



Original Research

Assessment of the cutaneous wound healing efficiency of acidic, neutral and alkaline bacterial cellulose membrane in rat

Parastoo Pourali¹ · Nasrin Razavianzadeh¹ · Leila Khojasteh¹ · Behrooz Yahyaei¹

Received: 21 November 2017 / Accepted: 5 June 2018 / Published online: 25 June 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Recent research was conducted to evaluate the healing efficiency of bacterial cellulose (BC) as a wound dressing in different pHs and its possibility of being a smart wound dressing that can indicate pHs. BC was produced by environmentally isolated bacterial strains. After washing the best achieved BC, it was floated in normal saline with different pHs with phenol red used as a pH indicator. Finally the wound healing effects of the acidic, neutral and alkaline BC membranes were evaluated in rat cutaneous wounds. Results showed that one of the isolates which its partial 16srRNA genome had 95% similarity with *Gluconacetobacter intermedius*, had the thickest layer. The microscopic and macroscopic evaluations showed that the acidic BC had the best healing activity. Although the color of the films remained unchanged during the experiments because they were transparent and thin, these changes could not be easily seen. This suggests the use of thicker films such as the ones which are cross linked with some materials (e.g., sterile gauze). In conclusion the pH can affect the healing ability of natural BC and acidic pH had the best wound healing efficiency. In future it is better to use the acidic BC instead of natural one for different wound healing purposes.

1 Introduction

Successful wound healing process depends on several endogenous and exogenous factors that directly or indirectly involve in the regeneration process. One of the important factors that may have impact on the metabolism of wounds is the pH values [1].

The intact skin surface usually has acidic milieu due to the keratinocyte secretions like some types of fatty acids and amino acids. This acidic pH varies between male and female and is altered by the age, but it is usually reported to be around 4–6 [1]. This pH is altered due to the damage of the surface layers of the skin and lack of stratum corneum. The underlying tissues of the skin have normal pH around 7.4. This alternation in the pH milieu has some circumstances. For example some pathogenic bacteria can simply colonize in pHs higher than that of the normal pH values of skin, which will consequently affect the normal healing

process. Also, it has been shown that antibiotic therapy may be altered by the variations in the pH values. It was reported that if the pH milieu of the wound area become alkaline, then its healing will be decreased. For example chronic wounds which have alkaline pH are poorly responsive to different ways of treatments [1].

There are three phases in the normal wound healing process: inflammatory phase, proliferative phase and remodeling phase. In the first phase due to the injury and production of different cytokines, neutrophils and macrophages invade the wound site and due to their activity, the pH value of the site becomes acidic. This pH has an important role in the cell function and its ability to respond to the immunological factors. This process is followed by the second one, proliferative phase. Angiogenesis, formation of extracellular matrix, epithelial tissue, collagen bundles and etc., are seen in this phase. Finally in the last phase, by the activity of different enzymes such as proteinase, the healing is achieved. In all the aforementioned stages, the pH value is very important factor. For example the enzymatic activity completely depends on the pH value and each alternation in this item could impact the appropriate wound healing process. Bacterial infections usually take place in higher pH values, which is induced by their enzymatic activity.

✉ Behrooz Yahyaei
behroozyahyaei@yahoo.com

¹ Department of Medical Sciences, Shahrood Branch, Islamic Azad University, Shahrood, Iran

In situations where the pH milieu of the wound cannot be stabilized correctly, the bacterial enzymatic activity will proceed resulting to pain, pus formation, and etc., For example it is proven that the colonization of the pathogenic bacteria such as *Staphylococcus aureus* easily take place in pH values around 7.5–8. Hence one of the therapeutic techniques is the modification of the wound pH by some agents such as topical application of acetic and citric acids which could stop the bacterial colonization [2].

Therefore the use of the new generation of wound dressing with both abilities of maintaining the acidic pH milieu and indicating pH of the wound will help toward a better understanding of the healing process.

Wound dressings based on their action are classified into three major groups that are named passive, interactive and active dressings. In the passive dressing group, there are some agents such as sterile gauze which are waterproof and cover the wound surface. In the interactive dressing group, there are some products which not only cover the wound surface but also maintain the water, permeable to the atmospheric oxygen and are barrier against pathogenic microorganisms. Hyaluronic acid, alginate and collagen are some examples which help re-epithelialization and granulation of the wound area. Finally the bioactive dressing group is developed from the interactive one which delivers antibiotics and other drugs to the wound site and by alternating in the wound chemical microenvironment helps the best healing process [3]. There is another classification that on the basis of the dressing nature, the dressings were divided into natural and synthetic types [3].

