC treated membranes were then placed in 0.25 mg/ml COL (in 0.1 M acetic acid) to react at 4 °C for 24 h. Afterwards, these membranes were treated by the Soxhlet extraction with methanol for 24 h to remove residual EDC. The reaction is given as scheme 2 (1) in Fig. 1. The resulting samples were −40 °C freeze-dried using freeze-dryer for 2 h.

Chitosan grafted membranes were first treated with 0.2 mM glutaraldehyde at 25 °C for 30 min, and were washed 3 times with PBS and double-distilled water, then the membrane was dried in an oven at 65 °C. These glutaraldehyde treated membranes were then placed in 0.25 mg/ml COL in 0.1 M acetic acid and reacted at 25 °C for 30 min. Afterwards, these membranes were treated by the Soxhlet extraction with methanol for 24 h to remove residual glutaraldehyde. The reaction is given as scheme 2 (2) in Fig. 1. The resulting samples were −40 °C freeze-dried using freeze-dryer for 2 h.

2.6. Determination of surface grafting density

The surface density of carboxyl group from AA or COL were determined by dyeing with 0.01 g/ml of C. I. Basic Blue 17 (Chroma-Gesellschaft GmbH, Müster, Germany) at pH 10 and 30 °C for 5 h. After dyeing, the membrane was rinsed with adequate double-distilled water, followed by soaking in 0.1 mM NaOH to remove adsorbed dye molecules. Finally the associated dye molecules were desorbed in 50%(v/v) acetic acid. The dye concentration was determined at 633 nm using a spectrophotometer (UV 3101 PC, Shimadzu, Tokyo, Japan) and calculated from the calibration curve.

The surface density of amino groups of ChS on the membrane surface was determined by dyeing with 0.01 g/ml of C.I. Acid Orange 7 (Tokyo Kaseo Kogyo Co., Ltd., Japan) at pH 3 and 30 °C for 5 h, and then rinsed with adequate double-distilled water, followed by 1 mM HCl to remove adsorbed dye molecules, and finally by 1 mM NaOH to release the associated dye molecules. The dye concentration was determined at 485 nm and calculated from the calibration curve (Kato & Ikeda, 1996).

2.7. Measurement of water contact angle

The water contact angles of the surface-modified polyester were measured with a contact angle goniometer (DSA 100, Krüss GmbH, Germany). A piece of 1×1 cm^2 membrane was stick on a glass slide and mounted on the goniometer. The drop size was 0.01 ml. After water dropping, the membrane was incubated in 65%RH and 20 °C for 10 min before taking the value of contact angle. Each point was averaged from 6 measurements.

2.8. Protein adsorption

A piece of membrane of 1×1 cm^2 was immersed in 5 ml of 0.5 mg/ml of HSA or HPF, and was shaken at 100 rpm and 37 °C for 12 h. The membrane was gently taken out and rinsed 5 times with PBS. Then the membrane was placed in a glass bottle with 1 wt% aqueous solution of sodium dodecyl sulfate (SDS) and shaken for 60 min at room temperature to remove the protein adsorbed on the surface. A protein analysis kit (MicroBCA protein assay reagent kit, Pierce, Rockford, IL, USA) based on the bicinchoninic acid (BCA) was used to determine the concentration of the proteins in the SDS solution (Rebeix, Sommer, Marchin, Baude, & Duc, 2000).

