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Biocompatibility Study with Baby Hamster Kidney Cells on Acetobacter Xylinum Pellicle for Diabetic Foot Laceration

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ABSTRACT

Diabetes mellitus, commonly known as diabetes, is a persistent metabolic condition marked by elevated blood glucose levels. This condition's global prevalence has been consistently increasing which lead to various complications, including impaired wound healing. Diabetic ulcers arise from a convergence of elements, encompassing hindered blood circulation, neuropathy (nerve impairment), and weakened immune reactions triggered by elevated blood sugar levels. These ulcers typically manifest on the feet and lower limbs. This research fabricated the *Acetobacter xylinum* pellicle as hydrogel membrane for diabetic foot laceration. The cytotoxicity test was carried out using the MTT assay method to determine the cytotoxicity level of hydrogel membranes made from *Acetobacter xylinum*. Moreover, the swelling test was done to support the result. The findings of this study revealed that the dehydrated hydrogel membrane derived from bacterial cellulose and processed through oven drying exhibited a toxicity level of less than 50% across all samples. Additionally, the swelling test indicated a rise in water absorption when the membrane was combined with collagen material.

1. INTRODUCTION

The high prevalence of diabetes has made it one of the significant challenges in global health. Besides causing blood sugar-related issues, diabetes often impacts various organs, including the skin. Diabetes wounds are a common complication faced by diabetes patients, especially in the advanced stages of the disease. These wounds typically appear on the feet or other body parts and have a high level of difficulty in healing. It also can become serious problems that threaten lives if not handled properly.

A person with diabetes faces a 15%–20% chance throughout their life of developing a diabetic foot ulcer [1]. Keeping wound sites moist is vital and presents a challenge in wound management. The contemporary approach to wound healing has shifted from a dry wound bed to a moist wound healing model, making balanced moisture the optimal condition for tissue regeneration [2].

Research on the use of materials with high moisture levels, such as hydrogel or gel membranes, in addressing necrotic wounds or ulcers, has become an interesting focus of study. Several previous studies have experimented with various types of materials for this purpose, including hyaluronic acid supplemented with antibacterial compounds such as Zinc Oxide Nanoparticle (Zn NPs) [3]. Apart from hyaluronic acid, some studies have also utilized other types of hydrogel made from materials like collagen, chitosan, and PEG (Polyethylene glycol) and bacteria cellulose [4].

Drawing upon advancements in materials science, scientists have attained impressive command over the characteristics of hydrogels. Recent breakthroughs, Paramadini et al., 2015 and Angtika et al., 2018 in biofabrication techniques have enabled the creation of intricate hydrogel structures, mimicking native tissue architectures for applications in tissue engineering. That research fabricated *Acetobacter xylinum* pellicle which turned into Bacteria Cellulose (BC) membrane and tested the mechanical properties. However, they have not studied its biocompatibility yet [5], [6].

According to another research, BC is truly remarkable material that holds great promise in the field of tissue engineering. It is a type of cellulose produced by certain bacteria through a fermentation process. The unique structure of bacterial cellulose, composed of an interconnected network of nanofibers, closely resembles the extracellular matrix found in natural tissues. This similarity makes it an excellent candidate for tissue engineering applications [7]. It also offers several advantageous properties, including its exceptional biocompatibility, high water-holding capacity, and mechanical strength. These characteristics make it ideal for promoting cell adhesion, proliferation, and tissue regeneration [8].

Moreover, collagen variation represents essential protein found in the human body and plays a significant role in the structure of skin tissues. As a biodegradable material, both bacteria cellulose and collagen also eliminates the need for surgical removal after transplantation, reducing potential complications. With ongoing research and advancements, bacterial cellulose continues to show great potential in revolutionizing tissue engineering approaches, paving the way for improved therapies and regenerative medicine [5], [9].

This research will focus on the biocompatibility properties of bacterial cellulose combined with collagen through the immersion method. The assessment of biocompatibility plays a vital role in determining the safety and efficacy of wound dressing materials for medical purposes. It is imperative to guarantee that the chosen materials for wound dressings do not provoke harmful responses or hinder the natural wound healing process during their design and development. Referring to previous studies, this research is able to provide insights regarding biological testing methods on hydrogels using BHK Kidney cells.

2. METHOD

The study carried out in this research employs a solidly established experimental approach, with the goal of producing practical observations and cultivating a more profound comprehension of the topic at hand. By employing a well-organized series of controlled protocols and adjustments, our team of researchers proactively intervened in both the creation of hydrogels and the assessment of their potential toxicity.

