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Research Article

Fabrication and Evaluation Wound Dressing Based on Bacteriocellulose Derived from Kombucha along with Bioactive Glass

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Abstract

Introduction: Cellulose is the most abundant natural biopolymer and one of the most popular environmentally friendly materials that bacterial species can produce. Due to the unique characteristics of bacteria cellulose, such as using a very low-cost culture medium for growth, creating a suitable wet environment in the wound bed, and the ability to adapt to various wound conditions, diversity in size, high porosity, non-toxicity, Biocompatibility, high strength, and resistance, being impervious to bacteria were used in this study.

Methods: In this research, we designed, fabricated, and evaluated biocompatible and strong wound dressings with maximum ideal characteristics. These wound dressings were based on bacteriocellulose (derived from kombucha) and bioactive glass seeds. We investigated the biochemical and physical properties of the wound dressing, such as Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (FE-SEM), tensile strength, swelling, contact angle, toxicity (MTT), and antimicrobial properties. Based on the results, the prepared wound dressing was selected for in vivo evaluation on rats. We used microscopic and histological (hematoxylin and eosin staining) techniques and wound surface measurement to evaluate wound healing on days 7, 14, and 20 after treatment.

Results: The results obtained from FE-SEM show the three-dimensional structure of bacterial cellulose. The FTIR examination of the wound surface shows its hydrophilic solid properties; bioglass nanoparticles in the bacterial cellulose substrate strengthen and improve its mechanical properties. Bacteriocellulose has a relatively good swelling ability and creates a moist and suitable environment in the wound area; it also makes it easier to separate the wound dressing from the wound environment and is a suitable absorbent for wound secretions. It has biocompatibility. In the examination of the in vitro performance by measuring the degree of wound closure as an indicator of the effectiveness of the treatment, it was shown that the treatment of the wound with a bacteria cellulose dressing treated with bioglass particles led to the wound healing after 20 days.

Conclusion: The findings show that this wound dressing has suitable capabilities such as biocompatibility, high mechanical strength, non-toxicity, creating and maintaining moisture on the wound's surface, antimicrobial properties, Appropriate flexibility, and the ability to absorb wound secretions. Therefore, this dressing has good potential for the successful and targeted healing of wounds, especially burns.

Keywords: Wound Dressing, Bacteriocellulose, Kombucha, Bioactive Glass.

Introduction

The human epidermis constitutes the external envelope of the corporeal form and is the foremost component of the integumentary system. Its multifaceted role encompasses several vital functions, including thermoregulation, prevention of desiccation, sensory guidance, and provision of a mechanical barrier to safeguard against the incursion of minuscule microbial entities and pernicious environmental factors. These adversarial factors encompass radiation, mechanical trauma, and thermal and chemical burns.[1] A wound, in essence, constitutes any form of physical lesion or laceration that arises from physical, thermal, chemical, or thermic injuries inflicted upon the integument. Such injuries culminate in the rupture of the integument layers, resulting in the dissipation of structural integrity and cohesion, thus giving rise to the division or severance of the integument [2].

The process of wound healing and convalescence is a multifaceted and intricate undertaking. It is an automatic response to injury and transpires through a nexus of interactions between cytokines, growth factors, blood constituents, and the extracellular matrix within the biological organisms. The inception of this process becomes evident post-injury.[1-3]

The paragon of wound dressings endeavors to reestablish the customary conduct of healthy skin. A spectrum of parameters is considered when designing and fabricating wound dressings. These encompass the preservation of protein and fluid content within the wound, the facilitation of exudate drainage, the prevention of infection by exogenous microorganisms, flexibility, non-toxicity, hypoallergenic, and a heightened level of biocompatibility.[1]

In contemporary society, the prevalence of bacterial infections has emerged as a pressing concern. Consequently, the imperative for creating and fabricating antibacterial biomaterials, encompassing synthetic and naturally derived polymers, has gained paramount significance.[4]

Bioactive wound dressings, a pivotal component in this context, are crafted from an array of natural polymers, including collagen, hyaluronic acid, cellulose, chitosan, alginate, and elastin, among others, as well as synthetic polymers like polyurethane, polycaprolactone, polyvinyl alcohol, and more. These materials contribute significantly to the augmentation and expeditious rejuvenation of epithelial tissue and collagen synthesis, thus pivotal in wound healing.[5]

Cellulose, the most abundant natural polymer, has been a subject of extensive research in recent decades, with the intriguing possibility of its biosynthesis by bacterial species6 Bacterial cellulose, while sharing a chemical identity with plant cellulose, exhibits notable disparities in purity, macromolecular properties, and physical attributes. Contrasting plant cellulose, bacterial cellulose boasts remarkable purity because it lacks lignin, hemicellulose, and pectin, facilitating heightened polymerization and crystallization. In producing bacterial cellulose by cultivating Acetobacter selenium bacteria, the resulting cellulose particles, derived from dynamic and static culture media, exhibit dimensions under 60 nanometers.[7]

Bacteriocelluloses are natural polymers characterized by fibers one hundred times smaller than those found in plant cellulose. These materials offer a host of desirable attributes, including biocompatibility, high porosity, non-toxicity, favorable physical properties, appropriate surface chemistry, and mechanical resilience. Moreover, their utilization is cost-effective, involves a low-cost culture medium, and creates an optimal moist environment within wound sites.

These properties allow them to adapt to diverse wound conditions, facilitate cell proliferation, exhibit a range of sizes, maintain a suitable wound bed temperature, and offer resistance to bacterial colonization. In light of these attributes, the synergy of bacterial cellulose in hybrid material design and production holds significant promise for various applications, such as tissue engineering. It is a three-dimensional extracellular matrix that sustains cell continuity and governs tissue structure and cellular behavior. Such capabilities make bacterial cellulose valuable in preparing, designing, and manufacturing hybrid materials, including wound dressings.[8]

Creating multiple bacteriocellulose composites represents a versatile biopolymer with enhanced physical and biological characteristics. For instance, aerogel composites, achieved through the amalgamation of bacteria cellulose and sodium fusidate, exhibit pronounced antibacterial efficacy and commendable mechanical and tensile strength. These composites serve a dual purpose and can aptly be denoted as wound dressings. [9-10]

By employing polycarboxylic acid, it is feasible to maintain the three-dimensional structure of bacteriocellulose even after desiccation. This preservation effectively prevents structural collapse and augments water absorption, viability, adhesion, and cellular proliferation within the material.[11]

Furthermore, bacteria cellulose, notable for its high porosity, demonstrates the capacity to facilitate antibiotic penetration, consequently reducing the presence of bone pathogens responsible for infections such as Staphylococcus aureus and Pseudomonas aeruginosa.[12] Incorporating various plant extracts, such as sage, into bacteria cellulose wound dressings confers antimicrobial properties.[13]

