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THE USE OF ELECTRICAL MICROCURRENTS IN TISSUE HEALING THROUGH MICROBIAL FUEL CELLS

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Abstract: In Brazil, more than fifteen million people are affected by diabetes, according to a survey by the Federal Council of Medicine. Diabetic wounds are one of the most critical consequences of this disease, with the main risks being tissue necrosis and limb amputation. In this sense, electrotherapy has gained a prominent role in tissue healing, mainly with the use of microcurrents, direct electrical currents of up to 1000 µA. Electrical stimulation through microcurrents triggers biochemical effects in biological tissues, which can restore tissue bioelectricity, stimulate protein and adenosine triphosphate synthesis, increase membrane transport of amino acids, as well as fibroblast synthesis, helping in tissue recovery injured. In this sense, this article sought to develop a treatment protocol, based on the use of electrical microcurrents, in the healing of chronic injuries, with emphasis on diabetic wounds. In order to safeguard the sustainability and financial accessibility of the electrotherapy treatment, the electricity used for the study was originated from a Microbial Fuel Cell. Microbial Fuel Cells (MCC) constitute an electrochemical system based on the degradation of organic compounds by a biological agent and the conversion of chemical energy into electrical energy. The CCM was adapted by the authors with accessible materials, reducing the impacts of greenhouse gas emissions. Using it provided an intensity of 2 to 10 microamperes, ideal for research and falling within the classification of electrical microcurrents. Therefore, the United Nations Sustainable Development goals were achieved in consolidating health and well-being, in addition to generating renewable and clean energy, through the Microbial Fuel Cell.

Keywords: MICROBIAL FUEL CELLS, HEALING, MICROCURRENTS.

INTRODUCTION

Worldwide, every 20 seconds, a lower limb is amputated due to complications from diabetes (IWGDF, 2019). The difficulty in healing resulting from diabetes, especially the development of diabetic wounds, is one of the consequences that most impacts the quality of life of patients affected by the disease, with high incidence rates, reducing the quality of life of patients and generating socioeconomic impacts. for family members and the healthcare system itself (OLIVEIRA et al., 2019). In this sense, electrotherapy has gained ground in the recovery of tissues damaged by chronic wounds. According to BORGES (2006), electrical stimulation through microcurrents can trigger biochemical effects in biological tissues, which can restore tissue bioelectricity, increase the permeability of cell membranes and the transport of amino acids, in addition to assisting in protein synthesis. Furthermore, after in vitro and in vivo analyses, it was confirmed that microcurrents accelerate protein and adenosine triphosphate (ATP) synthesis and increase membrane transport of amino acids, helping the recovery of injured tissue (CHENG, 1982).

Most of the studies compiled by KLOTH (2005) are restricted to in vitro applications, or applications with higher electrical current intensities, with high-cost resources. In this sense, this research sought to develop a treatment protocol with low-intensity microcurrents, in an accessible and sustainable way for patients with chronic diabetic wounds. To this end, a Microbial Fuel Cell (CCM) was developed, a device that operates similar to a conventional battery, which guarantees autonomous, accessible and sustainable electrical energy generation, appearing as an alternative capable of being applied for electrotherapeutic treatments. Added to this, the clean nature of CCM contributes to its suitability for the project, as it fits as a low-cost renewable energy solution, taking into consideration, the unsustainable use of fossil fuels in the world and climate change generated by pollution resulting from of non-renewable energy sources. This way, CCM also contributes to the solution of two Sustainable Development Goals (SDGs) of the United Nations: SDG 3, healthy life for the population, improving their quality of life; and SDG 7, creation of new renewable energy sources.

Thus, according to TICIANELLI; GONZALEZ (1988), CCMs constitute an electrochemical system responsible for the energy conversion of chemical compounds into electrical current. Based on the theoretical Fuel Cell model, such a device was developed in a completely sustainable way and with accessible materials, implementing its use for electrotherapeutic purposes, generating microcurrents through the brewer's yeast Saccharomyces cerevisiae for the degradation of organic matter. Therefore, over the course of ten weeks, the electrical production of the CCM was monitored and used for certain necessary electrotherapeutic purposes, reaching productivity of 2 to 10 microamperes.