Bacterial cellulose (BC) is a type of interactive dressing which is made by some bacterial strains such as some species of *Gluconacetobacter* and *Acetobacter*, and is a homopolymer of β (1, 4) linked D-glucose. This polymer is natural, water insoluble, non-toxic, biocompatible and biodegradable, with high ability of water maintenance. Furthermore this polymer is available due to the use of the bacterial strains as small factories. The nanodiameter produced mesh makes it a microbial barrier in the wound site [4]. Although it was proven that the acidic pH milieu of the wound site has better wound healing properties [2]; however there is no available report about the healing efficiency of the BC as a wound dressing with different pHs and the possibility of being a smart wound dressing which indicates the pHs. Hence in this study BC was produced by some bacterial strains that were isolated from environmental sources. After washing the best achieved BC, it was floated in normal saline with different pHs with phenol red used as a pH indicator. Finally the wound healing effects of the acidic, neutral and alkaline BC membranes were evaluated in rat cutaneous wounds.

2 Materials and methods

2.1 Isolation of the BC producing bacteria

Bacterial strains were isolated from different food sources such as home-made vinegar and fruit juices. 1 mL of each sample was cultured in Hestrin–Schramm (HS) broth medium and incubated at 30 °C in a static incubator for one week. Formation of the yellowish layer on the top of the broth was the first sign of BC formation. Using a microbiological loop, a loop from the surface of the broth was cultured by streak plate method into a HS agar medium and incubated in the aforementioned condition. The obtained colonies were sub cultured into HS broth flasks and after incubation, the flasks which had thicker layers were used for further studies [4].

2.2 Bacterial identification

Identification of the bacterial isolates was done using phenotyping and genotyping methods. For phenotyping, the API 20E (bioMerieux, France) kit [2] was used and for genotyping test polymerase chain reaction (PCR) with 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1389R (5'-ACGGGCGGTGTGTACAAG-3') as the forward and reverse primers was used. The DNA extraction method (i.e. boiling technique) and PCR conditions have been described previously [4]. Briefly 1 μ l (10 mM) of each primer, 0.4 μ l of dNTPs (10 mM), 1.4 μ l of MgCl₂ (25 mM), 1 μ l of the extracted DNA, 0.2 μ l of Taq polymerase (5 unit) and 2 μ l of 10 \times PCR buffer were used for each 20 μ l PCR reaction. The PCR program was done with initial denaturation at 94 °C for 2 min which was followed by 30 cycles of 94 °C for 1 min, 59.5 °C for 1 min and 72 °C for 1 min, and a final extension cycle at 72 °C for 10 min [5]. Finally the PCR products were sequenced in one direction using 63F primer and the obtained sequences were compared with the sequence database available on the National Centre for Biotechnology Information (NCBI) [4, 5].

2.3 Purification of BC film

Based on the obtained results, the best BC sample was first produced again and purified. For purification, the obtained BC film was floated in 3% NaOH solution overnight. In the next day, the BC film was washed using ddH₂O and floated again in ddH₂O (pH 7.0) until the pH of the water was adjusted to pH 7.0 [4].

2.4 Proving the production of BC membrane

In order to prove that the obtained layer was cellulose, enzymatic hydrolysis was performed. The combination of

endo- and exo-glucanase (0.05 mL of 0.5 U of each, Sigma-Aldrich, USA) suspended in 0.2 mL of 0.05 M sodium acetate buffer (pH 5.0) were added to the dried BC film and the mixture was incubated at 30 °C for 24 h in the shaker incubator at 100 rpm. In order to detect the presence of carbohydrates and reducing sugars, the suspension was filtered and subjected to the Molisch's and Benedict's reagents, respectively [4].

2.5 pH adjustment

The purified BC film with the thickness of about 2 mm was cut into 5 × 5 cm pieces, divided into three Beakers and floated in normal saline containing 0.0159 g/mL phenol red as indicator. This concentration equals to the amounts that are used in Dulbecco's Modified Eagle's Medium (DMEM). Using pH meter (PCE-228, USA), the pH of the solution of one Beaker was adjusted to 4.5, the next was adjusted to 7.5 and the last was adjusted to 9.5. The samples were incubated at room temperature overnight and the obtained colored BCs were used for *in vivo* analysis.

2.6 *In vivo* analysis

2.6.1 Macroscopic analysis

For wound healing assessment, 32 Wistar rats (female, 7 weeks old, 180–200 g weight) were purchased from Pasteur Institute of Iran. The rats were kept in 12 h dark and 12 h light cycles, with food and water *ad libitum*, at 25–28 °C, from 10 days before and 21 days of the studies in separate cages. In the first day of the studies they were randomly separated into four groups (n = 8): one group was the control and remained without any treatments. The three remaining groups were treated by the acidic, neutral and alkaline BC films, separately. The rats were anesthetized using ether, and after shaving and disinfecting their skin one full-thickness circular wound (8 mm in diameter) was created on their dorsal body surface and each group

received its own treatment. Each day, the color of the BC film and the size of the open wounds were measured, the contraction percentages were calculated using following formula:

The wound contraction percentage (%) = [size of the wound (mm) (first day-test day)/size of the wound (mm) first day] × 100.

The obtained results were compared with each other using one-way analysis of variance (ANOVA) test in SPSS software version 22. P-values less than 0.05 (p < 0.05) were regarded as significant [6].