Additionally, wound dressings containing bioactive glasses release biologically active ions that increase angiogenesis, wound healing, and immunogenesis. Such wound dressings release biologically active ions such as borate, silica, copper, and zinc to improve healing conditions for soft tissue such as skin. By mimicking the physical structure of the extracellular matrix through the activation and positive regulation of factors such as fibroblast growth factor receptor precursor, vascular cell adhesion factor, vascular endothelial growth factor precursor, and fibronectin receptor beta subunit, they provide essential prerequisites for effective wound healing. In this context, wound dressings reinforced with bioactive borate glasses have successfully treated chronic wounds. [14]

In light of these considerations, extensive research endeavors have been undertaken to advance the production of bacteria cellulose-based composites. These composites incorporate a variety of constituents, including nanoparticles, both natural and synthetic polymers, antibiotics such as tetracycline, ciprofloxacin, ceftriaxone, and ampicillin, and biological molecules such as enzymes, hormones, and amino acids.[15]

Moreover, comprehensive investigations have been conducted to assess the therapeutic properties of diverse wound dressings. These investigations encompass multiple parameters, including blood clotting, long-term durability, wound exudate absorption capacity, biocompatibility, mechanical and tensile strength, hydrophilicity, thermal stability, inhibition of bacterial proliferation, non-toxicity, cost-effectiveness, antibacterial properties, as well as moisture and gas absorption capabilities. These efforts aim to pave the way for the development of various materials and applications. Notably, these applications range from producing advanced-function wound dressings to targeted drug delivery systems and innovations in bone tissue engineering to cartilage repair. Clinical trials have corroborated the effectiveness of bacteriocellulose dressings when coupled with antibiotics, bioglass, silver particles, and similar agents. These specialized dressings have proven highly efficacious in enhancing and expediting wound healing, particularly in burns and surgical wounds.[16-17]

This research involves processing bacteriocellulose obtained from Kombucha to make wound dressings. Acetobacter xylinium is the primary bacterium present in Kombucha, which has the power to form a floating cellulose network during fermentation. This thin membrane on the liquid surface has many chemical, molecular, mechanical resistance, and non-toxicity characteristics, the essential characteristics of an optimal wound dressing. Such wound dressings are designed to facilitate the regeneration of damaged skin tissue and reduce the risk of infection in invasive medical procedures.

In addition, in this study, Bioactive glass particles, whose effects have recently been confirmed with suitable combinations on angiogenesis, immunogenicity, and bacterial infections, have also been used to evaluate the therapeutic effectiveness of bacteriocellulosic wound dressings compared to bacteriocellulosic dressings treated with bioactive glass.

Methods

Cultivation of Kombucha

The initial step to prepare the kombucha solution involved brewing 15 grams of black tea in one liter of boiling water for 19 minutes. Subsequently, 70 grams of sucrose (expressly, Naghsh Jahan white sugar) was incorporated into the brewed tea. Standard kombucha pieces, cultivated in the Baqiyatullah University of Medical Sciences laboratory, were rinsed with a quantity of cooled, boiled water. These cleaned pieces were then introduced into the sweetened, cooled tea, maintaining an ambient temperature range of 10-30 degrees Celsius.

The concoction was transferred to an incubator set at 27 degrees Celsius for 5 to 7 days, away from direct light. Over this incubation period, bacteriocellulose layers developed at the interface between the air and the liquid. After 7 days, a distinct layer of kombucha enriched with bacteriocellulose had formed. This layer was carefully isolated, with any surplus medium removed through centrifugation. Subsequently, the layer was subjected to multiple washes with deionized water.[18]

Decontamination of Kombucha

Separating bacteria and yeasts from the bacteriocellulose is imperative after the cultivation process. A 0.1 M NaOH solution was employed to achieve this. The samples underwent sonication treatment at a temperature of 90 degrees Celsius for 10 minutes. Subsequently, they were subjected to a series of rinses with deionized water, each repetition encompassing ten cycles. Finally, residual water was extracted through centrifugation at 5000 rpm for 15 minutes.[18]

Kombucha Decolorization

The specimen was immersed in a 1.5% sodium hypochlorite solution for 2 hours to achieve sample decolorization. Subsequently, it underwent five steps, each involving immersion in deionized water for 3 hours. This process yielded a thoroughly decontaminated white sample without bacterial or yeast presence.[19]

Figure 1: Decolorized bacteriocellulose extracted from kombucha.

Determining the Type of Bioactive Glass and Adding It to Bacteriocellulose

In the course of this research, Bioactive Glass 45s5 (Tofigh Daru Company) was introduced. The immersion method was used to incorporate bioactive glass into a bacteriocellulose matrix. Specifically, bacteriocellulose was immersed in a 1% bioactive glass solution (dissolved in double-distilled water) within a shaker incubator. This immersion procedure was executed twice, with durations of 10 and 20 minutes, each conducted at a rotational speed of 25 revolutions per minute.[20]

Drying the Produced Bacterial Cellulose

These layers were meticulously positioned within small containers to desiccate the layers of bacteria cellulose and those treated with bioglass using a Freeze-Dryer. Subsequently, the containers were introduced into the machine for the drying process. As a result of this procedure, the layers underwent substantial thinning, adhering tightly to the plates and exhibiting a notably high level of transparency.[18]

Structural Characterization Tests

Field-Emission Scanning Electron Microscopy (FE-SEM)

A Scanning Electron Microscope XL device was utilized to assess the structural and morphological characteristics of the bacteriocellulose. Scanning electron microscopy was employed after the samples were dried in a Freeze-Dryer and a thin layer of gold was deposited on their surfaces.

Fourier Transform Infrared Spectroscopy (FTIR)

To scrutinize the chemical bonds within the bacteriocellulose wound dressings treated with bioactive glass, Fourier Transform Infrared Spectroscopy (FTIR) was conducted using the BUKER-Equinox 55 device. Spectra were recorded with a 1.4 cm^-1 resolution and 16 scans. The test specimens employed in this method were powder-prepared through freeze-drying.

Mechanical Tensile Testing

The mechanical tensile strength of bacteriocellulose wound dressings, an essential mechanical property, was examined to ensure their suitability for therapeutic applications. Bacteriocellulose samples were prepared in three distinct states and treated with bioactive glass for 10 and 20 minutes. These samples were prepared in dimensions of three centimeters by half a centimeter and securely fastened between the jaws of a testing machine.

Contact Angle Assessment

Contact angle analysis can gauge a surface's hydrophilicity, hydrophobicity, and photocatalytic properties. To measure the contact angle, 60 mg of deionized water at 25 degrees Celsius was dispensed onto the bacteriocellulose samples, both untreated and treated with bioactive glass, for 10 and 20 minutes, using a microsampler. Subsequently, the contact angles were promptly measured by a system equipped with a CCD camera for drop visualization and software for contact angle measurement on the target surface.

