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Research paper

Bacterial cellulose membranes as drug delivery systems: An *in vivo* skin compatibility study

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ABSTRACT

Bacterial cellulose (BC) is a highly pure form of cellulose, produced in the form of a swollen membrane by several bacteria that demonstrated to be able to modulate the skin release of model drugs. In the present study, the skin irritation potential of BC was evaluated in human subjects. BC membranes with and without glycerin (acting as plasticizer) were tested. No significant differences were observed for transepidermal water loss (TEWL) measurements in comparison with negative control, 2 and 24 h after patch removal, which is an indicator of an absence of barrier disruption. Similar results were found for erythema. Clinical scores were zero at both times for all volunteers, with the exception of five volunteers that exhibited weak reactions. BC with glycerin provided a skin moisturizing effect statistically higher than the negative control ($p = 0.044$), which was not observed for BC alone. The good skin tolerance found after a single application under occlusion reinforces the putative interest of BC membranes as supports for drug topical delivery. Besides modifying the mechanical properties, the inclusion of glycerin results in a skin moisturizing effect which could be clinically relevant for the treatment for skin diseases characterized by dryness, such as psoriasis and atopic dermatitis.

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1. Introduction

Bacterial cellulose (BC), also known as biocellulose, is an extracellular polysaccharide produced by several bacteria of the genera *Glucoacetobacter*, *Agrobacter*, *Sarcina*, among others, in the form of a wet membrane (~99% water) in the interface air/culture medium [1]. Bacterial cellulose presents high purity and water absorption capacity, as well as unique mechanical properties, good permeability, and resistance to degradation [2–4]. Most of these properties arise from BC peculiar tridimensional nanofibrillar network. Due to its singular properties and biocompatibility [2,5], BC has triggered considerable interest in several fields, but particularly in the biomedical area, as for example, in wound dressing for skin burns [2,5] and in artificial blood vessels for microsurgery [6]. Moreover, the peculiar nanofibrillar structure of BC should represent a suitable macromolecular support for inclusion of drugs and therefore for the development of specific controlled release systems. Previous studies documented the ability of BC

membranes to modulate the release and bioavailability of model drugs for percutaneous administration [7,8], and hence, they were proposed as supports for topical or transdermal drug delivery. Therapeutic feasibility is dependent on the skin compatibility of these supports; however, little information is available concerning the skin compatibility and irritation potential of BC-based biomaterials. Cytotoxicity studies have been conducted with human cells [9–11]. However, reports regarding *in vivo* biocompatibility are rather scarce and mainly used mice surgeries [9,11,12]. Clinical tests have been conducted with commercial biocellulose films mainly for wound healing effect [13] which account for their dermal compatibility, but this parameter has not been objectively characterized. Only one study described the absence of skin irritation when a biocellulose mask was applied for 24 h to the arm of human volunteers [14]. To our knowledge, the present study is the first report of human skin irritation evaluation of BC membranes under occlusion. Patch testing after single application is a widely used procedure to evaluate acute irritant reactions [15]. In this methodology, occlusion is achieved by means of an aluminum chamber in order to exaggerate exposition conditions. A 24 h patch test with a follow-up reading at 24 h after patch removal is recommended as optimal procedure [16]. The evaluation of irritant reactions can be accomplished with objective and

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subjective methods. Visual scoring, although subjective, can be a sensitive, reliable, and reproducible method [17]. Several bioengineering techniques have been developed to provide objective and quantitative data. Transepidermal water loss (TEWL) is considered to be the first choice to evaluate slight skin reactions as it detects disrupted epidermal barrier that results in a higher loss of TEWL [18]. However, water barrier function and water evaporation from the skin are neither felt nor seen, and therefore, findings based on TEWL measurements are indirect and not automatically clinically relevant [19]. In this work, the evaluation of irritant reactions was carried out with a combined approach in order to cover the different features of skin irritation. The aim of the present study was to evaluate the skin irritant potential of BC membranes (with and without glycerin) and characterize their skin moisturizing effect.