2.6.2 Microscopic analysis

Four days of the 21 days of the experiments were chosen for sampling: Days 3, 7, 14 and 21. In each sampling day, two rats from each group was euthanized and skin samples (2 × 2 cm²) which were taken from the wound regions were obtained and fixed in 10% formalin. After paraffin embedding, sections of the obtained blocks were achieved using microtome device (HHQ-1508R Rotary Microtome, China) and were used for hematoxylin and eosin (H&E) staining. The obtained microscopic slides from all the rat tested groups were analyzed and compared with each other for the criteria such as: levels of the infiltration of the inflammatory cells, collagen bundles formation, re-epithelization, angiogenesis, fibroplasia and proliferation of the fibroblast cells [7].

3 Results

3.1 Isolation of the BC producing bacteria

A total of 21 different food sources were used, and almost on the top of all the broth the yellowish layers after one week were formed and the thickest films were further purified. Figure 1 shows the obtained BC films after isolating the bacteria and sub culturing.

Fig. 1 The obtained bacteria isolated in HS agar and broth. **a** HS agar and **b** Flasks contain BC membranes after isolating the bacteria and sub culturing. Each flask contains one bacterial isolate

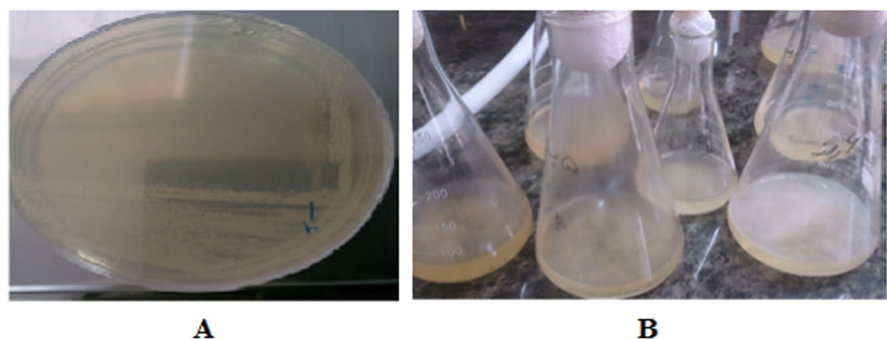


Table 1 The obtained sequencing results of the isolated bacteria

No	Accession number	Description	Maximum identity (%)
1	NR_042762.1	<i>Gluconacetobacter swingsii</i> strain DST GL01	99
2	NR_026435.1	<i>Gluconacetobacter intermedius</i> strain TF2	95
3	NR_041295.1	<i>Gluconacetobacter oboediens</i> strain DSM 11826	99
	NR_026513.1	<i>Gluconacetobacter europaeus</i> strain DES11	97
4	NR_026325.1	<i>Acetobacterium carbinolicum</i> strain DSM 2925	100
	NR_026323.1	<i>Acetobacterium woodii</i> strain DSM 1030	95
5	NR_026329.1	<i>Acetobacterium bakii</i> strain DSM 8239	98
6	–	Unknown	–

3.2 Bacterial identification

In this step, 6 of 21 isolates were identified using phenotyping and genotyping methods. Phenotyping analyses revealed that all the isolated bacteria were non spore forming Gram negative rods and belong to *Gluconacetobacter* and *Acetobacterium* genera. Genotyping test revealed the species of the bacterial isolates. Table 1 shows the obtained sequencing results of the six bacterial isolates using the Blastn program available on NCBI. From this table, the sequencing data of one strain that was identified by the phenotyping method as a strain of *Acetobacterium* was not available and was represented as unknown.

3.3 Purification of BC film

Based on the obtained results, the best BC producing strain was chosen. In this study the BC membrane that was produced by *Gluconacetobacter intermedius* strain TF2 had the thickest layer, and the produced layer was used for purification and *in vivo* studies. The transparent BC membrane after treatment by 3% NaOH solution and achievement of its normal pH (around 7) is shown in Fig. 2.

3.4 Proving the production of BC membrane

Enzymatic hydrolysis using endo and exo-glucanase showed the presence of carbohydrates (appeared as the red ring on the top of the vial after addition of Molisch reagent) and reducing sugars (appeared as orange color after addition of Benedict reagent) in the extracted BC film.

3.5 pH adjustment

In order to adjust the pH, the purified BC films were floated in aqueous solutions of phenol red that was used as an indicator. Changes in the color of the BC films were achieved due to the pH alternations. The color of the acidic BC was orange (pH 4.5), the color of the neutral BC was red (pH 7.5) and the color of the alkaline one was pink (pH

9.5). Figure 3 shows the changes in the color of the BC membranes with different pHs.

3.6 *In vivo* analysis

3.6.1 Macroscopic analysis

As previously mentioned, after treating the wounds with the acidic, neutral and alkaline BC films each day during the experiments, the color of the BC film and the size of the open wound were measured and the contraction percentages were calculated. Results show that the color of the BC films in each group remained constant but because the BC films were transparent, these changes could not be easily seen. Table 2 shows the obtained contraction percentages in the four tested groups.