In Vitro Performance Evaluation

Investigation of Antimicrobial Properties

The Disk Diffusion test was employed to assess and compare the antibacterial attributes of the specimens. During this assessment, Gram-positive bacteria, specifically Staphylococcus aureus, and Gram-negative bacteria, such as Escherichia coli, were cultured under sterile conditions at 37 degrees Celsius. Subsequently, after a 24-hour incubation period, sections of bacteriocellulose and bacteriocellulose treated with bioactive glass were affixed to individual plates for durations of 20 and 10 minutes. Following a further 24-hour incubation at 37 degrees Celsius, the presence of halos surrounding the wound samples was examined, serving as an indicator of their antibacterial efficacy.

Investigation of Cytotoxicity

Cytotoxicity assessment is the most prominent method for evaluating cell viability and determining the impact of various compounds, medications, or supplements on cellular health. In this study, the in vitro biocompatibility of the fabricated wound dressings was explored through their interaction with human fibroblast cells procured from the National Center for Genetic and Biological Resources of Iran. This examination was conducted under three distinct time intervals while employing a culture medium containing DMEM without FBS. Cellular survival rates were evaluated at each of these intervals.

The cell viability test involves a colorimetric approach, wherein the reduction of a tetrazolium component to form an insoluble formazan product is quantified, primarily facilitated by the metabolic activity of living cell mitochondria. This experiment, which transpired across three phases, commenced by seeding 10 cells and 100 microliters of culture medium into each well. The culture plates were then placed within an incubator at 37 degrees Celsius, characterized by a controlled atmosphere of 5% oxygen and 2% CO2. Cellular viability and morphological aspects were observed through microscopic examination following incubation durations of 24, 48, and 72 hours.

Subsequently, the cell culture medium was aspirated, and 50 microliters of MTT solution (Sigma-Aldrich, USA) were introduced, after which a further incubation at 37°C ensued for 4-6 hours, thereby allowing the cells to metabolize tetrazolium. The supernatant was removed, and 500 microliters of dimethyl sulfoxide (Sigma-Aldrich, USA) were added to each well to dissolve the formazan crystals. The resulting samples were collected, and the optical density of each well, indicative of cell quantity, was measured using an ELISA reader (Stat Fox) at a wavelength of 570 nm. A linear correlation between cell count and the observed absorbance values was established for each cell type. (It is worth noting that an empty well served as the control in the culture medium) The calculation of cell survival percentages was accomplished using the following formula:

Formula 1

$$Survival\ Percenta = \frac{\text{Optical Absorbance of Each Treated Cell}}{\text{Average Optical Absorbance of Control Cells}} \times 100$$

Swelling test

Assessing swelling characteristics is a crucial aspect of formulating appropriate wound dressings. In order to investigate the swelling behavior of the samples (bacteriocellulose and bacteriocellulose treated with bioactive glass in 10 minutes and bacteriocellulose treated with bioactive glass in 20 minutes) their weight changes when exposed to PBS buffer. This PBS solution was prepared by dissolving 0.4 grams of sodium hydrogen phosphate (Na_2HPO_4) from the "Merck Company," along with 5.85 grams of sodium chloride (NaCl) and 0.6 grams of potassium dihydrogen phosphate (KH_2PO_4) from the "Merck Company." Subsequently, after a 24-hour observation period, each sample's Effective Water Absorption (EWA) values were determined using Formulas 2 to 3.

Formula 2

$$\text{Swelling Rate} = \frac{W_1 - W_0}{W_0}$$

Examination of In-Body Performance

An animal model was employed to assess the efficacy of the prepared wound dressings in wound healing. This method involved utilizing 12 male rats (weighing 200 ± 5 g) procured from the animal facilities at Baqiyatallah University of Medical Sciences, Tehran. The rats were housed individually in standard conditions, characterized by 12-hour light-dark cycles and a controlled temperature range of 22-28 degrees Celsius. To mitigate the potential influence of environmental stressors, the animals underwent a 14-day acclimatization period preceding the commencement of the experimental procedures. During this period, the rats had a pelleted diet and unrestricted access to water and food. Subsequently, the 12 rats were randomly allocated into two groups: One treatment group received control and dressings consisting of bacteriocellulose wound dressings and bacteriocellulose wound dressings treated with bioactive glass for 10 minutes, while the other group received control and dressings consisting of bacteriocellulose wound dressings and bacteriocellulose wound dressings treated with bioactive glass for 20 minutes. It is imperative to note that all procedures involving laboratory animals adhere strictly to the research ethics guidelines established by the Ministry of Health.

Wound Induction

Following a 14-day acclimatization period to the laboratory environment, the dorsal fur of the animals was carefully shaved. Subsequently, the targeted area was sanitized with 70% sterile ethanol. Under anesthesia induced by ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (5 mg/kg), circular skin sections measuring 8 mm in diameter were excised from the backs of the rats using a biopsy punch. The wound length was meticulously gauged with a caliper. The day of surgery was designated as day zero for this study. The above dressings were applied to the wounds to evaluate the progress of wound healing in the presence of bacteriocellulose dressing and bacteriocellulose treated with bioactive glass at 10 and 20 minutes after wound formation. In parallel, untreated wounds served as controls in each sample, and photographic documentation of the wound surfaces was performed. The extent of wound closure, indicative of treatment efficacy, was scrutinized. After the 7th, 20th, and 14th days, the mice were humanely euthanized for histological examination. Sterile scissors and forceps were employed to extract skin samples for a comprehensive investigation into wound healing progress and the underlying components contributing to this process.

Wound Management Procedure

Full-thickness wounds were induced on the animals' skin, after which each animal was individually housed in dedicated treatment cages. The wounds were securely bandaged and remained so until the predetermined evaluation days.

Macroscopic Wound Assessment

Over 21 days, immediate post-wound creation, and subsequently at 7, 14, and 20-day intervals, photographs of the wound site were captured to facilitate wound area measurements. The percentage of wound closure was calculated using the following formula:

Formula 3

$$\textit{Percentage of wound contraction} = \frac{\text{Area of the original wound} - \text{Wound area on the nth day}}{\text{Area of the original wound}} \times 100$$

where "n" designates the assessment day.

Data Analysis and Histopathological Examination

After 7, 14, and 20 days of the treatment regimen, the experimental mice were humanely euthanized to facilitate the procurement of tissue samples for histological scrutiny. Sterile scissors and forceps were employed to extract skin specimens, which were subsequently preserved in a 10% formalin solution. A sequence of alcohol solutions was used for tissue dehydration, culminating in embedding the specimens in paraffin wax. Subsequently, 6-micron sections were prepared and stained with hematoxylin and eosin (H&E). The objective behind the preparation of cutaneous sections was to explore microscopic alterations in skin tissue, assess shrinkage rates, and identify elements contributing to the wound healing process.

Data Analysis

SPSS 22 software was utilized for the data analysis, and the Kolmogrov-Smirnov One-Sample test was applied to evaluate the research data. This study maintained a significance level of $P \leq 0.05$ and a confidence interval of 95%. The Wallis-Kruskal test was employed to analyze the results of H&E staining, hematoxylin, and eosin staining, as well as wound closure outcomes.

