International Journal of Pharmaceutics 435 (2012) 83–87

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Bacterial cellulose membranes applied in topical and transdermal delivery of lidocaine hydrochloride and ibuprofen: In vitro diffusion studies

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ARTICLE INFO

Article history:
Received 18 October 2011
Received in revised form 5 January 2012
Accepted 5 January 2012
Available online 13 January 2012

Keywords:
Bacterial cellulose
Topical and transdermal drug delivery

ABSTRACT

Bacterial cellulose (BC) is a biomaterial with unique physical and mechanical properties that triggered considerable interest, but there are few studies addressing the use of such membranes for drug loading and controlled release. This study aimed to investigate the applicability of BC membranes in topical or transdermal drug delivery systems. To assess its therapeutic feasibility, the permeation through human epidermis of two model drugs (lidocaine hydrochloride and ibuprofen) in BC and other formulation systems was compared in vitro.

A uniform distribution of both drugs in the BC membranes was achieved. Diffusion studies with Franz cells showed that the incorporation of lidocaine hydrochloride in BC membranes provided lower permeation rates than those obtained with the conventional formulations. However, the results obtained with the lipophilic drug were quite different, since permeation of ibuprofen in BC was almost three times higher than that of the drug in the gel or in a PEG400 solution.

These results indicate that this technology can be successfully applied to modulate the bioavailability of drugs for percutaneous administration, which could be particularly advantageous in the design of delivery systems that have, simultaneously, the ability to absorb exudates and to adhere to irregular skin surfaces.

1. Introduction

The last decades have witnessed an increased interest in the use of biomaterials in healthcare products that is predominantly associated to their renewable nature, biocompatibility and biodegradability. An exceedingly wide assortment of biomaterials with suitable biological properties is on offer, but considerable attention has been drawn to polymers, and in particular, within one of its most profuse families-polysaccharides-, to cellulose.

Formed by repeated dimers of β-1,4 linked D-glucose units, cellulose reveals unique properties that make this material excel over other natural and synthetic polymers: hydrophilicity, broad chemical modifying capacity, and availability of distinct morphologies (Klemm et al., 2009). Nevertheless, morphology, properties and specific application fields are highly dependent on the source, i.e., "building process", of the cellulose type. Plants are the most prevalent suppliers of this material, but there are numerous algae and microorganisms that are also capable of fabricating this biopolymer. Bacteria from the *Gluconacetobacter* genus produce an extremely pure variety of bacterial cellulose (BC), in the form of a highly swollen membrane (Fig. 1), with around 99% water, on the culture medium surface (Klemm et al., 2001). The distinctive tridimensional and branched nano and micro-fibrillar structure prompted considerable interest in BC, and several applications in the biomedical area have been developed, mainly as wound healing membranes or as substitutes of natural skin (Czaja et al., 2006). Recently, a bioabsorbable bacterial cellulose incorporating cellulase enzymes has been developed (Hu and Catchmark, 2011). The same study also demonstrated that the mechanical properties of this material had tensile strength and extensibility similar to human skin. In another study, thin films of BC demonstrated to support the growth, spreading, and migration of human keratinocytes (Sanchavanakit et al., 2006).

The greatest advantage from the use of this material would be to combine its wound healing capacity, protective properties and the ability to absorb exudates with the release of therapeutically relevant drugs. However, to date, only a limited number of delivery systems have been attempted. A composite membrane for transdermal delivery of S-propranolol was developed based on the controlled pore functionalization of BC membranes

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doi:10.1016/j.ijpharm.2012.01.002