ANOVA results showed that there were significant differences among all the tested groups on different days of the experiments. As shown in Table 2 and Fig. 4, on day 3, the group which was treated by the alkaline BC had the maximum and the group which was treated by the neutral BC film had the minimum contraction percentage. On day 7, the group which was treated by the acidic BC had the maximum and again the group which was treated by the neutral BC film had the minimum contraction percentage. On days 14 and 21 the group which was treated by the acidic BC had the maximum contraction percentage but in these two days the minimum contraction percentage was seen in the group treated by the alkaline BC and the control one, respectively (Table 2, Fig. 4).

3.6.2 Microscopic analysis

The obtained skin sections after H&E staining were analyzed under light microscope and the levels of the infiltration of the inflammatory cells, collagen bundles formation, re-epithelization, angiogenesis, fibroplasia and proliferation of the fibroblast cells were evaluated. The results of the microscopic evaluations are represented in Fig. 5. As this figure shows, for the control group, on day 3, the level of fibroplasia was

Fig. 2 The transparent BC membrane with the normal pH after purification

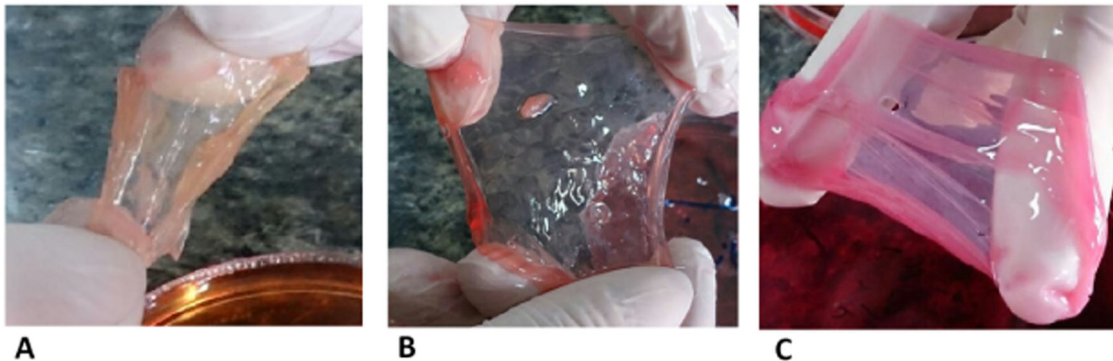
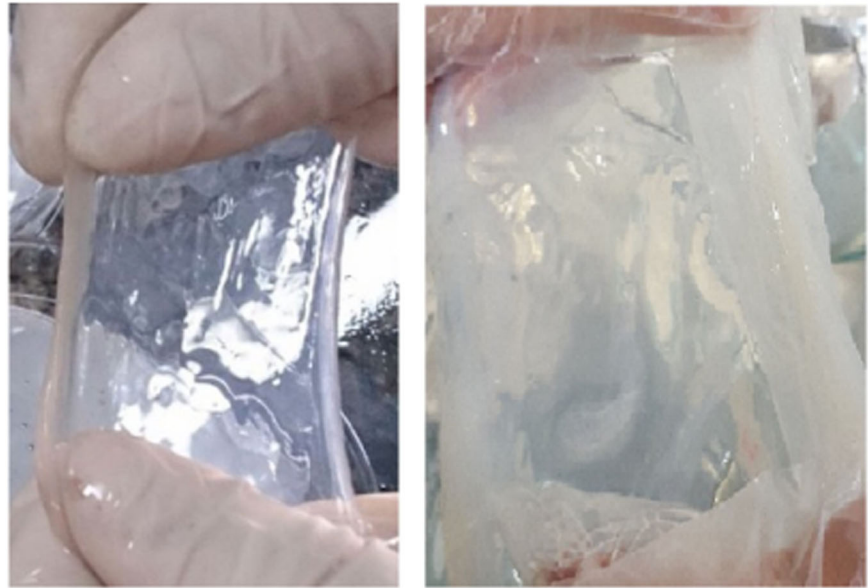


Fig. 3 The changes in the color of the BC membranes with different pHs. **a** Acidic BC film (pH 4.5). **b** Neutral BC film (pH 7.5) and **c** Alkaline BC film (pH 8.5)

suitable and the infiltration of inflammatory cells was seen. On Day 7, the level of collagen bundles was acceptable; fibroplasia and infiltration of inflammatory cells were seen. On day 14, the thickness of the collagen bundles were low. On day 21, the skin contents and the epithelial tissue were present, and the thickness of the collagen bundles was desirable. For the group treated by the acidic BC on day 7, the level of angiogenesis and the infiltration of the inflammatory cells in the wound site were high. On day 14, the collagen bundles with acceptable thickness were present. The healing process was going to be completed. The skin contents and the epithelial tissue were somehow completely formed. On day 21, the epithelial tissue with a thick stratum corneum was seen. For the group treated by the alkaline BC on day 7, the level of the infiltration of inflammatory cells and angiogenesis was high, and the level of fibroplasia was suitable. On day 14, again the level of the infiltration of inflammatory cells and fibroplasia was high but the level of angiogenesis was

Table 2 The wound contraction percentages in the four different groups; acidic, neutral and alkaline BC films and the control on different days of the treatments