Results

The electron microscope images in the current investigation reveal that cellulose fibers exhibit an intricate three-dimensional mesh structure characterized by uniform, rod-shaped pores. This intricate three-dimensional bacterial cellulose framework supports and promotes cell adhesion and proliferation. Moreover, this mesh structure can retain substantial volumes of water and facilitate the passage of nutrients.

Scanning Electron Microscope (SEM) images depict the presence of internal communication between particles, a factor of significant importance in incorporating bioactive glass nanoparticles. It is worth noting that bacterial cellulose demonstrates a finer structural profile when compared to plant cellulose fibers, which typically exhibit diameters ranging from 100 to 110 micrometers. (Figure 2-C)

In examining the wound dressing surface's infrared spectrum, cellulose, a polysaccharide characterized by its numerous OH groups, is a prominent feature. The notably strong and broad absorption observed at 3500-3500/1 cm is attributed to the stretching vibration of OH groups (hydrogen bonding between molecules at 3500-3000), underscoring the pronounced hydrophilic attributes of bacteriocellulose. Additionally, the robust absorption in the 1/cm 1065 region is linked to the stretching of the numerous C-O-H and C-O-C bonds within the molecular structure, particularly in the sugar ring. Furthermore, as the bioactive glass composite incorporates SIO_2, the absorbance at 1/cm 1100 indicates SIO_2 bonding. (Figure 2-B)

They should exhibit suitable tensile strength, flexibility, and elasticity for effective wound healing and successful dressing replacement. **Stress and strain curves were examined** to assess the mechanical strength of the prepared dressings. The results of the mechanical tensile test revealed that bacteriocellulose treated with bioactive glass for 20 minutes exhibited a lower Young's modulus compared to bacteriocellulose and bacteriocellulose treated with bioactive glass for 10 minutes. It is important to note that bioactive glass particles are characterized by their high strength and hardness. (Figure 2-D)

The findings of the contact angle test reveal that bacteriocellulose treated with bioactive glass for 20 minutes exhibits a shorter water absorption time and a reduced contact angle compared to both bacteriocellulose and bacteriocellulose treated with bioactive glass for 10 minutes. (Figure 2-A).

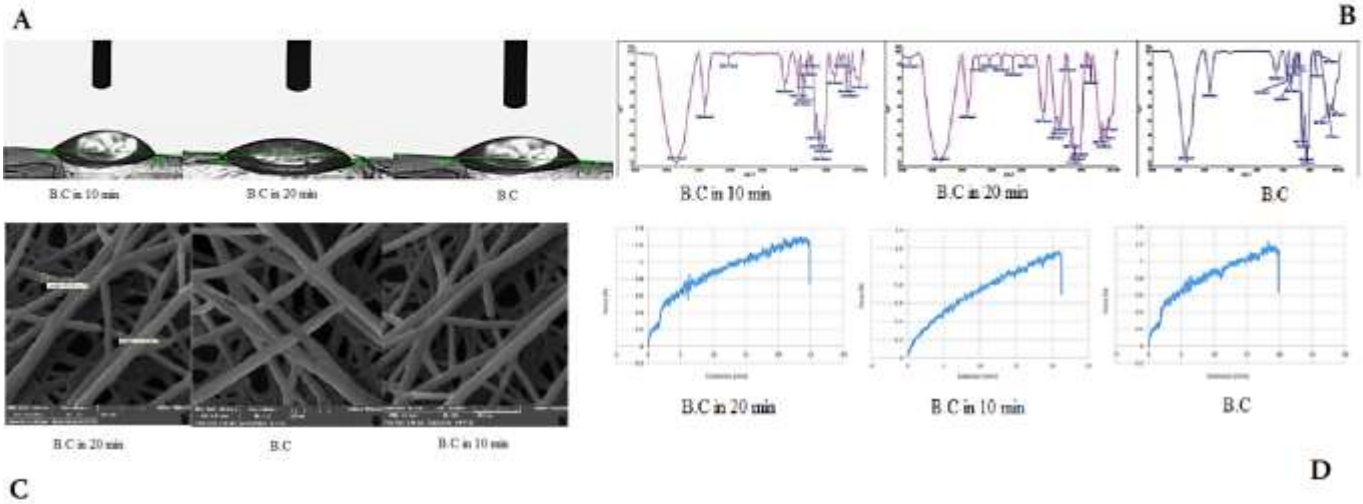

Figure 2: A: Contact angle test in all three samples: Bacteriocellulose (B.C) / Bacteriocellulose treated with bioactive glass in 20 minutes (B.C in20 min) / Bacteriocellulose treated with bioactive glass in 10 minutes(B.C in10 min) B: FTIR test in all three samples: Bacteriocellulose (B.C) / Bacteriocellulose treated with bioactive glass in 20 minutes (B.C in20 min) / Bacteriocellulose treated with bioactive glass in 10 minutes.(B.C in10 min) C: FE-SEM test in all three samples (Bacteriocellulose (B.C) / Bacteriocellulose treated with bioactive glass in 20 minutes (B.C in20 min)/ Bacteriocellulose treated with bioactive glass in 10 minutes(B.C in10 min)) that have nanofibers with almost the same thickness and uniformity. The average diameters of nanocellulose fibers are less than 100 nm. D: Tensile mechanical test in all three samples. (Bacteriocellulose (B.C) / Bacteriocellulose treated with bioactive glass in 20 minutes (B.C in20 min) / Bacteriocellulose treated with bioactive glass in 10 minutes B.Cin 10 min)).

Examination of in Vivo Performance

This surge in interest can be attributed to several factors, including the perceived safety of natural compounds, their widespread availability, and the ability to manage treatment courses meticulously. Upon scrutinizing the results of antimicrobial evaluations, it becomes evident that bacteriocellulose wound dressings treated with bioactive glass displayed remarkable antimicrobial efficacy when cultured alongside two bacterial strains, Staphylococcus aureus and Waschersia coli. Both Escherichia coli (a Gram-negative bacterium) and Staphylococcus aureus (a Gram-positive bacterium) exhibited susceptibility to bacteriocellulose wound dressings treated with bioactive glass, as manifested by the formation of a growth-inhibitory halo. It should be noted that bacteriocellulose, in its unaltered form, possesses limited inherent antibacterial properties. Notably, Fadakar and colleagues investigated the outstanding antimicrobial effects of bacteriocellulose in combination with sage extract, demonstrating its efficacy against various microorganisms, including Escherichia coli, Staphylococcus aureus, and Candida albicans. This finding aligns with the outcomes of the present study. (Figure 3-B)

During this investigation, many living cells underwent examination on wound dressing samples over 24, 48, and 72 hours. The results of the cytotoxicity assessment indicate that there exists no substantial disparity in cell survival rates among the groups cultivated on bacteriocellulose wound dressings treated with bioactive glass for 20 minutes, bacteriocellulose wound dressings treated with bioactive glass for 10 minutes, and unmodified bacteriocellulose. With time, cell proliferation and growth consistently persisted across all samples. Notably, incorporating bioactive glass into the wound dressings exhibited no discernible impact on the biocompatibility of the prepared wound dressings. (Figure 3-A).