2. Materials and reagents

Glucose (96% purity), glycerin (99.5%), and Na_2HPO_4 were purchased from Sigma–Aldrich. Yeast extract and bacteriological peptone were purchased from Himedia, and citric acid (99.6% purity) was obtained from Acros Organics. Glycerin solution (99%) was purchased from Fagron, and sodium lauryl sulfate (SLS, 99% purity) was obtained from Fluka. All other chemicals were of analytical grade. Patch test was performed with aluminum chambers (12 mm, Finn Chambers[®], Epitest) fixed with adhesive tape (Scanpore[®], Norgeplaster).

2.1. BC production and purification

BC membranes were produced using *Gluconacetobacter sacchari* [3]. The pre-inocula were prepared at 30 °C during 48 h, in static conditions, in Hestrin and Shramm (HS) liquid medium (20 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, 2.7 g/L Na_2HPO_4 , 1.15 g/L citric acid, agar, 15 g/L, pH 5), before inoculation (10% v/v) of 50 mL into 500 mL liquid production medium in Erlenmeyer flasks. The flasks were kept at 30 °C, in static conditions, for 96 h. After the incubation period, the BC membranes were withdrawn from the culture medium and treated with 0.5 M NaOH at 90 °C for 30 min. This procedure was repeated three times in order to eliminate attached cells [20]. Then, the membranes were washed with distilled water to remove components of the culture medium and other residues until its whitening and reaching pH 7.0.

2.2. Preparation of BC and BC-glycerin (BC-Gly) membranes

BC discs (7 cm diameter) were prepared from 8 mm thick wet membranes (~167 mg dry weight). BC discs for testing were placed in a Petri dish and dried at 40 °C in a ventilated oven for 16 h; BC membranes were previously drained down to 40% water content by hand-pressing between two acrylic plates at room temperature. BC-Gly membranes were prepared by soaking BC discs in 8 mL of an aqueous buffered solution (pH 7.4) of 1% glycerin, during 48 h at room temperature to assure complete absorption of glycerin. After the total absorption of the solution, BC-Gly membranes were placed over a Petri dish and dried as described above. The dried BC (about 5% residual water) and BC-Gly membranes (about 5% residual water and 32% glycerin) were kept in a desiccator until their use.

2.3. Characterization of BC and BC-glycerin (BC-Gly) membranes morphology and mechanical performance

Scanning Electron Microscopy (SEM) of the surface and cross-section of dried BC and BC-Gly membranes were performed using a Hitachi SU-70 instrument operating at 4 kV. BC and BC-Gly

membranes were placed in an appropriated steel support and covered with evaporated carbon. For cross-section analysis, the samples were previously broken with liquid nitrogen to expose appropriated cross-section regions.

Tensile assays were performed on an Instron machine 5966 Series, using a load cell of 500 N, operating at a deformation rate of 10 mm/min, under ambient conditions. At least 5 specimens were tested for each composite. Tensile strength, tensile modulus, and elongation at break were calculated using the Bluehill 3 Material Testing software.

2.4. In vivo evaluation

Fifteen healthy individuals (12 female and 3 male) with a mean age of 31.1 ± 9.9 years participated in this study, and written informed consent was obtained from all volunteers. BC and BC-Gly samples were tested. SLS (2% w/v) was the positive control, and an empty chamber was used as negative control. An aqueous glycerin solution was also tested. Test sites were randomized between volunteers. The products were applied in the inner forearm (Fig. 1), and the patches were removed after 24 h. The visual assessment of the degree of irritation was made at 2 and 24 h after patch removal and graded by an experienced dermatologist according to a 5-level reference scale [21]. In the same time intervals, TEWL measurements were performed with a Tewameter[®] (TM 210, Courage + Khazaka, Germany), and erythema was evaluated with a Colorimeter (CR-400, Minolta, Japan). Skin moisturizing effect was assessed with a Corneometer[®] (CM 825, MPA 9[®], Courage-Khazaka, Germany). All measurements were made in a draught-free room, with controlled temperature (18.6–22.5 °C) and relative humidity (41.4–60%). The volunteers were asked not to apply any topical products in the forearms 24 h before the beginning and throughout the test period. Additionally, solar exposure and use of occlusive clothes on the test area were forbidden. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and was approved by the ethics committee of the Faculty of Pharmacy of the University of Porto.