Test groups	Wound contraction (%) (mean \pm S.E.M)			
	Day 3	Day 7	Day 14	Day 21
Control	21 \pm 0.09	56 \pm 0.10	84 \pm 0.12	88 \pm 0.02
Acidic BC	20 \pm 0.08	77 \pm 0.25	98 \pm 0.07	99 \pm 0.00
Neutral BC	13 \pm 0.07	47 \pm 0.08	90 \pm 0.02	96 \pm 0.07
Alkaline BC	24 \pm 0.00	62 \pm 0.04	80 \pm 0.00	97 \pm 0.10

lowered. On day 21, the skin contents were not formed. The epithelial tissue was thin and the formation of collagen bundles was imperfect. For the group treated by the neutral BC on day 7, the level of fibroplasia was low but the level of the infiltration of inflammatory cells and angiogenesis was acceptable. On day 14, the epithelial tissue with thickness was completely formed. The collagen bundles were not formed

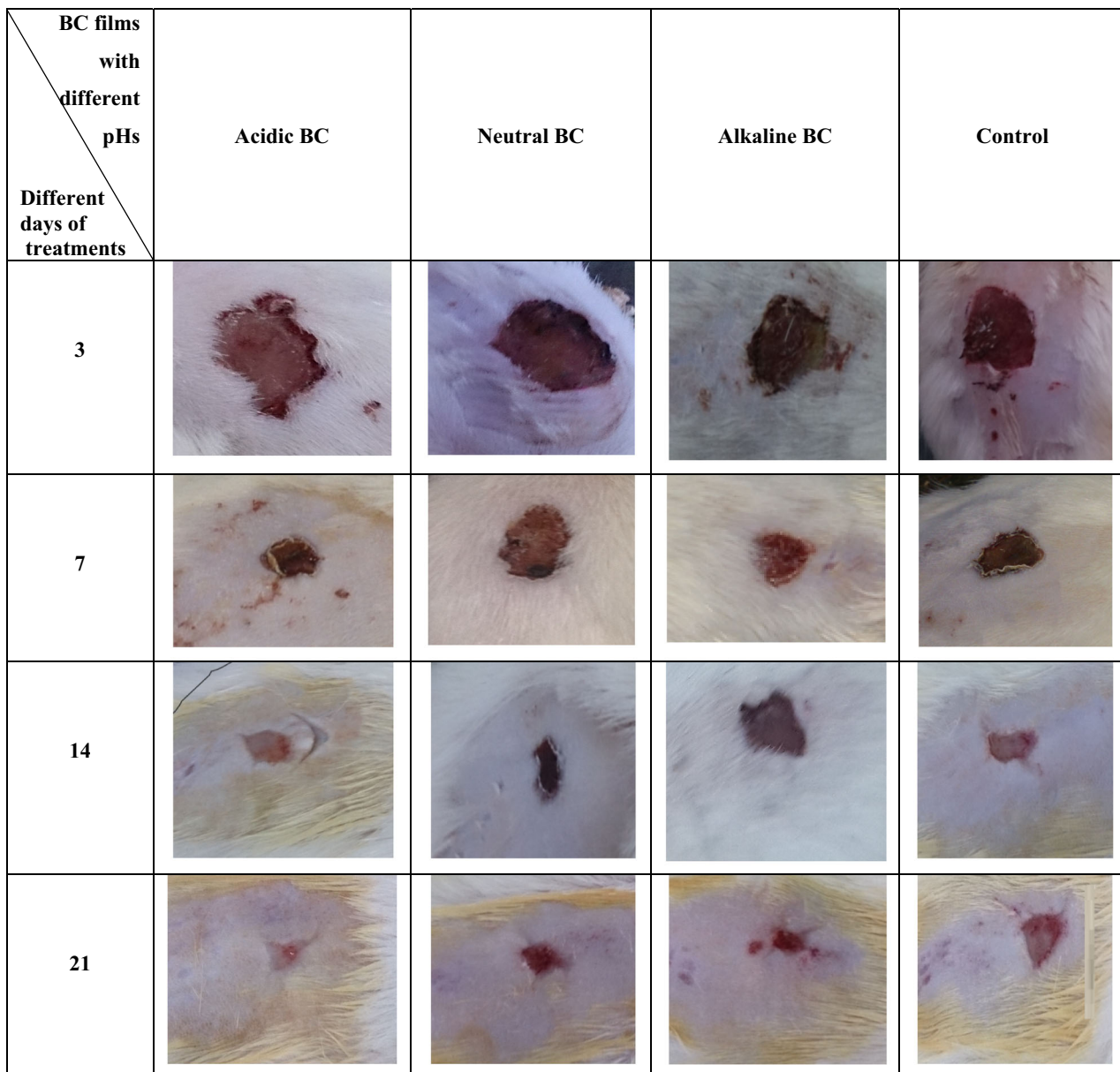


Fig. 4 Wound contraction percentages in four different groups; acidic, neutral and alkaline BC films and the control on different days of the treatments

and the infiltration of inflammatory cells and angiogenesis were present. On day 21, the thin epithelial tissue and stratum corneum were present. Some of the skin contents were seen and the collagen bundles with acceptable thickness were present.