Establishing and maintaining a moist environment on the wound surface is widely acknowledged as optimal for wound healing. Such an environment fosters cell growth, proliferation, and the creation of a conducive milieu for new tissue formation. It also activates a broad spectrum of biological molecules crucial for wound healing, including enzymes, growth factors, and hormones. Notably, bacteriocellulose exhibits a relatively robust swelling capability due to its predominant hydroxyl functional groups, which are highly hydrophilic. This property creates a moist and favorable environment within the wound area and facilitates the easy detachment of the dressing from the wound environment. Moreover, bacteriocellulose is an effective absorbent for wound secretions. The results of the swelling test indicate that all three samples exhibit nearly identical levels of swelling. (Figure 4).

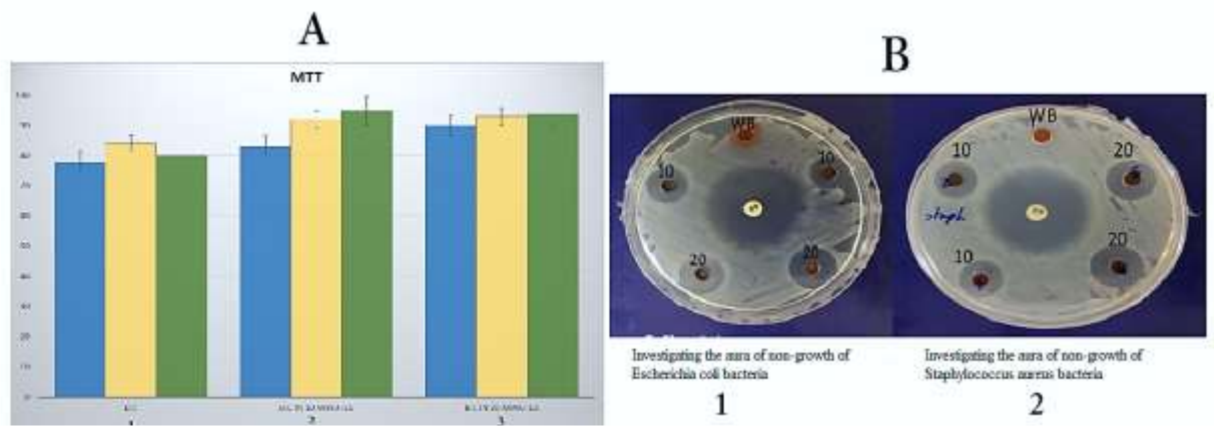

Figure 3: Figure 4: MTT test: 1) Bacteriocellulose (B.C) / 2) Bacteriocellulose treated with bioactive glass in 10 minutes / 3) Bacteriocellulose treated with bioactive glass in 20 minutes B: 1) Investigating the aura of non-growth of Escherichia coli bacteria (Investigation of antibacterial properties in all three samples and the control sample (WB) / 2) Investigating the aura of non-growth of Staphylococcus aureus bacteria(Investigation of antibacterial properties in all three samples and the control sample (WB).

Examination of in Vitro Performance

Given the favorable outcomes observed in terms of biocompatibility, coupled with the optimal physical and chemical properties, mechanical characteristics, adhesion properties, and cell proliferation rates established through non-toxicity assessments, bacteriocellulose wound dressings and bacteriocellulose treated with bioactive glass for 10 and 20 minutes were deemed suitable candidates for in vivo evaluations. These wound dressings were subsequently subjected to in vivo assessments using rat models.

The results derived from wound closure rate measurements, serving as an indicator of treatment efficacy and the reformation of the epithelial layer on days 7, 14, and 20, reveal that wound closure occurred at a more rapid pace in wounds treated with bacteriocellulose wound dressings treated with bioactive glass for 20 minutes compared to other wound dressings and the control sample (absent of dressing). By the end of the 20 days, the skin had healed without any trace of the wound, with all assessed criteria, including epithelial structure and the presence of skin appendages, displaying a discernible upward trajectory. (Figure 5-A)

According to the photographs taken at the same time intervals on the seventh day, the percentage of wound closure treated with bacteriocellulose treated with bioactive glass for 20 minutes was 55%, while this percentage for the wound dressed with bacteriocellulose treated with bioactive glass for 10 minutes was 40%. For the wound dressed with bacteriocellulose, the percentage was 35%, and for the wound without dressing, it was 25%. On the fourteenth day, the percentage for the wound dressed with bacteriocellulose treated with bioactive glass for 20 minutes was 80%, while the wound dressed with bacteriocellulose treated with bioactive glass shows 75% for 10 minutes, 65% for wounds dressed with bacteriocellulose, and 45% for wounds without dressings. On the 20th day, this percentage of the wounds dressed with bacteriocellulose wound dressings and treated with bioactive glass for 20 minutes was almost 95% healed (Figure 5-B).

Furthermore, in their comparative assessment of the bioactive properties of three distinct types of bioactive glass, they determined that the bioactive glass containing the 45S compound exhibited superior bioactivity and antibacterial attributes in contrast to the 49S and 58S compounds.[27] Their examination focusing on the therapeutic potential of S45 bioactive glass as a cellular scaffold, Bayno et al. corroborated the efficacy of bioactive glasses in facilitating the regeneration of soft tissues, providing mechanical support, and ensuring optimal biocompatibility when interfacing with living tissues. This favorable outcome stems from the release of soluble silica and other ionic species, which influence intracellular tissue proliferation. Consequently, utilizing this glass is deemed closer to achieving favorable outcomes in the context of the restorative process.

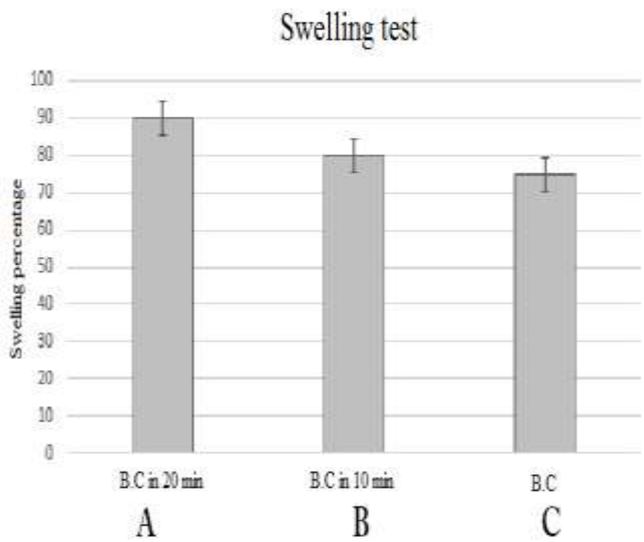

Figure 5: Swelling test: **A**: Bacteriocellulose treated with bioactive glass in 20 minutes / **B**: Bacteriocellulose treated with bioactive glass in 10 minutes / **C**: Bacteriocellulose (B.C).