Preparation of Pellicle

The process commenced by creating bacterial media from coconut water. Next, 300 mL of filtered coconut water, along with 1 gram of urea and 2.5 grams of sucrose, was subjected to heat until it reached the boiling point. Afterward, the cultured media was transferred to a fermentation container and allowed to cool. Once cooled, Acetobacter xylinum bacterial starter was introduced, initiating a 6-day fermentation period. The resulting pellicle was immersed in 1 mol/L NaOH at 80°C for 1 hour and subsequently boiled multiple times using deionized water [5], [10]. The sample then immersed in collagen solution for about 6 hours [5].

Cytotoxicity Test

The cytotoxicity test was carried out using the MTT assay method. Firstly, fibroblast cells were taken from liquid nitrogen storage (198°C) and immediately incubated in basic medium for 24 hours at 37°C. Next, the cells were washed with PBS and added with 1 m EDTA to loosen the cell attachment in the bottom of the dish. 80 µl of cell solution, 10 µl of FBS, and 10 µl of trypan blue in a 1.5 ml Eppendorf tube, the solution in the tube was transferred to a glass hemocytometer board. Cell count will be carried out on a glass board under an optical microscope with 40x magnification. The hemocytometer glass has a cell counting section which can be counted under an optical microscope.

Cells with a density of 2x 105 were included in 100 µL of eagles medium transferred into a 96-microwell plate according to the number of samples and controls. The sample is then put in a 96-microwell plate as much as 50 µL, and incubated for 24 hours at 37˚ C. After being incubated for 24 hours with the eagle media sample on the well plate, discard it, then wash it using PBS until the rest of the sample is removed from the well plate. The incubation stage was continued by administering 1 µl of MTT solution to each well plate which was immediately incubated again for 3 hours. Add 50 µL of

DMSO to MTT reagent to stop the reaction between MTT and BHK-21 cells. DMSO is added to the Microwell plate. DMSO was added to each well and then centrifuged at 30 rpm for 5 minutes.

After the centrifugation process, the optical cell density can be calculated using a tool commonly called Elisa Reader [11]. Equation 2.2 is used to calculate the percentage of living cells. According to Spielmann (2007), a material is said to be toxic if the percentage of living cells is stated to be less than 50%.

$$\% \text{ Living cells} = \frac{\text{AT} - \text{AM}}{\text{AUTl} + \text{AM}} \times 100\%$$

AT = Absorbance of the treated cell culture
AM = Absorbance of the media control
AUT = Absorbance of the untreated control

Swelling Test

Measurement of absorption rate: The membrane was weighed with an electronic balance, soaked in Saline Body Fluid (SBF) to dry quickly and weighed after 4 hours. The absorption rate (the average value of the six data in each group) is the accumulation according to the absorption rate according to equation 2.3 [12].

$$\%\text{Absorpsi} = \frac{\text{Post hydrating mass} - \text{Pre hydrating mass}}{\text{Pre hydrating mass}} \times 100\%$$

3. RESULTS AND DISCUSSION

Cytotoxicity Test

The cytotoxicity test assessed the degree of material toxicity towards living cells. This test conducted using the MTT Assay method and involved culturing BHK-21 (Baby Hamster Kidney) cells. The cells then incubate for 24 hours to obtain the visible result. The resulting cell cultures are then analyzed using an Elisa Reader to determine the percentage of viable cells in each treatment.

` Various factors can influence cell growth in a culture, including the growth media, environmental conditions. MTT Assay results affected by cell density, incubation time, and media composition [13]. To promote optimal cell growth, the culture requires a pH of 7.4, while the environmental temperature must be carefully maintained to ensure stability.

Table 1. Cell Viability Percentage Data

Sample	Optical Density (OD)	Cell Percentage (%)
Bacteria Cellulose (BC)	0,600	15,80%
BC-Collagen 0,4%	0,127	29,06%
BC-Collagen 0,5%	0,132	29,78%
BC-Collagen 0,6%	0,114	40,09%
BC-Collagen 0,7%	0,101	37,19%

Table 1. revealed the outcomes of the toxicity test for various samples. The SB sample displayed a percentage of living cells of 15.80%, while SB-Collagen 0.4% showed 29.06%, SB-Collagen 0.5% recorded 29.78%, SB-Collagen 0.6% exhibited 40.09%, and SB-Collagen 0.7% displayed 37.19%. Notably, all samples demonstrated a percentage of living cells below 50%. This could be attributed to the hygroscopic nature of bacteria cellulose, leading to water absorption, and consequently, adherence of the culture cells to the samples occurred [14].