4 Discussion

Previous studies have shown that the pH milieu of the wound area have an important role in the successful wound healing due to its direct or indirect effects on the

biochemical reactions [1]. It was shown that some natural activity in the wound site such as formation of pus is an important part of the wound healing because neutrophils invasion causes acidification of the wound site which helps the proliferation of the fibroblast cells, DNA synthesis and cell migration [1].

It was reported that the acidic pH had the best ability in the healing of the acute and chronic wounds [8]. At this pH the best control of the bacterial infection, antibacterial agent activity, and angiogenesis occurred. Therefore some researches tried to use acidic agents such as acetic acid, alginate acid, ascorbic acid and boric acid in the treatments

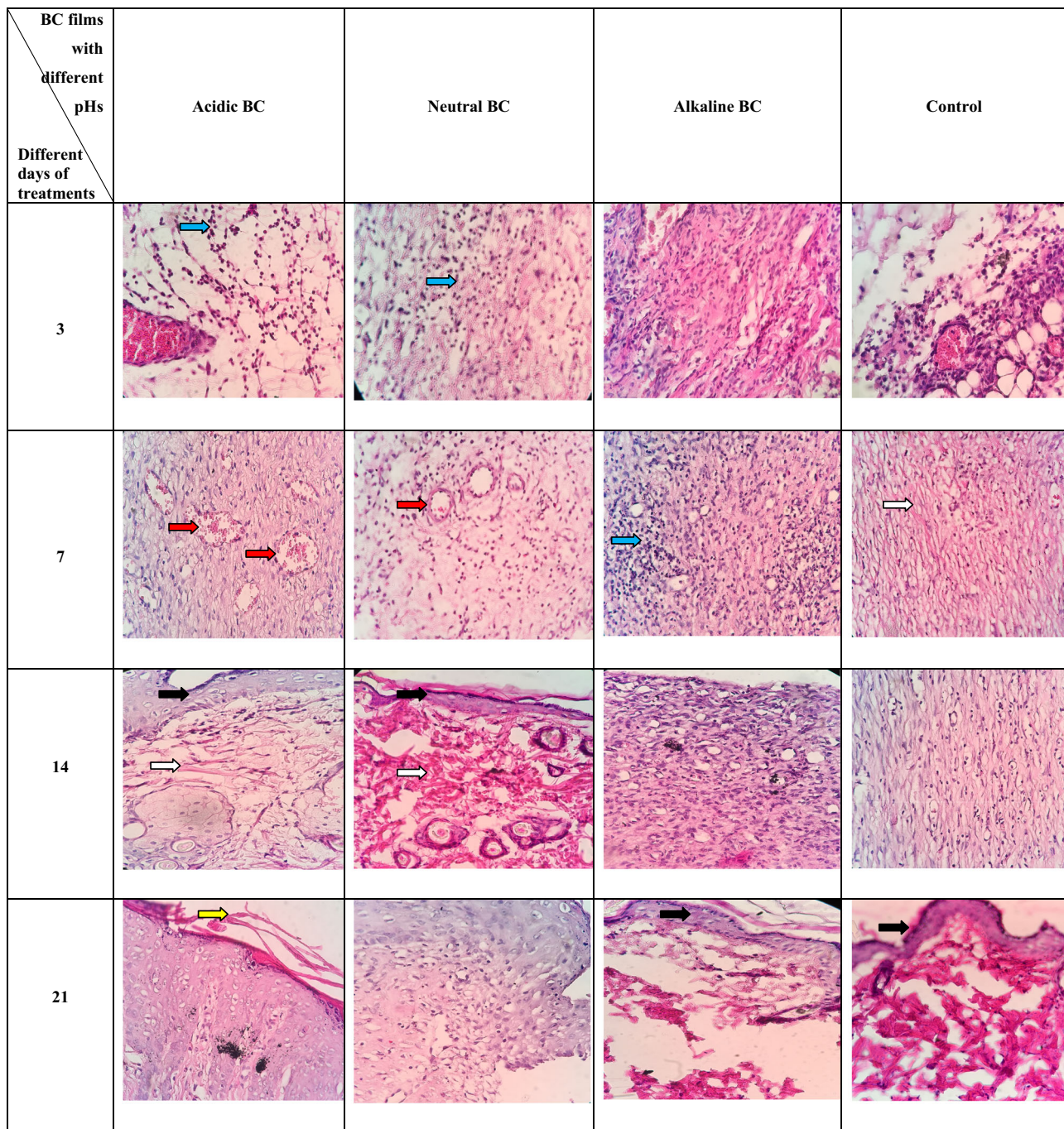


Fig. 5 Histological alternations of the wounds in the four different groups; acidic, neutral and alkaline BC films and the control on different days of the treatments. White arrows: Collagen Bundles, Black

arrows: Epithelium, Blue arrows: Inflammatory Cells, Red arrows: Angiogenesis and Yellow arrows: Stratum Corneum (H&E \times 400)

of wounds [8]. For example citric acid, boric acid and acetic acid were used for treatment the acute and chronic burns which were infected by drug resistant species of *Pseudomonas aeruginosa*. Other bacterial strains such as the species of *Escherichia*, *Proteus*, *Klebsiella*, *Acintobacter*, as Gram negative and *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococci*, and *Enterococci* as Gram

positive ones are involved in the chronic and acute wound infections [8].