2.5. Statistical analysis

Statistical evaluation of erythema, moisturizing effect, and TEWL data (variation from basal values) was performed using ANOVA. Post hoc comparisons with negative control were performed with Dunnett test. Statistical analysis was conducted with IBM SPSS Statistics 21 ($\alpha = 0.05$).

3. Results and discussion

3.1. Membrane characterization

BC and BC-Gly membranes were considered in this study, since glycerin is normally used as a plasticizer, to increase malleability, as well as the swelling (by promoting water holding and retention) of topical delivery systems [7,8]. Fig. 2 displays the visual aspect of BC and BC-Gly dried membranes. BC-Gly membranes are slightly more translucent than pure BC membranes and also quite homogeneous which clearly indicates a good dispersion of glycerin inside the BC nano- and microfibrils network surely due to the establishment of strong interactions between the two OH rich structures.

The surface and cross-section morphology of dried BC and BC-Gly membranes was assessed by SEM (Fig. 3). The surface micrographs of both BC and BC-Gly membranes showed the characteristic tridimensional nanofibrillar network of BC matrices; however, in BC-Gly, it is less compacted, certainly because of the presence of glycerin that limited the extent of collapse of the

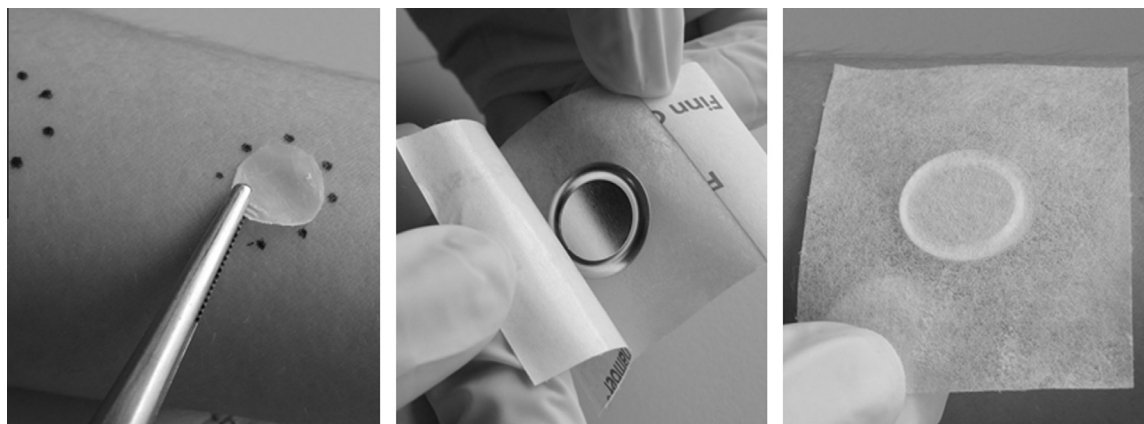


Fig. 1. Sequential steps of the patch test using Finn Chambers®.

tridimensional structure of BC during drying. Glycerin is retained between the cellulose nanofibers, reducing the intermolecular attraction forces and therefore increasing the distance between them. This behavior is particularly noticeable on the cross-section images where the typical lamellar morphology is much more expanded for BC-Gly rather than for BC.

In terms of mechanical properties, as intended, the addition of glycerin to the BC membranes increased considerably their malleability as observed by the increment in the elongation at break and by the corresponding decrease in the Young modulus of BC-Gly when compared to BC (Table 1). This feature is quite significant in terms of the clinical application of BC membranes since bendable type materials are easier to fix and to use.

3.2. *In vivo* tests

Significant differences were observed for TEWL and erythema for the positive control (SLS), and the irritation scores were different from zero for all volunteers, for both measuring times. These findings confirm the validity of the procedure undertaken. Regarding BC membranes, no significant differences were observed for TEWL measurements in comparison with negative control, 2 and 24 h after patch removal (Fig. 4), which is an indicator of an absence of barrier disruption. Similar results were found for erythema (Fig. 5). Clinical scores were zero at both times for all volunteers, with the exception of five volunteers that exhibited weak reactions (scores 1 or 2). From these, two volunteers also presented weak reactions to the negative control (Table 2). None of the individuals reported subjective irritation symptoms like stinging or itching. Taken together, these results confirm the good skin tolerance of both BC and BC-Gly membranes.

