RESEARCH PAPER

A Comparative Evaluation of the Surface Roughness and Hydrophilicity of Bacterial Cellulose Membrane Derived from Different Culture Media: An In Vitro Study

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ABSTRACT

Background: : Collagen membranes are widely used in Implant Dentistry for Guided Bone Regeneration (GBR), but their high cost and unpredictable degradation, have led to the search for alternative biomaterials such as Bacterial Cellulose (BC). BC is a naturally derived biomaterial and has advantages over the collagen membrane, like high purity, mechanical strength, and excellent biocompatibility. The comparison of the surface roughness and hydrophilicity of bacterial cellulose membrane derived from different culture media is the main objective of this study. **Materials and Methods:** In this in vitro experimental study, a total of 42 BC samples were synthesized using a specific strain of *Komagataeibacter*in three different media: Hestrin–Schramm (HS) medium, watermelon peel extract medium, and pineapple peel extract medium. Samples were categorised into three groups based on the medium used, and then, each group was subdivided for surface roughness and hydrophilicity; the Atomic Force Microscopy (AFM) was used for surface roughness assessment and contact angle measurements were used for hydrophilicity evaluation. The SPSS software (version 23.0) and one-way ANOVA test were used for Statistical analysis, and a p-value < 0.05 was considered a statistically significant value. **Results:** BC derived from HS medium demonstrated the highest surface roughness ($Ra = 216.12 \pm 7.8$ nm) and lowest contact angle ($14.10° \pm 0.95$), indicating superior hydrophilicity. Watermelon peel-derived BC showed moderate roughness and hydrophilicity, while pineapple-derived BC exhibited the lowest roughness and comparatively higher contact angles. **Conclusion:** In accordance with the results, BC synthesized using the HS medium exhibited optimal surface characteristics for GBR applications. Watermelon peel-derived BC demonstrated promising properties as a sustainable alternative.

Keywords: Atomic force microscopy, Bacterial cellulose, Cellulose Membrane, Contact angle, Guided bone regeneration, Hydrophilicity, Surface roughness.

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INTRODUCTION

Cellulose is a natural biopolymer widely utilized in various scientific and industrial domains. Apartfrom plant-derived cellulose, certain bacterial strains such as *Agrobacterium,Aerobacter,Achromobacter,Azotobacter,Rhizobium,Komagataeibacterxylinus*,*Komagataeibacterhanseni,*etc. are capable of synthesizing Bacterial Cellulose (BC), which is an extracellular cellulose. The properties of BC such as high purity, absence of lignin and hemicellulose, and a unique nanofibrous network structure provide excellent mechanical strength, high crystallinity, and superior water-holding capacity, making BC as a highly recommendable material for biomedical and dental usage including wound dressings, tissue engineering scaffolds, Guided Tissue Regeneration (GTR), periodontal wound dressings, Guided Bone Regeneration (GBR) [1–3].

In implant dentistry, guided bone regeneration (GBR) is a highly effective and established technique that utilizes collagen membranes to facilitate bone formation without soft tissue invasion [4]. Although the collagen membranes are widely used, their high cost and unpredictable degradation have led to the search for alternative biomaterials. BC has emerged as a highly valuable alternative biomaterial for collagen membrane; however, its large-scale production is limited by the cost of conventional media such as HS medium. Recently, some studies investigated the utilization of agro-industrial residues like watermelon and pineapple peel fruit peels, as cost-effective alternative culture media for BC production [6–8].

The efficacy of BC membranes is significantly influenced by surface characteristics, particularly surface roughness and hydrophilicity, which affect protein adsorption, cell adhesion, and proliferation [5]. The bacterial strain and culture conditions influence the BC characteristics, such as surface roughness and hydrophilicity. Although several studies have investigated the production of BC, limited studies are available related to surface characteristics of fruit peel-derived BC and its application in guided bone regeneration in implantology. So, further studies are needed to assess the surface characteristics of fruit peel-derived BC. Therefore, the aim of the present study was a comparative evaluation of the surface roughness and hydrophilicity of BC derived from watermelon and pineapple peel extracts media with the BC derived from HS medium and there by assessing its suitability for GBR applications.

MATERIALS AND METHODS

In this in vitro experimental study, 42 BC membrane samples were synthesized using HS medium and two alternative media derived from watermelon and pineapple peels over a period of three months. The BC membrane samples were categorized into three groups: Group 1 (BC prepared from HS medium) used as a control, Group 2 (BC prepared from Watermelon peel extract), and Group 3 (BC prepared from Pineapple peel extract). Each group was further divided into 2 equal subgroups for surface roughness and hydrophilicity analysis (n = 7). In this study, no human or participants are involved, ethical approval was not needed.