2.9. In vitro antibacterial test

Four strains of bacteria used in this work. Methicilin resistant *S. aureus* (MRSA; *S. aureus*−1) of acute abscess infection, *S. aureus* strain-2 (*S. aureus*-2) of wound infection culture due to suture, and *Pseudomonas aeruginosa* (ATCC 10145) are common infectious bacteria found in hospitals. Entrohemorragic *E. coli* O157:H7 is a pathogenic infectious bacterium found in recent years. *S. aureus*-1 and *S. aureus*-2 are Gram-positive bacteria, while *E. coli* O-157:H7 (ATCC 43894) and *P. aeruginosa* are Gram-negative bacteria. Frozen preserved stock was thawed at room temperature, and then 0.1 ml were pipetted and streaked into quadrant on sheep blood agar plate (Difco Laboratories, USA), and cultured at 37 °C overnight. Afterwards, a single colony was scraped with a loop and swabbed to a 15° slant medium (10 ml of nutrient agar) and incubated at 37 °C. After culturing for 18–24 h, 20 ml of PBS, which contains 72 ml of 0.2 M Na_2HPO_4, 28 ml of 0.2 M NaH_2PO_4, 0.5 g NaCl and 2 g Tween 80 in water (1 l) was added. After mixing, 1 ml of the solution was moved into 9 ml of nutrient broth (concentration = 8 g/l), and mixed with a vortex mixer. The solution was then diluted with PBS to $1.5\pm0.3\times10^5$ cell/ml, and placed in flasks (6 samples of 0.4 g/sample for each group). After incubating at 37 °C for a period of time up to 24 h, 20 ml of PBS were added and stirred for 30 s. Consecutive dilute solutions were prepared by taking 1 ml of the previous solution and mixed with 9 ml of PBS. From this solution, 1 ml was transferred to a 50-ml centrifugal tube, mixed with 15 ml of nutrient agar (at 45 °C), poured into a 9-cm plate, let it cooled down, and incubated at 37 °C for a period of time up to 24 h. The number of survival bacteria was then counted.

2.10. Cell culture

L929 Fibroblasts (obtained from Food Industry Research and Development Institute, Hsinchu, Taiwan) were grown in a medium supplemented with a minimum essential medium alpha medium (Gibco, USA) with 0.1 mM non-essential amino acids, 1 mM sodium pyruvate and 90% Earle's balanced salts (Sigma, USA), 10% horse serum in tissue culture flasks in a CO_2 incubator at 37 °C. Confluent monolayers were propagated by trypsinization (0.25% trypsin, 0.02% EDTA) and replating at 1:2 dilution. The cells were maintained in a complete medium replenished every day. After 3-day culture, the culture observation was carried out using an inverted light microscope (Axiovert 25 CFL, Carl Zeiss, Jena, Germany). At confluency, cells were harvested and subcultivated in the same medium (Freshney, 2000).

2.11. Cell attachment

Study of cell attachment was on two groups of six 3-cm polystyrene Petri dishes without biomaterial deposit: untreated PHBV, and modified membranes. Each specimen (1×1 cm^2) was thoroughly washed with PBS. Fibroblasts, 17,000 cells/cm^2, were seeded on top of each film, and cultured at 37 °C for 3 h. Unattached cells were removed by washing with PBS, and the number of attached cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) test (Freshney, 2000).

2.12. Cell proliferation

Modified membranes were stick with vacuum grease to the bottom of six 3-cm polystyrene Petri dishes without biomaterial deposit to prevent them from floating in the growth media. Fibroblasts, 350 cells/cm^2, were plated on the specimens, and the medium was changed every 24 h during incubation in a CO_2 incubator. Cell proliferation on each specimen was determined after 5 days. To remove unattached cells, specimens were gently washed with PBS. The attached cells were separated from the substrate by incubation in 50 μl of 0.25% w/v trypsin solution for 10 min at 37 °C, and 100 μl of media was added. After centrifugation, cells were placed in the fresh medium. An aliquot of the resulting cell suspension was stained with trypan blue and counted by using a Neubauer hemacytometer (Freshney, 2000; Park et al., 2000a) on an inverted light microscope.

3. Results and discussion

3.1. Surfaces modification of membranes

The surface modification was based on the schemes described in Fig. 1. The surface density of grafted functional groups on each specimen in this work was summarized in Table 1. We found out that the optimal condition for treating PHBV was 20 min of ozone treatment followed by 1 h grafting at 65 °C in 10 wt% AA. This resulted in 0.26 nmol/cm^2 of carboxyl groups. After esterification for 5 min in 1N HCl, the surface density of amino groups was 0.85 nmol/cm^2 for ChS.