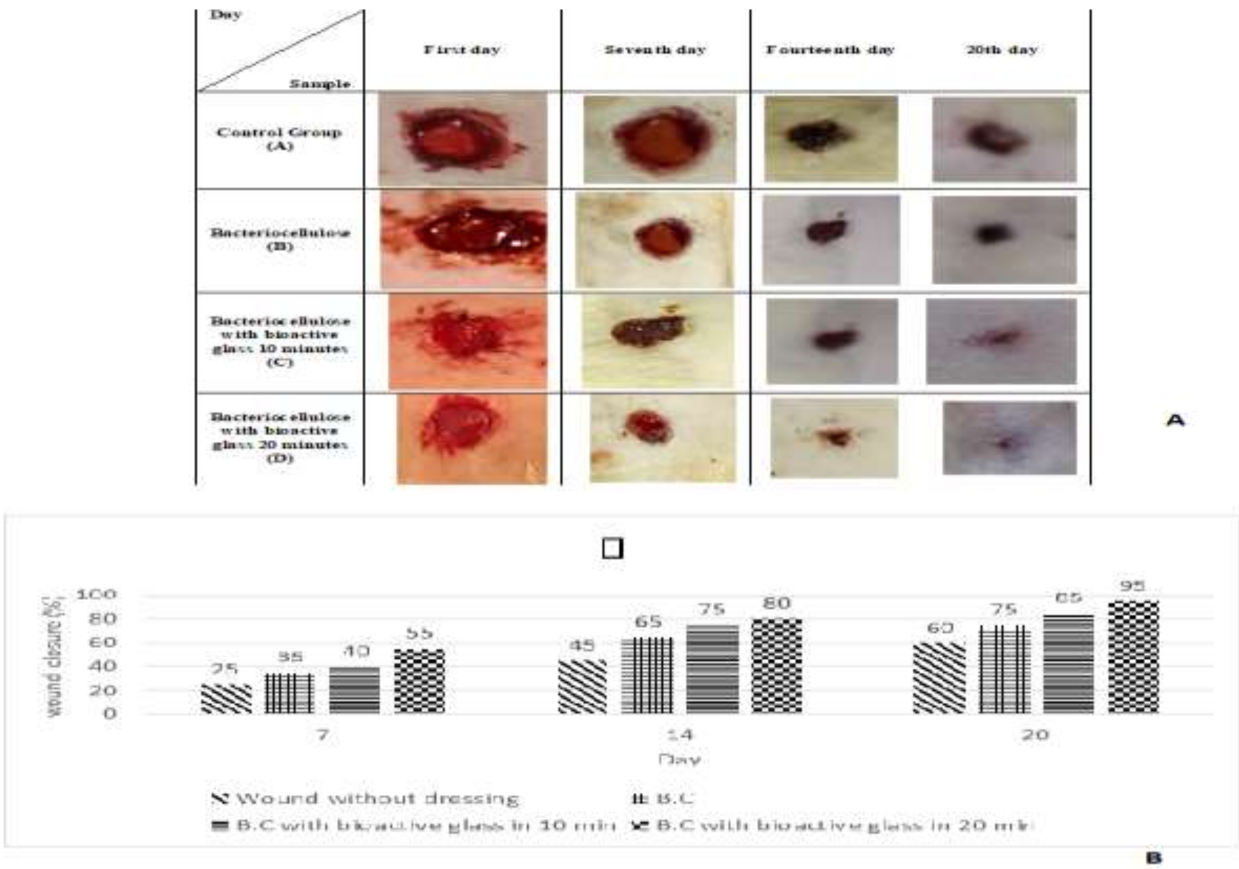

Figure 6: A: Examination table of the wounds created on the tested rats: A: Wound without dressing (control) / B: Bacteriocellulose (B.C) /C: Bacteriocellulose with bioactive glass 10 minutes / D: Bacteriocellulose with bioactive glass 20 minutes. B: Wound closure comparison diagram between all four tested groups over 20 days: Wound without dressing / Bacteriocellulose (B.C) / Bacteriocellulose with bioactive glass 10 minutes / Bacteriocellulose with bioactive glass 20 minutes.

Preparation of Hematoxylin and Eosin (H&E) Stained Slides

To assess wound healing progress and the efficacy of wound dressings, we utilized skin specimens obtained from wounds on tested rats. Our investigation involved the examination of histologically stained wound sections. Specifically, we scrutinized wounds treated with bacterial cellulose dressings and bioactive glass for 20 and 10 minutes and wounds left undressed (the control group). This evaluation was conducted on days 7, 14, and 20 following the initial injury. Our analysis yielded the following observations: On the 20th day, wounds dressed with bacteriocellulose wound dressings and treated with bioactive glass for 20 minutes displayed a conspicuous and complete formation of the epithelial layer. Furthermore, skin appendages along the wound's edge were evident, with noticeable dermal regeneration and angiogenesis. These findings collectively indicated a comprehensive wound-healing process. In stark contrast, the control group exhibited only a feeble development of the epithelial layer across the entire wound.

Skin appendages were notably absent within the bacteriocellulose dressing group, yet the epithelial layer was fully formed. In the case of bacteriocellulose dressings treated with bioactive glass for 10 minutes, we observed the emergence of a moderately developed bud tissue with a limited number of epithelial appendages. These results underscore the considerable impact of treatment duration and dressing type on wound healing, with the combination of bacterial cellulose and bioactive glass for 20 minutes proving particularly efficacious in promoting wound closure and tissue regeneration (Figure 6).