Isolation and production of bacterial cellulose membrane

Isolation and production of BC membrane was performed in accordance with the method described by Kumbhar J.V et al. (2015) [7].

Approximately 50 g of pineapple peels were collected from fruit vendors, thoroughly washed, and immersed in 50 mL of water comprising 5% table sugar and allowed to ferment for 7 days under static conditions in a container covered with muslin cloth, leading to the formation of a gelatinous pellicle on the surface of the medium. The pellicle was then aseptically transferred into HS culture medium. The HS medium components help in the cultivation of cellulose-producing bacteria. The cellulose-producing bacteria broth was streaked onto HS agar plates and incubated for 3 days at room temperature. To obtain pure isolated cultures, the colonies were subcultured on fresh HS agar plates. After 3 days, the bacterium isolated from the fermented fruit peel medium was identified as *Komagataeibacterhansenii*MCM B-967 based on characteristics such as colony morphology, Gram staining characteristics, pellicle formation, calcofluor staining, and standard biochemical methods, including catalase, oxidase, acetate oxidation, nitrate reduction, gelatin liquefaction, and indole production.

Isolated *Komagataeibacterhansenii MCM* B-967 bacterial colonies were inoculated into 100 mL of HS media with a pH of 5 in 250 mL Erlenmeyer flasks and incubated for 48 hours at room temperature under static conditions. After 48 hours of incubation, a thin gelatinous pellicle developed at the air–liquid interface. The thickness of the pellicle increased during further incubation, suggesting BC production. The pellicles were harvested after 7 days of incubation and washed thoroughly with water for the removal of residual media substrates. Purification was done by treatment with 2% NaOH at 90 °C for 60 minutes. . To achieve a neutral pH, the pellicle was washed repeatedly with distilled water.

For watermelon peel and pineapple peel media preparation, 100 g of fruit peels were collected, thoroughly washed, and homogenised in 100 mL of distilled water, then filtered, extracted, and boiled for 20 minutes. Upon cooling, the final growth media were prepared by incorporating 5% table sugar (carbon source), 0.7% ammonium sulfate (nitrogen source), and 0.02% cycloheximide (antifungal agent). By adding 0.1 NaOH or 0.1 M HCL, the media pH was adjusted to 5.0 and transferred into 250 mL Erlenmeyer flasks. The media was inoculated with 10% seed culture and incubated for 7 days under static conditions at room temperature. After 7 days, the BC pellicles were harvested and purified in the same way as the HS media.

After purification and neutralization, each BC hydrogel was frozen at –80 °C for 4 h and then freeze-dried (lyophilized) for 48 h at a condenser temperature of –55 °C under a vacuum <0.1 mbar.

The Atomic Force Microscopy (AFM) analysis (Anton Paar Step 700, Switzerland) in contact static mode was used for the evaluation of surface roughness over a scan area of 10 µm × 10 µm.Area Roughness Parameters such as Arithmetic mean height (Sa), Root mean square roughness (Sq), Maximum peak height (Sp), Maximum valley depth (Sv),Peak-to-peak height (Sy). Line roughness parameters, including arithmetic average height (Ra), RMS roughness (Rq), Maximum roughness height (Ry), and max peak height (Rp), were assessed.

The surface hydrophilicity of the BC membranes was evaluated using the sessile drop method using deionized water and phosphate-buffered saline (PBS). A 5 µL droplet was analyzed using MB-Ruler 5.3 software to measure contact angles and coordinate points. To ensure high measurement precision and account for potential surface heterogeneity, measurements were performed in triplicate at five distinct locations on each substrate, with contact angles determined using the standard tangent method at the liquid-solid-gas triple point.

The SPSS software (version 23.0) and one-way ANOVA test were used for Statistical analysis, and a p-value < 0.05 was considered a statistically significant value.

RESULTS

Surface Roughness

AFM analysis results showed that all groups produced BC with nanostructured surfaces. The HS group exhibited the highest surface roughness with prominent height variations (Ra = 216.12 ± 7.8 nm), followed by the watermelon group (Ra = 128.18 ± 7.5 nm), while the pineapple group showed the lowest values (Ra = 48.15 ± 5.2 nm) (Figure 1-3).

The quantitative analysis of roughness parameters showed that Sa, Ra, Sq, Rq, and Ry values of HS medium were 210.73 nm, 216.12 nm, 271.51 nm, 270.22 nm, and 1186.9 nm, respectively, which suggested that the HS medium-derived cellulose membrane had higher surface roughness. The watermelon derived membrane showed moderate roughness values (Sa: 127.11 nm, Ra: 128.18 nm, Sq: 164.61 nm, Rq: 158.03 nm and Ry: 557.35 nm) and pineapple derived membrane showed lowest surface roughness values (Sa: 50.15 nm, Ra: 48.15 nm, Sq: 63.35 nm, Rq:58.55 nm and Ry: 247.87 nm) (Table 1, Graph 1).