BC-Gly membranes provided a skin moisturizing effect, statistically higher than the negative control ($p = 0.044$) 2 h after patch removal which was not observed for BC alone (Fig. 6). Occlusion

provided by aluminum chambers may have potentiated this effect; however, the moisturizing ability observed was similar to that exhibited by an aqueous solution of glycerin, a well-known moisturizer widely used in skin care preparations. Besides, occlusion of the untreated site did not induce a relevant increase in skin hydration (Fig. 6). Skin hydration decreased 24 h after patch removal. The persistence of the moisturizing effect could be evaluated after long-term application. The role of glycerin in skin moisturization is well documented [22] and has been traditionally attributed to its highly hygroscopic nature associated with the presence of three hydroxyl groups. Glycerin present in BC-Gly membranes diffused into the stratum corneum and retained water in this skin layer. Among other biological actions, anti-irritant properties and recovery of the skin barrier functions have also been reported for this polyol [23]. For these reasons, formulations containing this moisturizer have been tested in the treatment for dermatosis with damaged barrier such as atopic dermatitis and in the treatment of dry skin conditions such as winter and senile xerosis [24–26]. As a consequence, it may be reasonably postulated the application of BG-Gly membranes in the therapy of skin lesions where moisturization ameliorates the clinical symptoms. This effect can also be complement with the therapeutic action of a drug loaded on BC membrane such as an anti-inflammatory or an antipruritic compound. Besides therapeutic application, BC membranes have been studied for cosmetic use [14]. It was reported that a facial BC mask applied for 5 min promoted an increase in skin moisture. This effect was attributed to the water content of the mask that augmented the water uptake by the skin. This mechanism cannot explain the effect observed in this study since the BC membranes used were previously dried and their water content was low ($\leq 10\%$). In the above mentioned study, the majority of the volunteers rated the cellulose mask comfortable to use [14]. The mask also adhered well to the skin. Consumer/patient compliance is an important issue regarding clinical efficacy. It has been reported that failure to comply with prescribed topical treatment can be as high as 60%, especially in chronic dermatosis such as psoriasis [27]. Poor cosmetic acceptability has been recognized as one of the reasons for non-adherence [27]. The results obtained in the study with a cellulose mask [14] cannot be fully transposed to the system studied in this work, since different preparation methods were used. However, it provides good indicators regarding cosmetic acceptability of BC-based preparations.

The ability of BC membranes to modulate the release of drugs can reduce the number of applications which offers a more simple and convenient therapeutic regimen especially considering chronic dermatosis. Membrane flexibility given by glycerin is highly valuable considering the need for adaptation to skin irregular

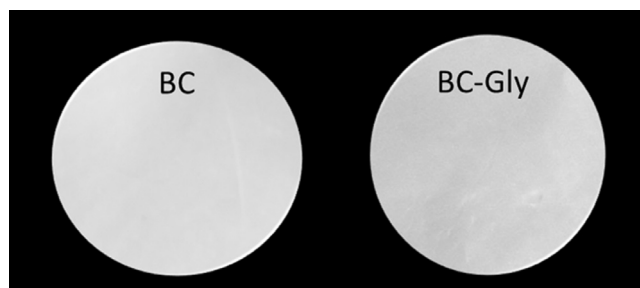


Fig. 2. Visual aspect of dried BC and BC-Gly membranes.