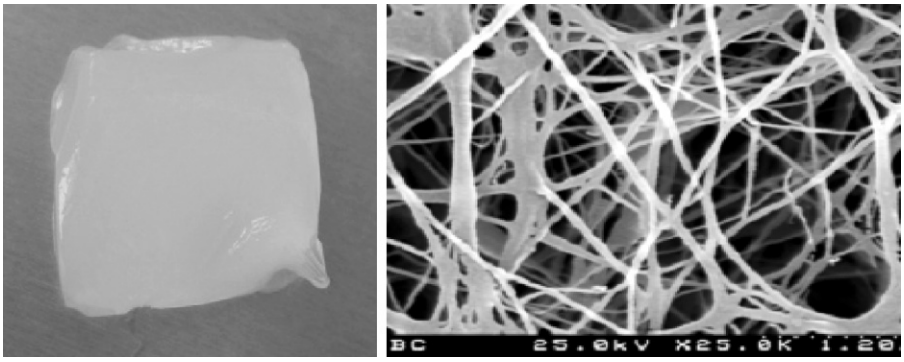

Fig. 1. Visual aspect and SEM micrograph of bacterial cellulose (25,000 × magnification).

using a molecularly imprinted polymer (MIP) layer synthesis (Bodhibukkana et al., 2006). Another research group developed a transdermal patch for selective controlled delivery of the active S-enantiomer from racemic propranolol mixture, and evaluated its performance in vivo using Wistar rats (Roongnapa et al., 2008).

Biocellulose systems also have potential to be employed in topical formulations and surpass some of its constraints. The use of topical formulations is inevitably linked to lack of reproducibility of the drug dose and to the loss of material because of contact with garments or surfaces. In a topical delivery system based in BC the dose can be precisely defined by the area of membrane applied to the skin, and the characteristics of such a system prevent any loss of drug after application. Finally, BC could represent a much more cosmetically appealing alternative to oily based formulations, improving patient compliance.

Advantages in the use of BC films could additionally be extended to transdermal drug delivery systems. Most transdermal patches are manufactured by superimposing different materials therefore, a system composed of fewer, or even a single layer could simplify the preparation procedure and lower production costs (Padula et al., 2003).

The aim of this study was to further investigate the potential of BC membranes as systems for topical or transdermal drug delivery. Lidocaine hydrochloride and ibuprofen were chosen as model hydrophilic and hydrophobic drugs, respectively. A drug loading process in BC membranes was developed for both molecules, and conventional formulations were employed for comparative purposes. A systematic in vitro diffusion study using Franz cells was conducted, using human epidermal membranes, and results were correlated where possible.

2. Materials and methods

2.1. Materials

Ibuprofen (98%), lidocaine hydrochloride monohydrate (99.5%) and glycerol (99.5%) were purchased from Sigma–Aldrich, St. Louis, MO, USA. Solvents and other reagents were of analytical grade (Sigma–Aldrich, Steinheim, Germany). BC membranes (99% water content) were produced using the bacteria *Gluconacetobacter sacchari* (Trovatti et al., 2011) and conventional culture media conditions (Hestrin and Schramm, 1954).

Commercially available formulations were used for comparative purposes. Lidonostrum® (Sociedade Nostrum, Lisbon, Portugal) contains 2% (w/w) of lidocaine hydrochloride in a water based hydroxypropylmethylcellulose gel formulation, and Nurofen® (Reckitt Benckiser Healthcare Ltd, Lisbon, Portugal) contains 5% (w/w) of ibuprofen in an ethanol and hydroxypropylcellulose based gel. A 2% aqueous solution of lidocaine hydrochloride and a 5% solution of ibuprofen in PEG400 were prepared and also used as comparative formulations.

2.2. Preparation of BC-lidocaine and BC-ibuprofen membranes

2.2.1. BC-lidocaine

Wet BC membranes were weighted and 50% of their water mass was removed by pressure. Drained BC membranes with 6 cm × 4 cm × 0.8 cm dimension were soaked in 5 ml of an aqueous buffered solution (pH 7.4) of lidocaine hydrochloride (2%) and glycerol (1%) and shaken at 100 rpm and 30 °C for 1 h, to allow the complete absorption of the solution. After the total solution absorption, the BC membranes were placed over a Petri dish and dried at 40 °C in a ventilated oven for 16 h.

2.2.2. BC-ibuprofen

The water of BC membrane was replaced by ethanol before the incorporation of ibuprofen. After five solvent exchanges, the BC membrane (6 cm × 4 cm × 0.8 cm dimension) was weighted and 50% of its mass (ethanol) was removed by pressure. The membrane was immersed in 5 ml of alcoholic ibuprofen solution (1%) and shaken at 100 rpm and 30 °C for 1 h, to allow the complete absorption of the solution. After the total solution absorption, the BC membranes were placed over a Petri dish and dried at 50 °C in a ventilated oven for 16 h.