Hydrophilicity

The results revealed that the mean average contact angles of the HS group, watermelon, and pineapple groups were 14.76° ± 0.70, 47.4 ° ± 7.39, and 54.08 ° ± 6.69, respectively. The HS group exhibited higher surface wettability than the watermelon and pineapple groups. The surface wettability among the three groups showed statistically significant results (p=0.001) (Table:2).

The droplet width of the HS medium group, watermelon group, and pineapple group were (416.45 ± 40.32 pixels), (384.50 ± 48.56pixels), and (341.91± 32.67 pixels). The HS Medium group revealed the greatest droplet width compared to the watermelon group and pineapple group, indicating a higher degree of surface interaction compared to the narrower droplets. The droplet width among the three groups showed a statistically significant difference (p=0.02) (Table:3).

DISCUSSION

Collagen membranes are widely used in Implant Dentistry for Guided Bone Regeneration (GBR), but thier high cost and unpredictable degradation,have led to the search for alternative biomaterials such as Bacterial Cellulose (BC). BC is a naturally derived biomaterial and has advantages over the collagen membrane, like high purity, mechanical strength, and excellent biocompatibility [1-3]. The efficacy of GBR membranes is significantly influenced by surface characteristics, particularly surface roughness and hydrophilicity, determining the biological performance of biomaterials [5].

In the present study, HS-derived BC exhibited surface roughness within the optimal nano roughness range known to enhance osteoblast adhesion and activity [5,9–11]. The watermelon-derived BC also demonstrated values within this range, suggesting its suitability for supporting cellular interactions. The lower roughness observed in pineapple-derived BC may limit cell adhesion but could be advantageous in applications requiring barrier function with minimal tissue integration.

Hydrophilicity is another critical factor influencing biomaterial performance. The superior hydrophilic nature of HS-derived BC is beneficial for protein adsorption and cell adhesion in dental implants. Although fruit peel-derived BC showed moderate hydrophilicity, these values remain within ranges reported to support cellular activity. The contact angles of all three groups are below 90°, which indicates hydrophilic surfaces and contact angles below 60° are associated with favorable protein adsorption and cell interaction [12–14]. These variations in hydrophility can be attributed to culture media nutrient composition, which influences bacterial metabolism and cellulose fibril organization [7,8].

*Komagataeibacterhansenii*was utilized in this study because of its well-established ability to synthesize highly pure extracellular cellulose with excellent physicochemical and structural properties. The organism produces a nanofibrillar cellulose network possessing high crystallinity, water-holding capacity, mechanical strength, and biocompatibility, making it suitable for biomedical and tissue engineering applications [15–17]. Studies conducted by Gorgieva S and Blanco Parte FG demonstrated that the physicochemical characteristics of BC synthesized by Komagataeibacterhansenii species depend on the culture medium and fermentation conditions 16,18.

Watermelon peel and pineapple peel-derived media can be used as alternative substrates for BC synthesis because these fruit wastes contain fermentable sugars, vitamins, and minerals that support bacterial growth and cellulose production. The usage of agro-industrial fruit waste is a sustainable and cost-effective approach for bacterial cellulose production while simultaneously reducing environmental waste burden. Studies by Kumbhar JV and Blanco Parte FG reported successful BC synthesis using fruit-based substrates with improved yield and favorable cellulose characteristics [7,18].

According to this study, HS medium-derived BC has superior surface characteristics and higher surface wettability. Watermelon peel-derived BC demonstrated promising properties as a sustainable alternative.

CONCLUSION

In accordance with the study results, among all three groups, HS medium-derived BC exhibited optimal surface characteristics, such as superior surface roughness and hydrophilicity, for GBR applications. Watermelon peel-derived BC demonstrated promising properties, indicating its potential as a sustainable and cost-effective alternative for HS medium. But the present study is limited to surface roughness and hydrophilicity. However, further studies evaluating other mechanical properties, biological response, and in vivo performance are necessary.