With the relevance of the project, a Case Report procedure was developed, through a partnership with the General Hospital of the Army of Salvador, for chronic diabetic wounds, and more specifically, treatments for the diabetic foot. Soon, we intend to expand the scope of the research to carry out the clinical testing period with the Case Report modality.

MATERIALS AND METHODS

The research was divided into two methodologies: one related to the assembly of the CCM, and the other related to the application of the electrical microcurrents produced. In general, the Fuel Cell is made up of two interconnected poles, one positive and one negative, which are responsible for establishing the difference in electrical potential and allowing the passage of microcurrents. Furthermore, one of these poles, the anode, must contain a microorganism capable of promoting the release of electrons, which will be directed to the cathode. Therefore, for the first prototype built, it was decided to use Saccharomyces cerevisiae brewer's yeast as biological material, due to the fact that, according to OLIVEIRA et al. (2018), has high energy productivity, an optimal growth temperature close to the environment, is non-pathogenic, has an affordable cost and can be kept in a solid state for a long time. Added to this, its ease of obtaining was one of the decisive factors in favor of using yeast instead of bacteria and other microorganisms that are less accessible.

In order for the yeast to carry out its alcoholic fermentation and release electrons as a product, a substrate suitable for its proliferation was defined on the CCM anode, based on its concentration in carbohydrates and its accessibility, since it was desired to preserve the affordable character of the Fuel Cell. Therefore, we chose to use humid soil, given that it is rich in organic matter, nutrients and inorganic molecules, such as water, and provides ideal conditions for the fungus to decompose the material and its consequent fermentation, as well as It is quite abundant in arboreal locations in the most diverse regions of the planet.

Finally, for the cathode, it was also necessary to use a solution that would encourage the passage of electric current when the two poles were joined. Therefore, it was decided to add a mixture of mineral water and sodium chloride (NaCl), which, when dissolved in an aqueous medium, releases Na^+ and Cl^- ions, forming an electrolyte solution that is a good conductor of electricity.

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When the internal components of each CCM container were defined, the external structure of the first prototype was built, consisting of simpler and homemade materials, in order to attest to the accessibility of the project. For the poles, two plastic boxes were used, connected by a polyvinyl chloride (PVC) pipe, which represents a salt bridge. In the case of CCM, the function of this structure is to maintain the stability of the device and represent an alternative to the traditional and less accessible proton transport and semi-permeable membranes already used in Fuel Cell models built in the past. It is worth noting that, according to LEHNEN (2014), such components can be replaced without energy losses by a salt bridge. This way, the PVC pipes were filled with NaCl to make them saline and copper wires were added to the plastic containers, so that they came into contact with their respective solutions and allowed the passage of electric current. With the structure completed, the free electrons released at the anode with the fermentation carried out by the yeast are captured by the copper wires and, due to the presence of an electrolytic cathode, taken to their ends.

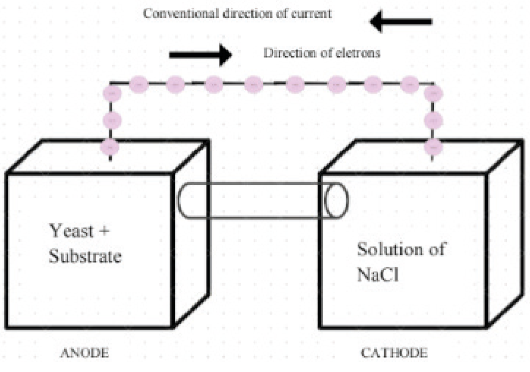


Figure 1: CCM model, analyzing the passage and direction of the electric current. Source: Prepared by the authors: ARARIPE, REIS & SANTOS, 2023.

Figure 2: Image of the CCM prototype, developed by students at Colégio Militar de Salvador (CMS), BA, Brazil. In the container with a green lid, the substrate containing the yeast, humid soil and other organic compounds. In the container with a yellow lid, a solution of mineral water and NaCl dissociated in water. Source: Prepared by the authors: ARARIPE, REIS & SANTOS, 2023.