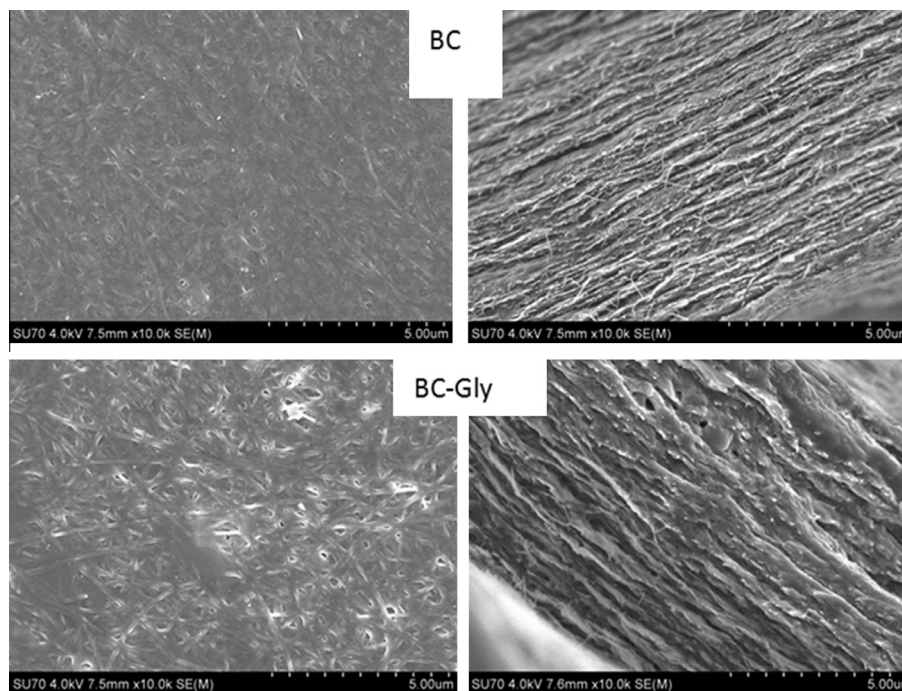


Fig. 3. Surface (left) and cross-section (right) SEM images of oven dried BC and BC-Gly membranes.

Table 1

Young modulus, tensile strength and elongation at break of BC and BC-Gly membranes, obtained from mechanical tensile assays.

Sample	Young modulus (MPa)	Elongation at break (%)	Tensile strength (MPa)
BC	19132.32 ± 1286.15	2.26 ± 0.73	271.93 ± 62.06
BC-Gly	9412.06 ± 747.70	6.17 ± 0.73	360.30 ± 51.00

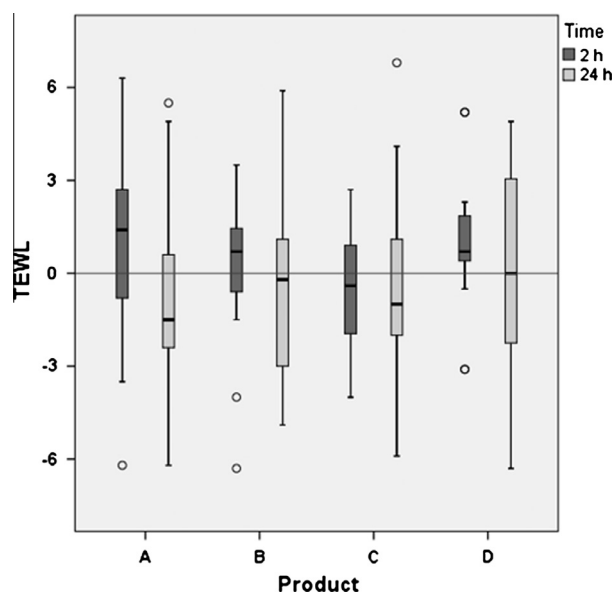


Fig. 4. TEWL measurements 2 h and 24 h after patch removal. (A) BC membrane; (B) BC-Gly membrane; (C) Glycerin solution, and (D) Negative control. Outliers are represented as circles.

areas. The membranes can also be cut (or is situ conformable) into the desired shape and dimensions to fit the target area.

