Study of the safety of topical application of bacterial nanocellulose membranes modified with Poly-L-Lysine-Cholesterol: phase I clinical trial

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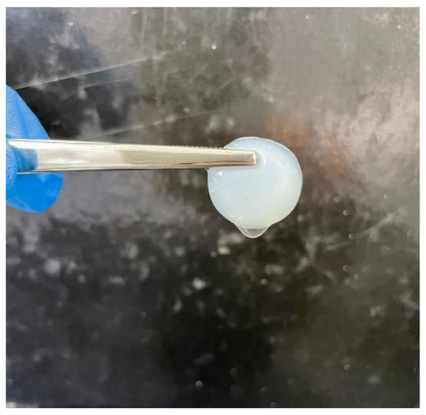
INTRODUCTION

Bacterial nanocellulose (BNC), also known as bacterial cellulose (BC) or microbial cellulose (MC), is found in the form of a translucent and gelatinous film, with a diameter in the range of 20-100 nm. This membrane has a pure nanofibrillar structure and can be synthesized by bacteria such as *Gluconacetobacter hansenii* and *Gluconacetobacter xylinus* (Figure 1).

Figure 1 – Hydrated NCB membrane



Source: Prepared by the author

In the pursuit of functional devices for topical applications, a study showed a novel modification on the surface of BNC using the adsorption of the Poly-L-Lysine-Cholesterol (BNC-PLC) polymer. PLC has been developed and studied, showing promising results in cell adhesion, proliferation, and differentiation as well as potential antimicrobial action when applied to the BNC surface.

AIM

Therefore, the present study aimed to develop a phase 1 clinical trial in humans to evaluate the use of the membrane of BNC-PLC on healthy patients' intact skin.

METHODS

Clinical study, triple-blind and randomized

(Study protocol number 5.448.480 approved by the Ethics Committee of Federal University of Juiz de Fora, Brazil)

Target population

- 20 healthy individuals, aged in the range 18-60 years, of both sexes, who met the inclusion criteria;
- Written informed consent was required

Analysis of variables

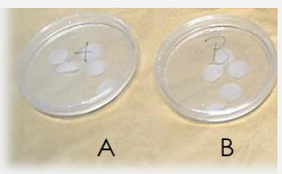
- Demographical data;
- Blood and urine samples were collected for analysis on the 1st (first) and 30th (last) days;
- Investigation of the presence of local inflammatory reaction.

Intervention

- The BNC-PLC membranes were applied to the right forearm of the volunteers, three times a week, during a period of one month;

✓ Membranes were divided into 2 groups: A and B;

✓ Volunteers were randomly assigned to groups.



Source: Prepared by the author

Group A NCB n=10

Group B NCB n=10

- Prior to the membrane application, skin site was cleaned with ethanol 70%;
- Membrane was covered with a transparent film;
- In order to evaluate the occurrence of local inflammatory reaction, the skin was assessed at every dressing change;
- Clinical evaluation of the skin site – follow-up;
  - Photograph.

Figure 2 – Application of membranes

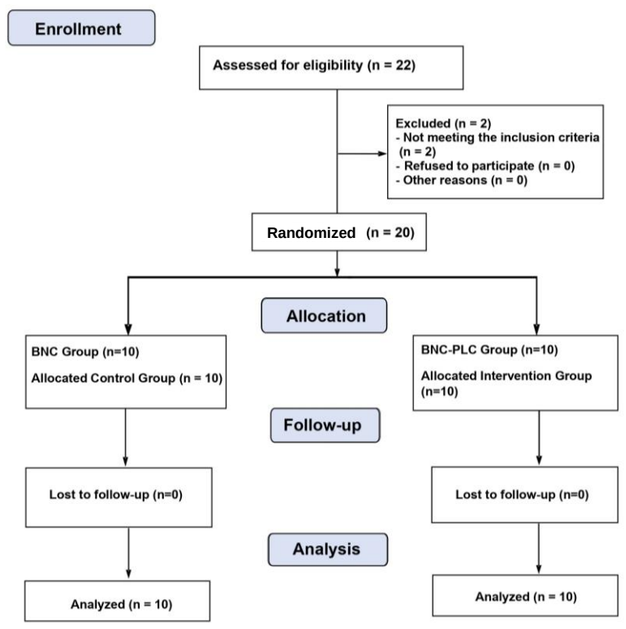
Source: Prepared by the author

Statistical analysis

Student's T test, and Chi-square were used
Differences were considered statistically significant when $p \leq 0.05$

RESULTS

A CONSORT chart showing the flow of patients during the trial:



Frequency of adverse effects:

Adverse effects		Pruritus			Dermatitis			*p* values
		With alteration		Without alteration	With alteration		Without alteration	
Occurrence		N	%	N %	N	%	N %	
BNC-PLC	0	0	10	0	6	60	4 40	0,087
Control	4	40	6	60	7	70	3 30	1,000

BNC-PLC: Bacterial Nanocellulose incorporated with Poly-L-lysine-cholesterol; **N**: number of participants. Chi-square test: $p \leq 0.05$ is considered statistically significant.

- After statistical analysis, it was found that the monocyte count was the only divergent value between the BNC-PLC and Control groups in the D1 collection, in which p=0.04 indicated statistical significance. However, the test results were within the laboratory normality standards (4-10%) for all participants in this collection, showing that the volunteers in both groups were similar.
- Statistical significance was found between the mean values for segmented neutrophils found in blood samples from the BNC-PLC and Control groups (p=0.04). However, the tests that showed slight alteration (<70%) relative to the laboratory normality standard were found in samples from volunteers in the control group.
- In the BNC-PLC group, some values for bilirubin (p=0.003) and potassium (p=0.004) were slightly elevated according to the laboratory reference range (bilirubin: 0.3-1.2%: potassium: 3.5-5.1%). However, these results occurred in the D1 test collection, which preceded the application of membranes; therefore, they are not related to the application of the BNC-PLC membrane.
- The evidence of allergic reactions in individuals during the initial phase of the clinical trial was related to the use of polyurethane film initially used in the membrane application and it was confirmed by the resolution of signs and symptoms after changing the adhesive dressing.

Baseline demographics:

Sample characterization, Clinical study phase I

Variables	n	%
Age (years)		
18-30	7	35
31-50	13	65
Gender		
Male	4	20
Female	16	80
Skin color		
White	12	60
Non-white	8	40
Status		
Single	14	70
Married	5	25
Divorced, separated or widowed	1	5
Smoking Habit		
Never	20	100
Ex-smoker	0	-
Yes	0	-
Alcohol consumption		
Never or almost never	13	65
At least once a week	7	35
Every day	0	-
Co-morbidities		
No	16	80
Yes	2	10
Hospitalization prior to the study		
No	19	95
Yes	1	5
Previous disease history		
No	17	85
Yes	3	15
Current use of medicines		
No	9	45
Yes	11	55
History of high blood pressure		
No	20	100
Yes	0	0
Body mass index		
Underweight	1	5
Normal weight	9	45
Overweight	5	25
Obesity	5	25
Integrity of forearm skin		
No	0	0
Yes	20	100
Treatment group		
BNC-PLC	10	50
Control	10	50

CONCLUSION

There was no clinical evidence of toxicity of BNC-PLC biomembranes applied in skin of healthy volunteers, suggesting that it is safe and can be further investigated for application in advanced tissue repairing processes.

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"I have no conflict of interest"

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