In total, 220ml of mineral water, 100g of humus soil, 11.5g of yeast and 36g of NaCl were used, which allowed the production of microcurrents of 0.08 V, corresponding to 80 mV. With this positive result, a second prototype was built with more sophisticated materials and larger quantities of components to try to increase the production of microcurrents and the biosafety of the CCM. In this, the construction methodologies were maintained, as well as the use of yeast as a biological agent, humus soil and saline solution for both the salt bridge and the cathode.

The first change was the replacement of plastic containers with two water purifying filters without their inner part, resistant and opaque. These maintained the purpose of storing the components of each pole, however, this time, with a more suitable seal for the application of the prototype in a hospital environment. Furthermore, as the volume of the filters was greater than that of the plastic containers, the quantities of components could be increased according to initial planning. Finally, it is still worth highlighting that the non-transparency of the filters is one of the factors that contributes to the well-being of patients who undergo treatment, since they do not see the decomposing organic matter, which could not be pleasant.

The second change was the addition of coconut water to the humus soil at the anode to together constitute the yeast substrate. Due to the fact that coconut water is a natural source rich in glucose and is very accessible in Brazil, especially in Bahia, it was considered an alternative to enhance the supply of sugars for yeast. Another relevant change was the choice of a specific typing of the *Saccharomyces cerevisiae* used: Ca-11 yeast, as it was recommended for small productions and could provide better energy use.

Furthermore, the purifying filters were adapted to allow the exit of carbon dioxide (CO2) released as a product of the alcoholic fermentation carried out by the yeast. Therefore, an aquarium hose was used, positioned at one of the anode outlets to drain the gas. Even though it was not hermetically closed, the CCM did not suffer any risk of external contamination, since the curved positioning of the hose prevented the entry of foreign microorganisms from the atmospheric air.

It is also worth noting that, with the replacement of plastic containers with purifying filters, the PVC structure that previously constituted the salt bridge also underwent changes. In the new prototype, the connection between the two poles was established by a second aquarium hose connected to the free ends of the cathode and anode. This was filled with the saline solution, as well as closed with small cotton balls at its ends so that the contents were retained in the tube. Finally, the structure was covered with tape to provide greater support. Furthermore, in order to facilitate the transport of the CCM, a metal support was adapted, with circular branches to allow the two filters to be coupled to it.

Another change made to the prototype was the use of an aquarium pump to oxygenate the anode and boost electrical productivity. Even with fermentation occurring in the absence of oxygen gas, the aeration process, commonly used for the fermentation of beers and wines, aims to adequately prepare the microorganism, adapting it to the temperature and allowing its reproduction to occur. Furthermore, it was made sure that the object was battery-powered to preserve autonomy for energy production. This way, aerobic preparation is carried out before the fermentation process, increasing CCM productivity.

Finally, it was also decided to use alligator clips connected to the ends of the copper wires, in order to facilitate the application of microcurrent treatment when these occurred. Therefore, such structures would be connected to disposable electrodes, which, in turn, would be installed at the ends of the patient's wound, thus allowing the passage of electrical current. In this prototype, in total, 1L of mineral water, 500ml of coconut water, 375g of humus soil, 50g of yeast and 72g of sodium chloride were used, divided equally between the cathode and the salt bridge.

With the new prototype, the deduction that there would be an increase in electrical productivity was proven, since the CCM, over the course of two hours, was able to generate electrical microcurrents of electrical voltages and maximum intensities that varied from 0.1 V (or 100 mV) and 1 μA up to 0.14 V (or 140 mV) and 2 μA. After creating comparative tables between case reports from research by KLOTH (2005) and GOLDMANN; POLLACK (1996), it was realized that such values could already be sufficient to demonstrate results with the application of microcurrents.

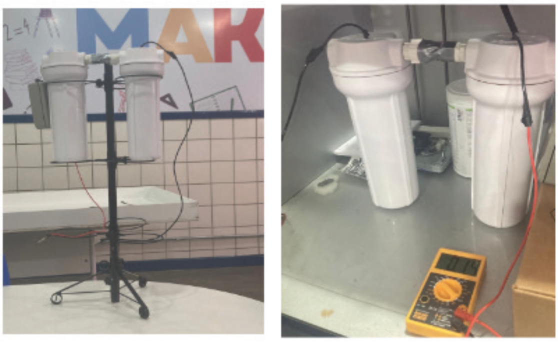


Figure 3: Images of the final prototype of the Microbial Fuel Cell, developed by students at Colégio Militar de Salvador (CMS) BA, Brazil, made with two purifying filters, and measurements obtained from the multimeter being tested on the prototype, developed by students at Colégio Militar de Salvador (CMS), BA, Brazil. The result shown on the multimeter display represents the electrical voltage corresponding to 0.14 volts. Source: Prepared by the authors: ARARIPE, REIS & SANTOS, 2023.