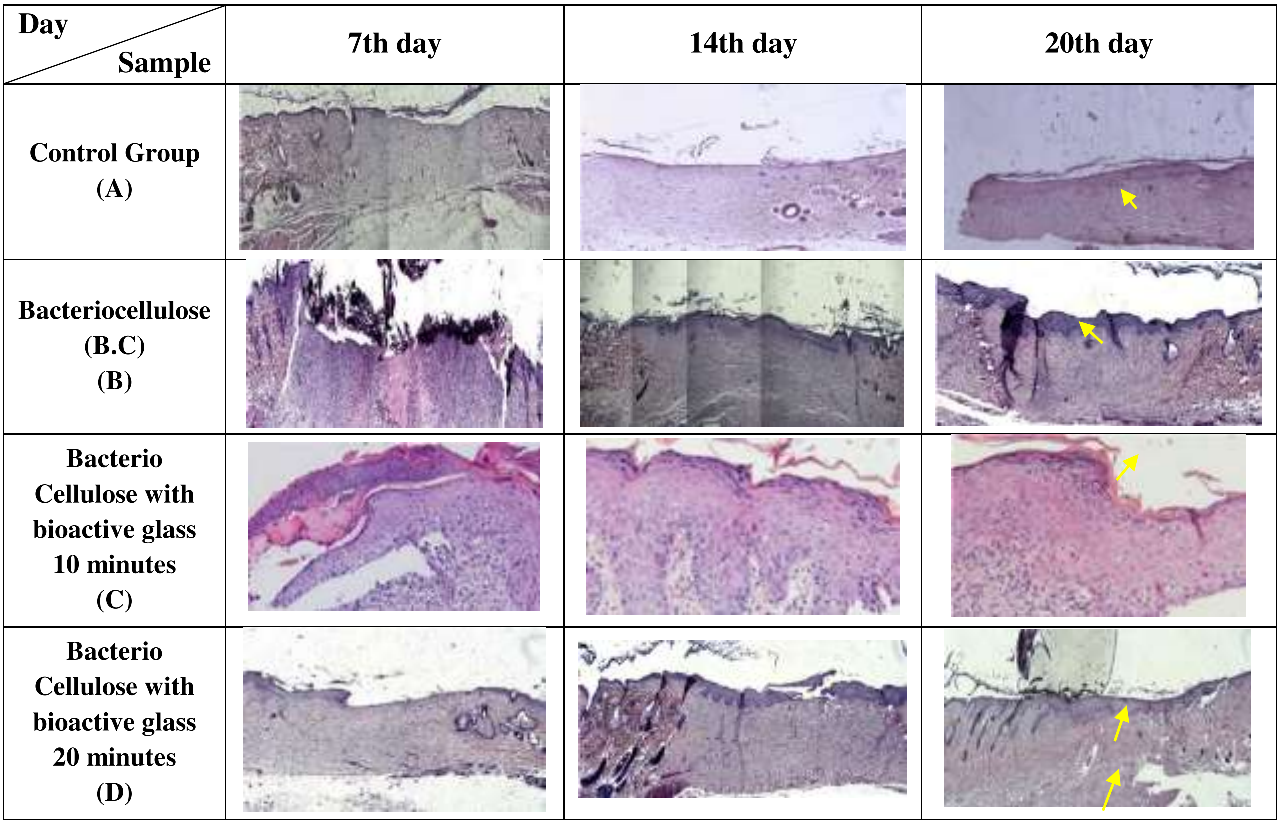

Figure 7: Pathological assessment of the quality of wound healing on days 7, 14, and 20 after surgery in the four tested groups (H&E, staining): A: Control Group, B: Bacteriocellulose, C: Bacterio Cellulose with bioactive glass 10 minutes, D: Bacterio Cellulose with bioactive glass 20 minutes.

Discussion

The skin, constituting the largest and outermost organ of the human body, serves as a primary defense barrier with crucial functions encompassing protection against physical, chemical, and biological agents, regulation of body temperature, and prevention of excessive loss of body fluids. It has three distinct layers: the dermis, epidermis, and hypodermis.[21]

Inadequate protection of wounds from external mechanical stress, inadequate management of wound secretions, insufficiency of proper wound surface moisture, microbial contamination, and infection all impede the wound healing process. These factors not only render wound healing conditions challenging but may also, in severe cases, culminate in fatality due to severe infection. One highly efficacious approach for addressing infected wounds involves the utilization of wound dressings designed to effectively curtail the proliferation of microbes both on the wound's surface and within the wound itself.

Bacterial cellulose has garnered substantial attention as a pristine biomaterial due to its unique attributes. These attributes encompass utilizing a cost-effective culture medium in its production and remarkable water absorption capacity. The prepared dressings' hydrophilicity and polarity were assessed by measuring water droplet contact angles on the membrane surface. Consequently, the bacteria cellulose wound dressing treated with bioactive glass for 20 minutes, featuring a lower contact angle, is notably more conducive to supporting cellular activities as a wound dressing.[23]

Cytotoxicity testing serves as a vital method for assessing cell viability on wound dressings and scaffolds in the context of tissue engineering, as the toxicity inherent to the substrate can hinder cell proliferation and lead to cell death over time. The results of the cytotoxicity assessment indicate that there exists no substantial disparity in cell survival rates among the groups cultivated on bacteriocellulose wound dressings treated with bioactive glass for 20 minutes, bacteriocellulose wound dressings treated with bioactive glass for 10 minutes, and unmodified bacteriocellulose. Notably, the incorporation of bioactive glass into the wound dressings exhibited no discernible impact on the biocompatibility of the prepared wound dressings. Siram Rama Krishna et al. and Raquel Portella et al. conducted independent research. Their investigations scrutinized the biocompatibility and non-toxic nature of bacteria cellulose dressings integrated with antibiotics, bioactive glass, and silver particles, among other elements. Moreover, they evaluated the therapeutic efficacy of these dressings through clinical trials, ultimately endorsing them as highly effective wound dressings capable of enhancing and expediting the healing process in scenarios such as burns and surgical wounds.[9-15]

Wound dressings must possess the capacity to retain their dimensional stability to safeguard damaged tissue from external forces and the pressure exerted by a moving patient. Furthermore, they should exhibit suitable tensile strength, flexibility, and elasticity for effective wound healing and successful dressing replacement. Consequently, including these particles within the bacteriocellulose matrix enhances the wound dressing's structural integrity and augments its mechanical properties. The augmented tensile strength can be attributed to the heightened bonding between hydroxyl groups and bioactive glass nanoparticles, along with the reduction in the size of internal pores under the influence of applied force. Researchers, including Roin and colleagues, have undertaken extensive investigations to advance the production of nanocomposites based on bacteriocellulose, incorporating various components such as nanoparticles and natural and synthetic polymers. Kohi et al. also obtained similar results in their research on the mechanical properties of bioactive glass, demonstrating its favorable impact on enhancing tensile strength.[16-22]

Creating a moist environment, in this case, the bacteriostatic agent has a relatively good swelling ability because its only active group is hydroxyl, which is very hydrophilic and creates a moist and suitable environment in the wound area, this property facilitates the easy detachment of the dressing from the wound environment. The results of the swelling test indicate that all three samples exhibit nearly identical levels of swelling. Leveraging this distinctive feature of bacteria cellulose, Khajavi and colleagues enhanced water absorption and cell survival rates, adhesion, and proliferation within bacteria cellulose by establishing cross-links.[11]