The dried BC-lidocaine and BC-ibuprofen membranes were kept in a desiccator until their use.

2.3. In vitro permeation studies

Human abdominal skin tissue from cosmetic surgery, obtained following informed consent was used to produce epidermal membranes. Ethical approval was provided by the Ethics Committee of the Faculty of Health Sciences of the Lusófona University After removal of the adipose tissue by blunt dissection, the epidermis was separated by immersing the skin in water at 60 °C for 1 min (Kligman and Christophers, 1963). It was then pinned on a corkboard, the epidermis was carefully peeled away from the dermis and mounted on filter paper, after which was stored in a freezer at −20 °C until required. Prior to the diffusion experiment the epidermis was defrosted and cut to appropriate size.

Permeation experiments ($n = 5$) with epidermal membranes were conducted on glass Franz type diffusion cells with a receptor volume of ∼4 ml and a diffusional area of 0.95 cm^2. The continuously stirred receptor medium was isotonic phosphate buffered saline (PBS, pH = 7.4). The receptor compartment was thermostated to 37 °C. A defined loading dose of lidocaine hydrochloride or ibuprofen in different systems was placed in each donor compartment (Table 1). In the case of the solutions and gel, a micropipette was used for this purpose. BC membranes were cut to a 0.95 cm^2

Table 1
Drug content and formulation amount applied in the permeation experiments.

	Lidocaine hydrochloride			Ibuprofen		
	% (w/w)	Amount	Dose (mg/cm^2)	% (w/w)	Amount	Dose (mg/cm^2)
Solution	2	400 μl	8.4	5	400 μl	21.1
Gel	2	60 mg	1.3	5	60 mg	3.2
BC membrane	50	8 mg	4.2	30	6 mg	1.9

size that fitted the surface area of the donor compartment and covered the entire epidermal interface. At the start of the experiment 100 μl of PBS were applied on the BC membranes. The diffusion experiments were performed under occluded conditions by sealing the donor compartment with microscope coverslips. At designated time intervals the receiver solution was withdrawn completely from the receptor compartment and immediately replaced with fresh and pre-thermostated PBS. Quantitative analysis of the drugs was performed on a UV-Vis spectrophotometer (Evolution 600, Thermo Scientific,UK) at 230 nm for lidocaine hydrochloride and at 225 nm for ibuprofen (Yuan et al., 2008).

Flux values for each individual diffusion experiment were calculated by monitoring the cumulative amount of drug diffused and measuring the slope of the graph once steady-state diffusion was reached. Additionally, the same data were fitted to the appropriate solution of Fick's second law (Eq. (1))

$$Q = (KH)C_{app}\left[\frac{D}{H^2 t} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{1}\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\pi^2 t}{H^2}\right)\right] \quad (1)$$

where Q is the cumulative amount of drug permeated per unit area, t is time, C_{app} is the applied concentration, H is the path length for drug diffusion across the *stratum corneum*, K is the partition coefficient between the skin and the applied formulation and D is the diffusion coefficient in the skin (Dias et al., 1999; Díez-Sales et al., 1991). This procedure led to the determination of each drug's partition parameter between the skin and the formulation (KH) and diffusion parameter in the skin (D/H^2), using a curve fitting computer program (Easyplot for Windows version 4.0). The permeability coefficient was then calculated, as the product of KH and D/H^2.

One-way ANOVA with post Hoc tests (Tukey's multiple comparison) were used in this study (SPSS Statistics 17.0, IBM Corporation, Somers, NY, USA). A 0.05 significance level was adopted.

3. Results and discussion

The visual aspect of BC, BC-lidocaine and BC-ibuprofen membranes and the SEM micrographs of the drug loaded BC membranes are shown in Figs. 1 and 2. It is clearly visible that the drug loaded membranes are homogeneous without the formation of drug aggregates on the surface. Furthermore it was observed that these membranes are quite flexible, mainly due to the plasticizing effect of glycerol, and therefore well suited for dermal application.