Some researches showed that the activity of the anti-bacterial agents such as silver [9], which were incorporated into the wound dressings in the acidic pH, were higher than the natural one. For example when alginate containing silver is used on the wound surface, the acidic pH of the dressing enhances the activity of the silver ions and better

wound healing will be obtained [10, 11]. It was reported that hyaluronic acid impregnated with silver nanoparticles had better antibacterial activity than the activity of each material alone [3].

Furthermore bacterial proteases are mostly active in alkaline environment where the products of enzymatic activity are toxic, therefore by the use of acidic materials this harmful effect will be reduced [8]. Moreover it was reported that acidic environment helps angiogenesis and epithelization processes of the wound area. For example the use of citric acid enhances epithelization, fibroblast regeneration and the blood circulation in the wound which consequently affects the healing process [12].

Furthermore the type of the wound dressing has a role in the healing process. Based on the type of the wounds, the used dressing will vary [9]. There are three main types of dressings including passive, interactive, and bioactive ones. The best examples of the natural dressing are honey with the healing activity in pH 3.5 [8] alginate, hyaluronate, and cellulose [13].

Cellulose is a natural polymer, water-insoluble substance, is the most abundant polymer in the earth, is usually combined with hemicellulose and lignin, and is extracted from plants. The extraction process is harsh, expensive and time consuming by the use of some chemical reagents to achieve the pure polymer. Also, it is produced by enzymatic reaction or chemosynthesis from glucose. Cellulose can be obtained through bacterial fermentation. There are several types of bacterial strains with the ability of cellulose production such as some species of *Acetobacter*, *Gluconobacter*, *Rhizobium*, *Sarcina*, *Agrobacterium*, *Pseudomonas*, *Alcaligenes*, *Aerobacter*, and *Achromobacter* [4, 14]. The produced BC is safe, with high purity, biocompatibility, cytocompatibility, histocompatibility, easily available with high water maintenance, ultrafine reticulated structure which acts as the bacterial barrier and has high mechanical strength [15, 16]. It has been reported that the production of this polymer takes place under aerobic and anaerobic conditions [17]. This polymer never dries in the wound area due to its high hydrophilic properties and its structure is similar to the extracellular matrix of the skin [16].

There are available reports about the usage of this polymer alone and in combination with some healing agents such as kaolin [18], silver nanoparticles [19], benzalkonium chloride, and silver sulfadiazine [16] in the wound site. Due to the natural pH of cellulose, it seems that its healing activity will be altered in different pHs, and up to date the wound healing ability of this polymer at different pHs is not fully understood. Recent research tried to answer these questions: Do different pHs have effect on the healing ability of the pure BC? Can BC, as the new generation of wound dressing, maintain the acidic pH milieu of the

wound site? Can this polymer be used as a pH indicator and as a smart wound dressing?

In the first step of this study, the BC producing bacterial strains were isolated from the natural fruit juices and vinegar. The bacterial strains with the highest ability of BC production (i.e., the thickest film) were subjected to phenotyping and genotyping identification tests. In this study, one isolate which its partial 16srRNA genome had 95% similarities with *Gluconacetobacter intermedius* strain TF2, had the thickest layer and was used for further analyses. After washing the layer using NaOH, it was cut into 5 × 5 cm pieces with the thickness of 2 mm and the films were adjusted at three pHs: acidic, natural and alkaline. The films were floated in the normal saline which contained phenol red at concentrations that were equal to DMEM. This means that the indicator was used in the concentrations that were nontoxic for cell culture (i.e., *in vitro* studies) and there was no need for cytotoxicity assay. If the phenol red is going to be used in higher concentrations, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay should be used to determine its nontoxic doses [20].

In order to analyze the ability of retaining the acidic pH milieu of the used wound dressing and understand the differences between each BC film with different pH, phenol red as a pH indicator was used. In previous researches, the glass microelectrode that was used on the surface of the wound was used in a routine method. Although this technique is used widely, the technique is time consuming. Hence many researchers attempted to achieve the new product with a fast, inexpensive and safe activity in the wound area [1]. Therefore, one aim of the recent research was to evaluate BC as a smart wound dressing, with the ability of indicating the pH.

Different films with different pHs were used in the rat cutaneous wound models and each day the extent of the wound area and the color of the BC films were evaluated. As the results indicated, the color of the BC films did not change during the experiment but because the BC films were transparent and thin, these changes could not be easily seen. As the macroscopic and microscopic assays results showed, there was a significance difference in the healing efficiency of the used BC films with different pHs. The acidic BC had the best activity in the wound site. This represents that if the BC to be used *in vivo* has acidic pH then it can act better in the wound site. Although the acidic BC had better wound healing activity, natural BC had acceptable wound healing properties too. Hence regulation of the pH could affect the BC activity in the wound area. Our results showed that the acidic environment made better angiogenesis and re-epithelization of the wound site which is in support of the previous research results that showed that in the acidic environment synthesis of the abnormal collagen in the wound bed decreased along the infiltration

of macrophages and neutrophils and increased in the fibroblast activity [8]. In the present research only in the group treated by the alkaline BC on day 21 formation of collagen bundles was imperfect which indicates the low healing ability of this type of biopolymer in the alkaline environment.