Table 1
Surface density of modified PHBV membranes ($n=5$, ±SD)

Sample	Surface density (nmol/cm^2)	
	Carboxyl group	Amino group
PHBV	NA	NA
PHBV-AA	0.26±0.01	NA
PHBV-ChS	0.06±0.01	0.85±0.05
PHBV-COL	1.79±0.10	1.17±0.08
PHBV-ChS-COL	0.58±0.02	0.42±0.02

By comparing the surface functional groups of PHBV-AA and PHBV-ChS, the esterification of ChS used about 77% of the carboxyl group on PHBV-AA. On the other hand, by comparing PHBV-AA and PHBV-COL, the immobilization of COL consumed about 66% of the carboxyl group on PHBV-AA (assuming the NH_2/COOH in COL is 79/115 (Miller & Gay, 1982) and only COL has NH_2). Because of the degree of reaction was less than complete, the ratio of amino to carboxyl group deviated from the theoretical ratio of 68.6%. Similar calculation suggests that more than 15% of the amino groups on PHBV-ChS-COL was from ChS, thus contributed to the antibacterial activity of the modified PHBV.

3.2. Hydrophilicity of surface-modified PHBV

The first event when biomaterials expose to blood is the adsorption of blood proteins such as globulin and fibrinogen. This may lead to the formation of thrombus. It is established that hydrophilic surface can reduce the protein adhesion (Park, Shim, Dewanjee, & Eigler, 2000b; Wang, Tan, & Kang, 2000). Since the thrombus formation begins with the adsorption of protein and platelets, efforts have been on controlling protein adsorption and platelet adhesion (Fujimoto, Inoue, & Ikada, 1993). In this work, we employed ChS, and COL to improve the hydrophilicity of PHBV.

The effect of surface modification on the hydrophilicity was illustrated by the contact angle shown in Table 2.

Table 2
Water contact angle and protein adsorption on the PHBV membranes ($n=4$, ±SD)

Sample	Water contact angle (°)	Amount of protein adsorption (μg/cm^2)	
		HSA	HPF
PHBV	53.0±0.6	98±6	26±2
PHBV-AA	31.9±0.4	67±4	22±1
PHBV-ChS	32.0±0.6	120±8	35±2
PHBV-COL	20.8±0.5	45±4	14±1
PHBV-ChS-COL	17.2±0.3	57±3	20±1

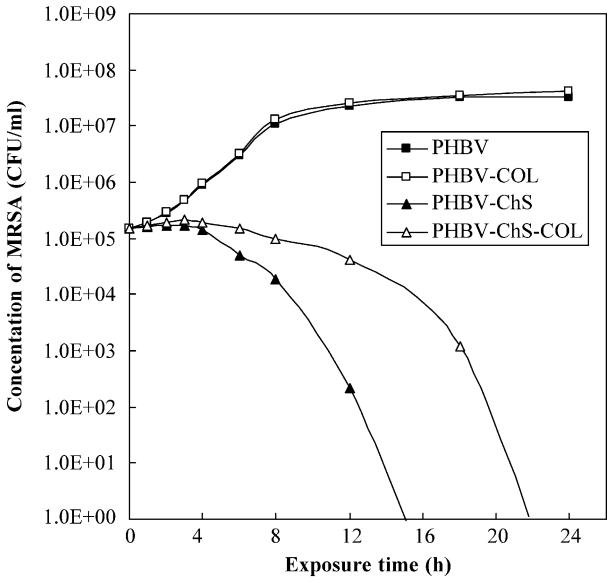

Fig. 2. Change in the viable cell number of MRSA with the exposure time.

Based on the contact angle results, the order of hydrophilicity was in the following order: PHBV-COL > PHBV-ChS-COL > PHBV-ChS ≅ PHBV-AA > PHBV.

The hydrophobic nature of the unmodified PHBV surface showed relatively higher contact angle than other samples. The contact angle of polymer surface can be decreased by introducing hydrophilic groups, such as peroxides, hydroxyl group, carbonyl, and carboxyl group after ozone oxidation (Kato & Ikeda, 1996). Because PHBV-COL has the highest surface density of both carboxyl and amino groups, its contact angle is the lowest among these samples. Considering that polyacrylic acid is soluble in water while ChS is not, and that the degree of dissociation of amino group is only 10% at pH 7, PHBV-AA and PHBV-ChS had similar contact angle, despite that the surface density of carboxylic group on PHBV-AA was less than the amino group on PHBV-ChS. The immobilization of COL introduced more functional groups and made the contact angle of PHBV-ChS-COL lower than that of PHBV-ChS.