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DECLARATIONS

Conflict of interest: The authors declares that there is no conflict of interest.
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Table 1: Table showing the Surface roughness parameters of bacterial cellulose membranes derived from different culture media

PARAMETER	HS	PINEAPPLE	WATERMELON
Sa	210.73	50.15	127.11
Sq	271.51	63.35	164.61
Ra	216.1	48.15	128.18

	2		
Rq	270.2 21	58.55	158.03
Ry	1186. 9	247.87	557.35

Table 2: Table showing the Average Contact Angle Analysis of Bacterial Cellulose Membranes derived from different Culture Media

	Mean	Std. Deviation	95% Confidence Interval for Mean		F value	P value
			Lower Bound	Upper Bound		
HS MEDIUM	14.76	0.7	13.02	16.5	93.16	0.001*
WATERMELON	47.4	7.39	29.04	65.76		
PINEAPPLE	54.08	6.69	37.47	70.69		

* Statistically significant; One way ANOVA

Table 3: Table showing the Droplet Width in Pixels of Bacterial Cellulose Membranes derived from different Culture Media

	Mean	Std. Deviation	95% Confidence Interval for Mean		F value	P value
			Lower Bound	Upper Bound		
HS MEDIUM	416.45	40.32	374.13	458.76	4.98	0.02*
WATERMELON	384.50	48.56	333.53	435.46		
PINEAPPLE	341.91	32.67	307.63	376.20		

* Statistically significant; One way ANOVA

Figure Legends:

Figure 1: AFM images of bacterial cellulose membrane derived from HS Medium

Figure 2: AFM Images of Bacterial Cellulose Membrane Derived from Watermelon Medium

Figure 3: AFM images of bacterial cellulose membrane derived from Pineapple Medium

Figure 4: Average Contact Angle Analysis of Bacterial Cellulose Membranes derived from HS Medium

Figure 5: Average Contact Angle Analysis of Bacterial Cellulose Membranes derived from Watermelon Medium

Figure 6: Average Contact Angle Analysis of Bacterial Cellulose Membranes derived from Pineapple Medium

Figure 7: Graph showing Surface roughness of Bacterial Cellulose from Different Media

Figure 1

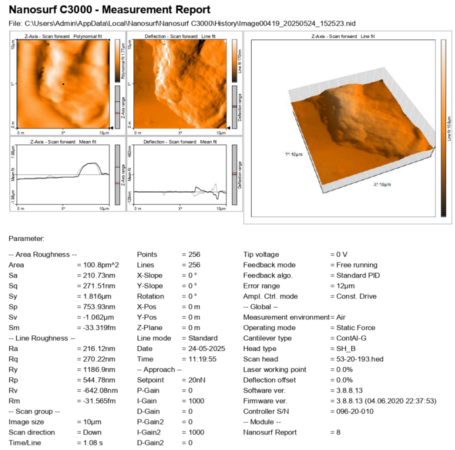


Figure:2

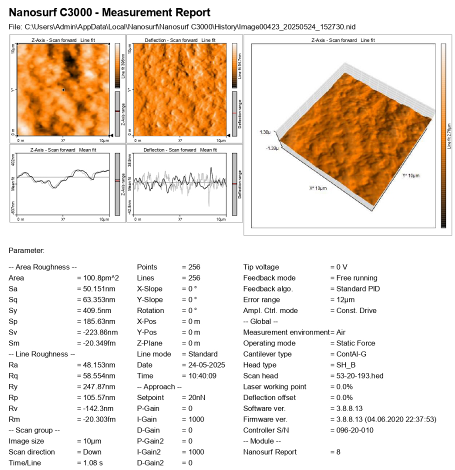


Figure:3

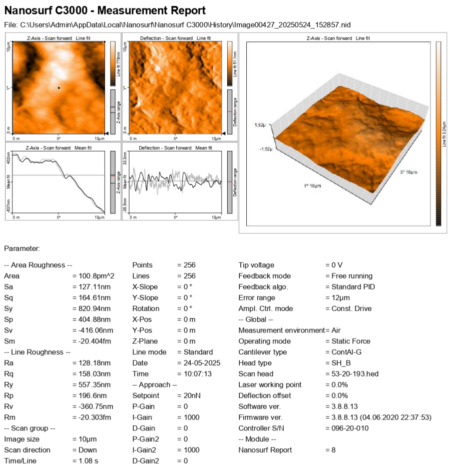
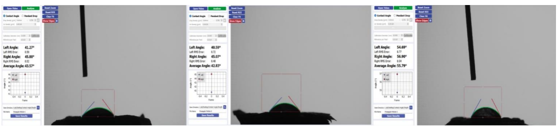


Figure: 4

Figure: 5



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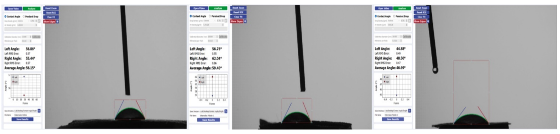

Figure: 7