5 Conclusions

In conclusion the pH can affect the healing ability of natural BC, and acidic pH had the best wound healing efficiency. In future it is suggested to use the acidic BC for different wound healing purposes. Furthermore the pH altered BC had the ability of maintaining the acidic pH milieu of the wound site. This conclusion was due to the stable color of the used film. Although the films had thin and transparent structures, their different colors (i.e., based on their pHs) were seen in the wound sites and the pH altered BC film can be used as a smart wound dressing in future. The dressing was closely stuck to the wound surface. By wound contraction, the cellulose layer detached slowly and finally separated. Because of the thin nature of the layers, it is suggested to use thicker films such as the ones which are cross linked with some materials such as sterile gauze in future [21].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Schneider LA, et al. Influence of pH on wound-healing: a new perspective for wound-therapy? *Arch Dermatol Res.* 2007;298:413–20.
- Schreml S, et al. The impact of the pH value on skin integrity and cutaneous wound healing. *J Eur Acad Dermatol Venereol.* 2010;24:373–8.
- Yahyaei B, et al. Production, assessment, and impregnation of hyaluronic acid with silver nanoparticles that were produced by *Streptococcus pyogenes* for tissue engineering applications. *Appl Biol Chem.* 2016;59:227–37.
- Pourali P, et al. Impregnation of the bacterial cellulose membrane with biologically produced silver nanoparticles. *Curr Microbiol.* 2014;69:785–93.
- Pourali P, et al. PCR screening of the *Wolbachia* in some arthropods and nematodes in Khuzestan province. *Iran J Vet Res.* 2009;10:216–22.
- Pourali P, Razavian Zadeh N, Yahyaei B. Silver nanoparticles production by two soil isolated bacteria, *Bacillus thuringiensis* and *Enterobacter cloacae*, and assessment of their cytotoxicity and wound healing effect in rats. *Wound Repair Regen.* 2016;24:860–9.
- Pourali P, Yahyaei B. Biological production of silver nanoparticles by soil isolated bacteria and preliminary study of their cytotoxicity and cutaneous wound healing efficiency in rat. *J Trace Elem Med Biol.* 2016;34:22–31.
- Nagoba BS, et al. Acidic environment and wound healing: a review. *Wounds.* 2015;27:5–11.
- Yahyaei B, et al. Production of electrospun polyvinyl alcohol/microbial synthesized silver nanoparticles scaffold for the treatment of fungating wounds. *Appl Nanosci.* 2018:1–10. <https://doi.org/10.1007/s13204-018-0711-2>.
- Vermeulen H, et al. Topical silver for treating infected wounds. *Cochrane Libr.* 2007;1:CD005486.
- Percival SL, et al. The antimicrobial efficacy of silver on antibiotic-resistant bacteria isolated from burn wounds. *Int Wound J.* 2012;9:488–93.
- Nagoba B, et al. Microbiological, histopathological and clinical changes in chronic infected wounds after citric acid treatment. *J Med Microbiol.* 2008;57:681–2.
- Queen D, et al. A dressing history. *Int Wound J.* 2004;1:59–77.
- Ross P, Mayer R, Benziman M. Cellulose biosynthesis and function in bacteria. *Microbiol Rev.* 1991;55:35–58.
- Esa F, Tasirin SM, Rahman NA. Overview of bacterial cellulose production and application. *Agric Agric Sci Procedia.* 2014;2:113–9.
- Kucińska-Lipka J, Gubanska I, Janik H. Bacterial cellulose in the field of wound healing and regenerative medicine of skin: recent trends and future perspectives. *Polym Bull.* 2015;72:2399–419.
- Ji K, et al. Bacterial cellulose synthesis mechanism of facultative anaerobe *Enterobacter* sp. FY-07. *Sci Rep.* 2016;6:21863.
- Wanna D, et al. Bacterial cellulose–kaolin nanocomposites for application as biomedical wound healing materials. *Adv Nat Sci Nanosci Nanotechnol.* 2013;4:045002.
- Wu J, et al. Silver nanoparticle/bacterial cellulose gel membranes for antibacterial wound dressing: investigation in vitro and in vivo. *Biomed Mater.* 2014;9:035005.
- Pourali P, et al. Biosynthesis of gold nanoparticles by two bacterial and fungal strains, *Bacillus cereus* and *Fusarium oxysporum*, and assessment and comparison of their nanotoxicity in vitro by direct and indirect assays. *Electron J Biotechnol.* 2017;29:86–93.
- Meftahi A, et al. The effects of cotton gauze coating with microbial cellulose. *Cellulose.* 2010;17:199–204.