3.3. Protein absorption

The effect of COL immobilization on the adsorption of proteins is shown in Table 2. From the results in Table 2, the order of the protein adsorption amount is PHBV-ChS > PHBV > PHBV-AA > PHBV-ChS-COL > PHBV-COL. After grafting with AA, the adsorption amount was reduced, while after the grafting of ChS, the adsorption amount was higher than the un-modified PHBV. On the other hand, after immobilized with COL, the adsorption amount was reduced to about 50–70% of that of the precursor.

The isoelectric points of albumin and fibrinogen are 4.8 and 5.5, respectively (Nishimura, Ikeuchi, & Tokura, 1980; Winterton, Andrade, Feijen, & Kim, 1986), thus these proteins carries negative charge in the normal blood circumstance (pH 7.4). Only PHBV-ChS was positively charged, thus negatively charged HSA and HPF were attracted. On the contrary, PHBV-AA, PHBV-COL, and PHBV-ChS-COL were negatively charged, thus negatively charged HSA and HPF were expelled.

In addition to electrostatic interaction, stereo hindrance also plays an important role in preventing protein adsorption. Although the surface densities of carboxyl group on those four negatively charged samples were close, those two COL-immobilizing samples adsorbed much less protein than PHBV-AA. Albumin has a heart-like shape with edges of $8.2 \times 7.0 \times 8.3$ nm and a thickness of 3.0 nm. Fibrinogen has a cylindrical shape with a diameter of 6.0 nm and a length of 45 nm (Basinska, 2001). Because HSA is much smaller than HPF, it can penetrate more easily into the COL layer, thus the adsorbed amount of HSA was higher (about 3 times in terms of μg or 15 times in terms of mole) than that of HPF.

3.4. Antibacterial activity

When using chitosan as biomaterial, it is able to prevent infection by inhibiting bacterial growth (Miller & Gay, 1982; Seo, et al., 1992;). The cationic amino groups of ChS can associate with anions on the bacteria wall, suppress its biosynthesis, disrupt the mass transport across the wall, and accelerate the death of the bacteria (Ikeda, Hirayama, Yamaguchi, & Tazuke, 1986).

The antibacterial activity was evaluated with those four clinical infectious bacteria described in Section 2.9. Fig. 2 shows the effect of grafting on the growth curves of MRSA. When contacting with untreated PHBV, the bacteria grew from 1.5×10^5 to 3.2×10^7 CFU/ml after incubating at 37 °C for 24 h. After COL immobilizing onto PHBV-AA, the bacteria grew to 4.19×10^7 CFU/ml. It has been reported that collagen may improve the adhesion of *S. aureus*, and allow bacteria to avoid host defenses (Lamba, Baumgartner, & Cooper, 2000). On the other hand, when contacting both PHBV-ChS, the concentration of bacteria reduced rapidly and died out after incubating at 37 °C for 24 h. After COL immobilizing onto PHBV-ChS, the reduction in the number of MRSA began to occur at 8 h, which was less than the 4 h of PHBV-ChS. However, after 24 h of incubation, all the bacteria died out for both PHBV-ChS and PHBV-ChS-COL. Similar results were observed for *S. aureus*-2, *P. aeruginosa*, and *E. coli* O-157:H7.

Table 3 compares the antibacterial activity of these modified PHBV membranes based upon the survival ratio after incubating at 37 °C for 12 h. Without grafting ChS, bacteria would grow at least 140% in 12 h. Therefore, we can conclude that membranes grafting with ChS can suppress the growth of all four bacteria used in this study. Our previous studies showed that the antibacterial activity of ChS is lower for *S. aureus* than for *E. coli* and *P. aeruginosa*. Table 3 shows that the order of antibacterial activity of ChS is *E. coli* > *P. aeruginosa* > *S. aureus*-2 > MRSA. Although the antibacterial activity did drop after grafting with COL,

Table 3
Bacterial survival ratio on the PHBV membranes after 12 h of incubation

	PHBV	PHBV-COL	PHBV-ChS (%)	PHBV-ChS-COL (%)
MRSA	146±65	166±71	0.144±0.027	27.6±3.6
S. aureus−2	299±82	349±88	0.043±0.008	8.1±3.1
E. coli	1274±424	1474±458	0.012±0.004	2.3±0.2
P. aeruginosa	831±334	956±363	0.025±0.006	4.5±0.8

the bacterial survival ratio of PHBV-ChS-COL was still less than 1%.