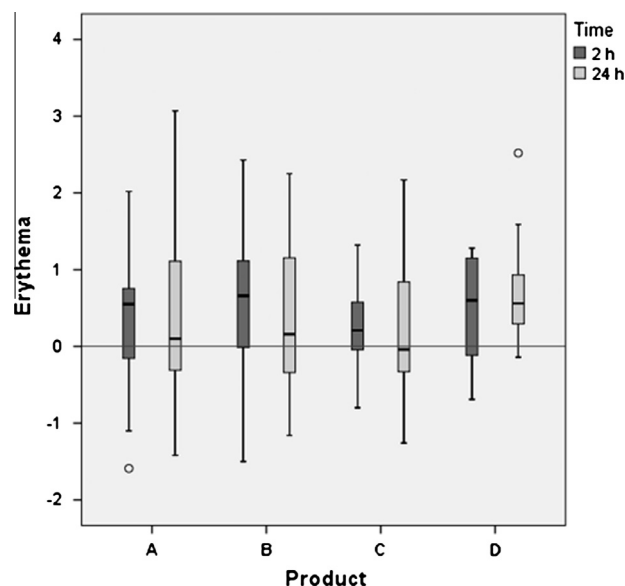


Fig. 5. Erythema measurements 2 h and 24 h after patch removal. (A) BC membrane; (B) BC-Gly membrane; (C) Glycerin solution, and (D) Negative control. Outliers are represented as circles.

Another critical issue regarding the use of topical semisolid formulations is the loss of material due to contact with garments or surfaces [8]. Regarding dose accuracy, for semisolids, the amount of drug used and application area are patient-dependent and therefore are not accurate and vary widely with the patient perception. In a topical delivery system based on BC, the dose can be precisely defined by the membrane area applied to the skin, and the characteristics of such a system prevent any loss of drug after application. Advantages over traditional transdermal patches can also be pointed out, such as a lower number of layers which render lower production costs.

Table 2
Irritation scores obtained in the patch test.

Score/Time	Untreated		BC		BC-Gly		SLS		Glycerin solution	
	2 h	24 h	2 h	24 h	2 h	24 h	2 h	24 h	2 h	24 h
0	13	15	11	14	11	14	0	0	14	15
1	2	0	3	1	2	1	1	2	1	0
2	0	0	1	0	2	0	4	2	0	0
3	0	0	0	0	0	0	0	2	0	0
4	0	0	0	0	0	0	3	6	0	0
5	0	0	0	0	0	0	7	3	0	0

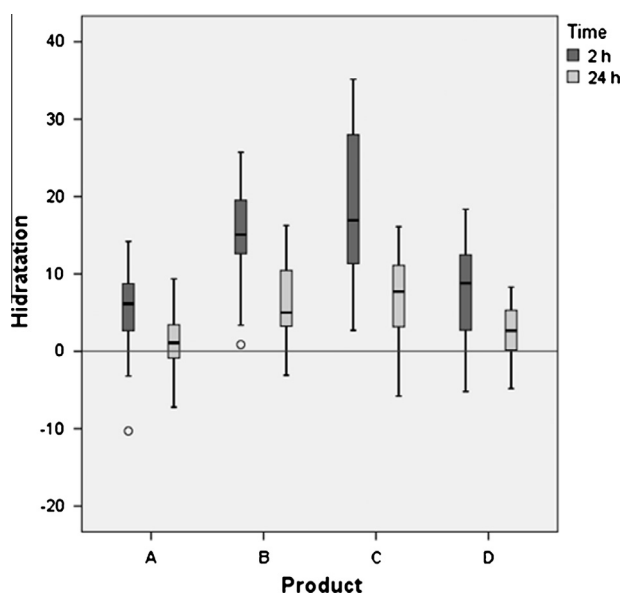


Fig. 6. Capacitance measurements 2 h and 24 h after patch removal. (A) BC membrane; (B) BC-Gly membrane; (C) Glycerin solution, and (D) Negative control. Outliers are represented as circles.

4. Conclusions

The good skin tolerance found after 24 h patch test reinforces the putative interest of BC membranes as supports for topical or transdermal drug delivery. It should be mentioned, however, that use tests are needed to confirm skin compatibility under normal use conditions. The inclusion of glycerin besides increasing malleability results in a skin moisturizing effect, which could be clinically relevant for the treatment for skin diseases characterized by dryness such as psoriasis and atopic dermatitis.

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