The permeation profiles of lidocaine hydrochloride and ibuprofen from the different systems through epidermal membranes are shown in Fig. 3. Depending on the formulation system, different flux values (J_s) were obtained, as seen in Tables 2 and 3. The highest lidocaine hydrochloride fluxes were obtained in the aqueous solution and the lowest observed in the BC. Statistically significant differences were not established between the results obtained with the gel and with the aqueous solution. However, the average flux values of lidocaine hydrochloride in BC were significantly lower than those obtained with the other two formulations. The results obtained with ibuprofen reveal a different trend, since the fluxes of the drug in BC membrane were almost three times higher than those observed in the gel or the PEG400 solution. Nevertheless, once again in these systems the permeation profiles and the drug fluxes were very similar.

A comparison of the different formulations based on the cumulative amount permeated after 8 h provides the same trends that were observed in the fluxes for both drugs (Tables 2 and 3). However, analysis of drug permeation after the same period in terms of percentage of applied dose shows that a high amount of lidocaine hydrochloride had already permeated from the gel, which might have caused drug depletion. This effect was not observed in the ibuprofen formulations. Nevertheless, the percentage of applied dose that permeated from the BC membrane was significantly higher than from the ibuprofen gel which, in turn, was higher than from the PEG400 solution.

The data from Fig. 3 can be analyzed into more detail using Eq. (1). Tables 2 and 3 show the values of KH, D/H^2 and k_p obtained for lidocaine hydrochloride and ibuprofen, respectively. All the observations above, established for the fluxes, are equally reflected in the permeability coefficients, i.e., significantly lower values were observed for lidocaine hydrochloride in BC and higher values were obtained when ibuprofen was in BC membranes. Analysis of variance of the other two parameters enables a more complete scrutiny. Significant differences were not established between KH calculated for lidocaine hydrochloride in the three systems, even though the values obtained for the aqueous solution were considerably lower. However, significantly higher D/H^2 were observed when water was used as vehicle. In the case of ibuprofen, inclusion of the drug in a PEG400 solution seems to have significantly influenced partition,

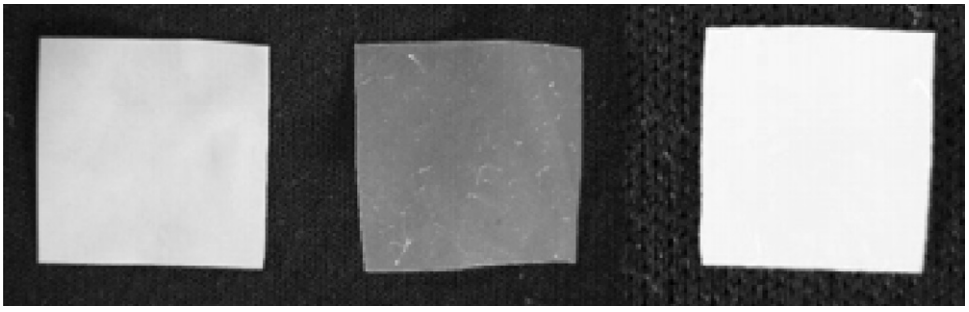

Fig. 2. Bacterial cellulose sheets (2 cm × 2 cm): pure (left), with lidocaine hydrochloride (middle), with ibuprofen (right).

Table 2
Skin permeation parameters and diffusion and partition data derived from Fig. 3a using Eq. (1). Mean values ± SD, $n = 5$.