The extracellular capsule of MRSA makes it more hydrophobic than capsule-less *S. aureus*-2. Bacterium with extracellular capsule carries less negative charges, and is less prone to be adsorbed on the positive-charged membrane surface. This makes MRSA less interactive with ChS grafted PHBV than *S. aureus*-2. For Gram-negative *E. coli* and *P. aeruginosa*, they have flagella on the structures external to the cell wall and thus have higher mobility. *P. aeruginosa* has less flagella than *E. coli*, thus is less mobile than *E. coli*. Futhermore, *E. coli* has fimbriae, which make the bacterium more adsorbable. Therefore, the grafting of ChS is more antibacterial to *E. coli* than other bacteria tested.

The results of this part of experiment indicates that by grafting ChS, PHBV can exhibit antibacterial activity, even after the additional immobilization of COL.

3.5. Cell attachment and cell proliferation

In general, hydrophobic polymers have been known as unfavorable for cell attachment unless modified to posses a hydrophilic surface with a higher surface energy and a correspondingly lower air–water contact angle (Evans & Steel, 1998). The effect of surface modification on the cell attachment is shown in Table 4. The order of attaching amount is PHBV-ChS > PHBV-ChS-COL > PHBV-COL > PHBV-AA > PHBV. The result suggests that positively charged ChS could improve the attachment of fibroblast. Chatelet et al. reported that the attachment of fibroblast increases with the decrease of the degree of acetylation of ChS (Chatlet et al., 2001). The degree of acetylation is equivalent to the amino group used up for immobilization of COL in our work. Thus their result is similar to our finding that higher surface density of amino group leads to more cell attachment. Immobilization of COL would result in less attachment, although still higher than the control. This is probably due to the negative charge and the stereo hindrance of COL molecule. Being hydrophobic, the fibroblast attachment for PHBV was the least among all those samples.

Table 4
Cell attachment and growth on the PHBV membranes ($n=6$, ±SD)

Sample	Relative cell attachment (%)	Relative cell growth (%)
Control[a]	100	100
PHBV	119±3	95±2
PHBV-AA	177±4	82±2
PHBV-ChS	384±6	86±2
PHBV-COL	309±5	173±12
PHBV-ChS-COL	334±5	130±9

[a] Polystyrene petri dish.

The effect of surface modification on the proliferation of fibroblast is also shown in Table 4. The order of cell proliferation is PHBV-COL > PHBV-ChS-COL > PHBV > PHBV-ChS > PHBV-AA. Many reported that COL grafted to polymers shows growth-promotion effect (Ishihara et al., 2001; Lee, Hsiue, Chang & Kao, 1996; Park et al., 2000a). This agrees with our finding that COL immobilization did improve the proliferation of fibroblast. On the other hand, ChS grafting can reduce the number of cells. Chatelet et al. suggested that chitosan seems to be cytostatic toward fibroblast: it is not cytotoxic, but inhibits cell proliferation (Chatlet et al., 2001). This is in agreement with our results of ChS grafting membranes.

4. Conclusion

The hydrophilicity of PHBV films can be improved by grafting ChS. Due to the introduction of amino group, the grafting of ChS can also endow PHBV with antibacterial activity against four pathogenic bacteria. On the other hand, being positively charged, ChS grafting shows higher adsorption for HSA (120 μg/cm^2) and HPF (35 μg/cm^2). By the immobilization of COL, the surface becomes negative charged, and reduces the protein adsorption to about 40% of that of PHBV-ChS.

Grafting ChS onto PHBV shows improvement in fibroblasts attachment, yet reduces the cell proliferation. Immobilization of COL without ChS can promote the proliferation of fibroblast to 181%. Grafting only ChS without COL can improve the attachment of fibroblast to 322%. On the other hand, by immobilizing both ChS and COL onto PHBV, the fibroblast attachment can be increased to 280% and the proliferation can be increased to 137%. In addition, PHBV-ChS-COL also exhibits the anti-bacterial activity against four pathogenic bacteria.

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