In recent years, researchers have increasingly focused on identifying natural compounds with antibacterial properties. Various classes of compounds have been effectively employed to inhibit bacterial growth in controlled laboratory settings. This surge in interest can be attributed to several factors, including the perceived safety of natural compounds, their widespread availability, and the ability to manage treatment courses meticulously. It should be noted that bacteriocellulose, in its unaltered form, possesses limited inherent antibacterial properties. The investigation of the antimicrobial results showed that the antimicrobial properties of the bacteriocellulose wound dressing treated with bioactive glass as cultured on a plate with two bacteria, Staphylococcus aureus, and Escherichia coli bacteria and Staphylococcus, compared to the bacteriocellulose wound dressing treated with Bioactive glass showed sensitivity so that the inhibitory effect on the growth of bacteria was observed in the form of a non-growth halo. Notably, Fadakar and colleagues investigated the outstanding antimicrobial effects of bacteriocellulose in combination with sage extract, demonstrating its efficacy against various microorganisms, including Escherichia coli, Staphylococcus aureus, and Candida albicans. This finding aligns with the outcomes of the present study.[13]

The small size of their fibers compared to plant cellulose, a three-dimensional structure of bacterial cellulose, can support and increase the adhesion of cells and their reproduction. Also, this mesh structure keeps large amounts of water and facilitates the passage of nutrients. SEM images show the existence of internal communication between particles, which plays a vital role in loading bioactive glass nanoparticles. It is worth mentioning that bacterial cellulose has a much finer structure compared to plant cellulose fibers, which have a diameter between 100-110 micrometers. These findings are consistent with those reported by Abbasi et al. in their research titled "Investigating the Properties of Reticulated Microbial Nanocellulose as a Novel Wound Dressing," where various assessments were conducted, including ATR-FTIR, MTT, SEM, water absorption, and in vitro and in vivo experiments.[11]

In the investigation of the infrared spectrum of the surface of the wound dressing, because cellulose is a polysaccharide and the presence of multiple OH groups has caused strong hydrophilic properties in bacteria cellulose, and where it is present in the structure of bioactive glass, the absorption is an indicator of SIO2 bond 1/CM1100. Notably, Azimzadeh et al. reported similar findings in their research, where they explored the synthesis and characteristics of bacteria cellulose produced by Acetobacter xylinum bacteria. They conducted a comprehensive analysis utilizing XRD, FTIR, MTT, and SEM techniques.[8]

Bacteriocellulose wound dressings and bacteriocellulose treated with bioactive glass in 10 and 20 minutes were also selected for in vivo evaluation, and the dressings were evaluated in vivo on rats. The results derived from wound closure rate measurements indicate treatment efficacy and the reformation of the epithelial layer after 20 days. The skin was repaired without leaving a trace of the wound, and all the considered criteria, including the epithelial structure and the presence of skin appendages during the repair, the particular significance is the antimicrobial role played by the bioactive glass, marked by its in vitro bioactivity and substantial antibacterial potential. Furthermore, the favorable surface topography supports cell survival, adhesion, and culture without adverse side effects. This, coupled with creating a moisture-rich environment facilitated by bacteriocellulose, offers an ideal setting for cell growth, multiplication, and enhanced migration, fostering optimal conditions for generating new tissue. Consequently, these factors have collectively contributed to the heightened efficacy of this wound dressing and an accelerated wound healing process. Notably, this achievement aligns with Wang et al.'s investigation into the latest advancements in bacterial cellulose's potential as a wound dressing and the formulation of antibacterial composites based on bacteria cellulose.[26]

Farag et al. delved into the impact of glass nanoparticles on the performance of bacteria cellulose, along with its biocompatibility, bioactivity, and antimicrobial characteristics, through the eco-friendly synthesis of bacterial cellulose/bioactive glass nanocomposites. Their findings substantiate that incorporating bioactive glass enhances the performance of bacteriocellulose. Moreover, it significantly elevates the biocompatibility and antimicrobial attributes of bacteriocellulose. This investigation underscores the potential for the widespread application of bacteria cellulose-bioactive glass composites in biomedical sectors, particularly in fields such as bone regeneration and wound healing, characterized by their unique properties and benign impact on human health.[29]

After preparing hematoxylin and eosin staining slides of the skin isolated from the tested rats to check the effectiveness of the bacteria cellulose wound dressing treated with bioactive glass in 20 minutes on the 20th day, the complete formation of the epithelium layer and the presence of some skin appendages on the edge and edge of the wound. Also, the epidermis and angiogenesis regeneration are more evident, and the wound is completely healed. This regeneration is while the sample on the same day in the control sample only had a weak epithelial layer formed on the entire wound. No skin appendages were seen in the bacterial cell sample, but the epithelial layer was formed entirely. In the sample of Bacteriocellulose treated with bioactive resin in 10 minutes, a relatively average bud tissue with few appendages shows a complete epithelial layer.

This inherent versatility positions bacterial cellulose as a compelling candidate for application in the medical domain, where it can serve as a biological material in tissue engineering, the fabrication of wound dressings, drug delivery systems, and various forms of biosensors, as well as in the development of energy storage devices. Furthermore, its potential extends to diverse sectors, including the environmental industry (encompassing water purification, filtration, and pollutant absorption), the food industry (encompassing additives and packaging), and the textile and cosmetics production sectors. Of recent note, it has come to light that bioactive glasses, characterized by their silicate, phosphate, and borate compositions, exhibit the capacity to induce the expression of critical genes, such as VEGF for angiogenesis, bFGF, fibronectin receptors, enhance skin cell activity, and augment collagen production during the wound healing process.

Conclusion

Inadequate protection of wounds from external mechanical stress, inadequate management of wound secretions, insufficiency of proper wound surface moisture, microbial contamination, and infection all impede the wound healing process. These factors not only render wound healing conditions challenging but may also, in severe cases, culminate in fatality due to severe infection. As a pristine biomaterial, Bacterial cellulose has garnered substantial attention due to its unique attributes.

Moreover, the diminutive size of its fibers relative to those of plant cellulose has augmented its appeal significantly. This inherent versatility positions bacterial cellulose as a compelling candidate for application in the medical domain, where it can serve as a biological material in tissue engineering, the fabrication of wound dressings, and drug delivery systems.

Hence, wound dressings crafted from bacteria cellulose treated with bioactive glass, notable for its affinity with both soft and hard tissues, have been subject to rigorous assessment. The outcomes of these evaluations have revealed remarkable biocompatibility, pronounced antimicrobial properties, adept wound secretion management, effective moisture retention, non-toxicity, commendable tensile strength, and impressive flexibility. Furthermore, in vivo experiments conducted on rats substantiate the conceptualization and efficacious functionality of this wound dressing in the context of skin tissue regeneration, expediting the wound healing process and notably diminishing the wound surface area in the cohort that received this specialized wound dressing.

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Conflict of Interest Disclosures

The authors declare no conflicts of interest.

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Authors' Contributions

Concept: M.N, SJ. D, Design: M.N., Analysis or Interpretation: M.N, B.B, Writing: B.B

Ethical Statement

All applicable international, national, or institutional guidelines for the care and use of animals were followed. The research protocol obtained ethical approval from Baqiyatallah University of Medical Sciences, designated by the code IR.BMSU.AEC.1399.00.

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