	Lidocaine hydrochloride					
	J_s (μg cm^{-2} h^{-1})	Cumulated dose Q_{8h} (μg)	Drug permeated 8 h (% applied dose)	KH (cm)	D/H^2 (h^{-1})	K_p (cm/h)
Aqueous Solution	47.9 ± 4.2	390.8 ± 30.5	4.64 ± 0.36	$6.33 \times 10^{-6} \pm 3.38 \times 10^{-6}$	4.87 ± 2.65	$2.40 \times 10^{-5} \pm 2.12 \times 10^{-6}$
Gel	40.8 ± 6.2	391.8 ± 37.5	31.10 ± 2.97	$5.12 \times 10^{-5} \pm 4.04 \times 10^{-5}$	0.72 ± 0.55	$2.04 \times 10^{-5} \pm 3.08 \times 10^{-6}$
BC membrane	31.4 ± 1.2	287.6 ± 26.8	6.83 ± 0.64	$2.76 \times 10^{-5} \pm 2.66 \times 10^{-5}$	1.07 ± 0.56	$1.58 \times 10^{-5} \pm 7.89 \times 10^{-7}$

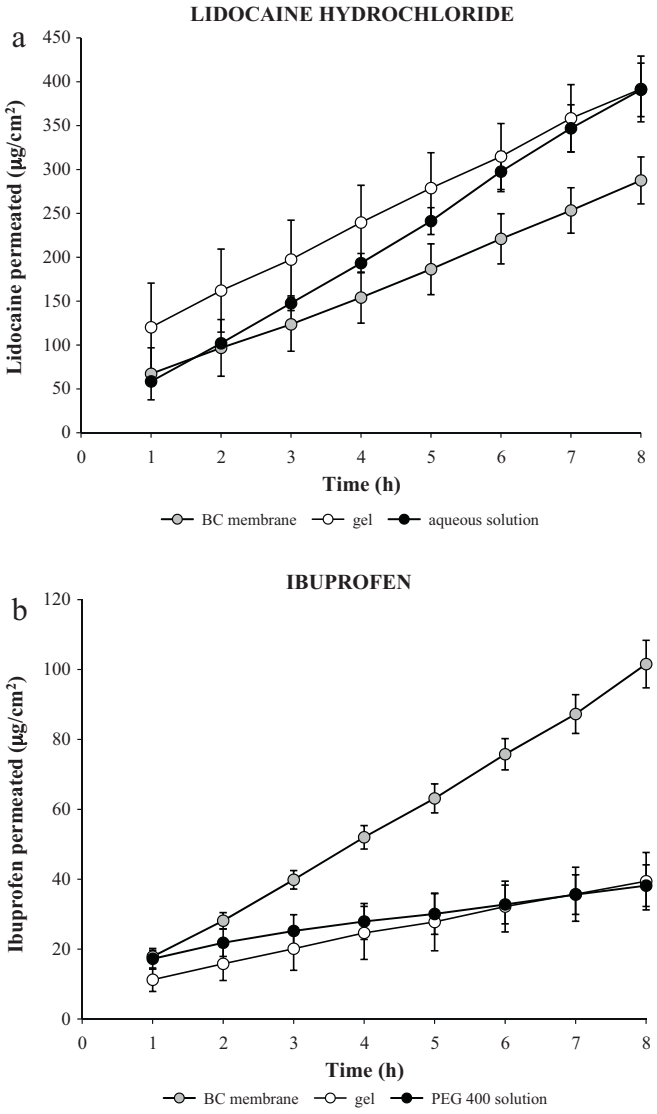

Fig. 3. Drug permeation profiles from the different systems across human epidermis. Mean values ± SD, $n = 5$.

whereas effects in diffusion were only evident for the BC membranes.

Alterations to the epidermal diffusional barrier observed with both the hydrophilic and the lipophilic drug could be attributable to the penetration enhancement effect of water. This substance was used as a vehicle for lidocaine hydrochloride and also as a contact medium between BC-ibuprofen and BC-lidocaine membranes and the skin. Increased tissue hydration appears to increase transdermal delivery, even though, and despite extensive research in the area, its mechanisms of action are unclear (Williams and Barry, 2004). Glycerol, which was used in the preparation of the BC-lidocaine membranes, is a well known humectant and plasticizer of the stratum corneum (Rawlings et al., 2002), and could potentially influence epidermal penetration by the mechanisms described previously. However, the amount of this substance in the membrane is probably too small for this effect to occur. Ethanol has the ability to change the solution properties of the stratum corneum by altering the chemical environment, and thus reduce the barrier capacity of this cutaneous layer (Bach and Lippold, 1998; Barry, 2001). Its presence in the ibuprofen gel and BC-ibuprofen membranes could potentially contribute to an impairment of the epidermal diffusional barrier. Nevertheless, it is a very volatile component and probably remains in vestigial amounts shortly after application time.