Based on the research findings, during the counting process using the Elisa Reader, the sample absorbed the media. It was leading to a false negative indication where some cells were not detected. In line with the study conducted by De Olyveira (2012) related to bacterial cellulose's cytotoxicity, it was observed that cells tended to grow abundantly on the membrane's surface. However, it should be noted that these cells would be removed later on after washing [15].

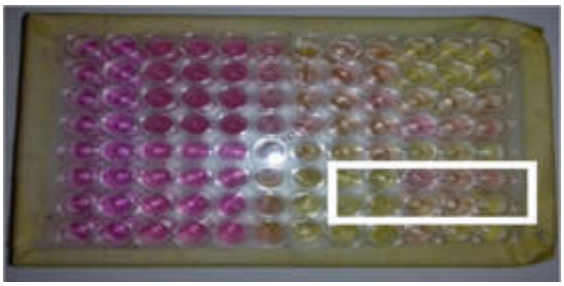

Figure 1. *Well-plate* with dominant purple color

Per the MTT Assay test guidebook from the American Type Culture Collection, several potential errors (troubleshooting) may arise during the MTT treatment. One commonly encountered issue in Table 4.4 is a low reading. This problem often occurs when the incubation time is insufficient, leading to a lack of dominant purple color when analyzing the quality using a light microscope. In some cases, certain cells may even necessitate a longer incubation period beyond 24 hours. Furthermore, this research emphasizes the need for further observations in the cytotoxicity test on bacterial cellulose. [15].

Swelling Test

The objective of the swelling test was to evaluate the sample's liquid absorption capacity. This test involved immersing all five samples in a solution of Simulated Body Fluid (SBF) for 1 hour to assess the SBF absorption properties of each sample. The intention was to determine whether the cytotoxicity was related to sample's swelling ratio. Upon analysis, the control sample demonstrated a swelling ratio of 59.86, while sample A exhibited a ratio of 60.547%. Sample B and Sample C both showed a swelling ratio of 74.34%, and Sample D had a swelling ratio of 59.87%.

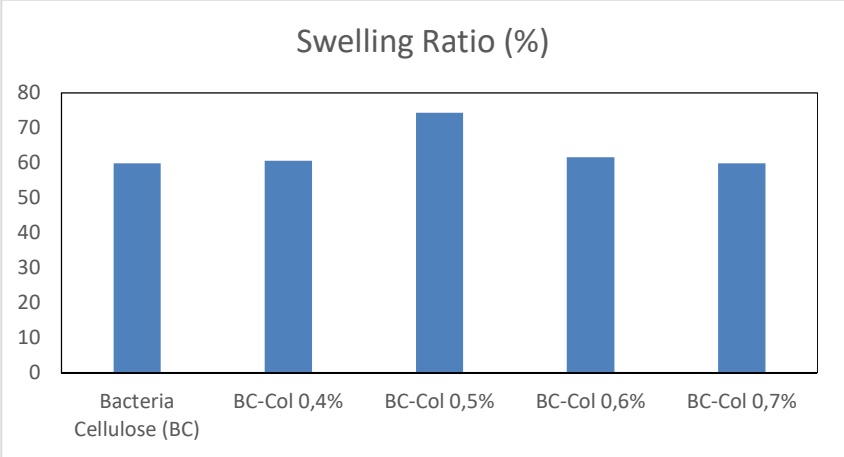

Figure 2. Swelling Ratio

The swelling test calculations revealed that the four treated samples exhibited a greater swelling ratio than the control sample. The introduction of collagen led to an increase in the swelling ratio, as depicted in Figure 2, owing to the abundant presence of hydroxyl groups, which greatly enhanced their hydrophilic properties [16]. This is consistent with the MTT Assay results, which state that the sample has a substantial water absorption rate, allowing it to absorb cell culture media.

4. CONCLUSION

This research concludes that the drying method using an oven needs to be reevaluated. Effective drying is required for bacterial cellulose hydrogel membrane samples made from Acetobacter xylinum pellicle. The results show that the cytotoxicity level has not been optimized yet even with the optimum drying process using oven and addition of collagen. Additional materials such as collagen affect the

hydroxyl bond of the material. Further research is needed to find the appropriate method to enable cell culture media. Nonetheless, this study can contribute valuable insights into using Acetobacter xylinum as a wound dressing material for diabetes-related conditions.

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