Additionally, results could be partially explained by the fact that the drugs, depending on the system, were applied in different amounts per unit area, which could partly clarify the variations observed in fluxes. Conflicting reports can be found in the literature describing the effect of dose level on drug permeation through skin (Brain et al., 2002) and, even though some studies have shown poor correlations between drug concentration and flux (Akhter and Barry, 1985), or even bioequivalence between topicals with different drug concentrations (Peltonen and Solberg, 1984) it is recommended that similar dose levels are used in comparative studies (SCCS, 2010).

On the other hand, results can be attributed to differences in the resistance opposed by the formulations to the diffusion of the drug, which will appreciably influence the drug release. This effect should be more significant in the gel and in BC membranes. The effect of gel viscosity on drug release is well known, and most studies have found inverse relationships between the viscosity of preparations and drug diffusion coefficients (A-sasutjarit et al., 2005). Drug mobility in aqueous dispersions of polymers is basically restricted by mechanical impediments of polymers and reductions in free volume with increases in medium viscosity (Lorenzo et al., 1999). Díez-Sales et al. (2005) have reported that, when gels were used,

Table 3
Skin permeation parameters and diffusion and partition data derived from Fig. 3b using Eq. (1). Mean values ± SD, $n = 5$.

	Ibuprofen					
	J_s (μg cm^{-2} h^{-1})	Cumulated dose Q_{8h} (μg)	Drug permeated 8 h (% applied dose)	KH (cm)	D/H^2 (h^{-1})	K_p (cm/h)
PEG400 Solution	3.0 ± 0.5	38.18 ± 5.95	0.18 ± 0.03	$4.41 \times 10^{-6} \pm 9.35 \times 10^{-7}$	0.14 ± 0.02	$5.98 \times 10^{-7} \pm 1.00 \times 10^{-7}$
Gel	4.0 ± 0.8	39.44 ± 8.22	1.25 ± 0.26	$2.38 \times 10^{-6} \pm 9.81 \times 10^{-7}$	0.40 ± 0.25	$7.99 \times 10^{-7} \pm 1.65 \times 10^{-7}$
BC membrane	11.9 ± 1.0	101.56 ± 6.80	5.37 ± 0.36	$1.38 \times 10^{-6} \pm 8.53 \times 10^{-7}$	1.67 ± 0.34	$2.37 \times 10^{-6} \pm 2.11 \times 10^{-7}$

skin permeation values were smaller than those of solvent systems, which could be attributed to the network structure created by the polymer that increases the length of the diffusional pathway. BC membranes have a complex tridimensional organization, which will certainly make the diffusion pathway of the drug tortuous and could be responsible by a global decrease on the drug release rate.

The results obtained with lidocaine hydrochloride are in agreement with the behavior described above, but the BC membranes loaded with ibuprofen had a different performance. Since the drug is lipophilic, and BC is essentially a hydrophylic environment, it seems reasonable to assume that the drug was released more rapidly due to weaker interactions with the BC ultrastructure. Further microscopic and dissolution studies will be required to fully support these assumptions.

4. Conclusions

The data for lidocaine hydrochloride and ibuprofen penetration through human epidermis, in different delivery systems, was analyzed to clarify the therapeutic feasibility of BC membranes in topical and transdermal delivery.

The permeation rate of lidocaine hydrochloride in BC membranes was lower than that obtained with the conventional formulations, which seems to indicate advantages in the use of the system to address pathologies that require hydrophilic drugs with a complex toxicological profile, requiring more long-term release of the drug.

High fluxes were achieved when a lipophilic drug was included in the BC membrane, which could be valuable in delivery systems that provide fast release for the treatment of acute conditions.

In summary, results indicate that this technology can be successfully applied to modulate the bioavailability of drugs for percutaneous administration, which could be particularly advantageous in the design of delivery systems that have, simultaneously, the ability to absorb exudates and to adhere to irregular skin surfaces.

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