However, after the analysis period, the aim was to optimize the resources used. Therefore, it was necessary to reduce the stipulated measures to adapt them to the capacity of the filters and guarantee the biosafety of the CCM. The adapted quantities were determined experimentally, considering the volume of the containers, the fermentation capacity of the yeast and the best use of resources to drive the electrochemical process.

Materials	Quantity
Mineral water	900 mL
Coconut Water	350 mL
Substrate (humiferous soil)	470 mg
Yeast: *Saccharomyces Cerevisiae* (Ca-11)	25 g
Sodium Chloride	90 g

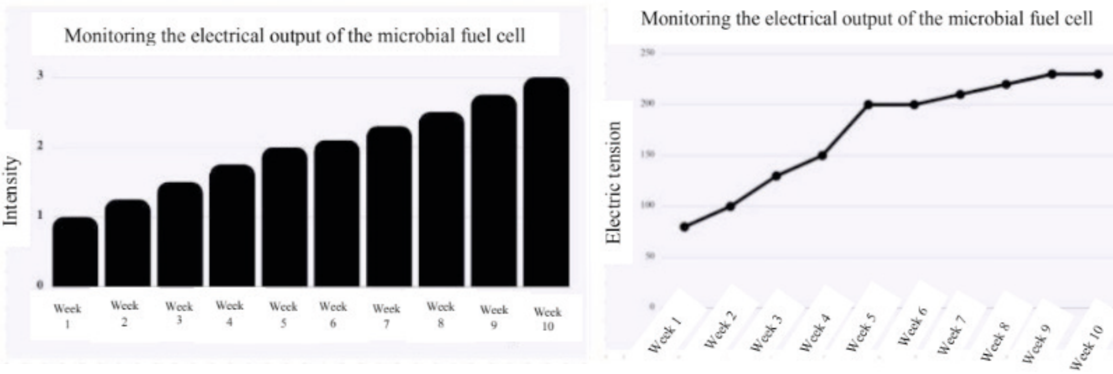
Table 1: Materials and quantities used in the CCM Definitive Model in the second test.

Source: Created by the authors: ARARIPE, REIS & SANTOS, 2023.

With this second test, microcurrents of voltages between 0.14 and 0.22 V and intensities of 2 to 5μA could be generated, fitting the specific standards sought by the study of the effects of microcurrents on human beings. The electrical production of the Microbial Fuel Cells was monitored for around 15 weeks in total, with the first 10 being evaluated periodically and with statistical analysis of the measured data. Intensity and electrical voltage values were defined through a weighted average of measurements obtained over 3 days each week analyzed. The compilation of all this data was carried out through tables and graphs shown on the following pages.

Based on them, another advantage of the final CCM prototype was noted: its durability. From the fifth week of monitoring the CCM supplied with the defined amounts of yeast and substrate, the electrical microcurrents remained stable or reached higher levels of intensities and electrical voltages. Three main reasons were attributed to this phenomenon: the long time the yeast was exposed to the substrate, which stimulated fermentation; aeration to favor the reproduction of microorganisms; and the implementation of coconut water to degrade glucose, expanding the fermentation process.

The second methodology developed, related to treatment with microcurrents, was based on studies on electrical healing properties compiled by KLOTH (2005). The healing process results from a series of metabolic events in the cell so that the injured tissue can restore itself, requiring an anatomical, histological, biochemical and immunological analysis of the human body. It is, therefore, a set of complex phenomena that aim to restore the morphological integrity and functionality of any injured tissue or organ. It consists of a coordinated chain of cellular and molecular events that interact so that



Graphic 1: Quantitative analyzes of the intensity of electrical current and electrical voltage in mA and µV, respectively, achieved by the Fuel Cell during the 10 weeks of monitoring. Source: Prepared by the authors: ARARIPE, REIS & SANTOS, 2023.

tissue reconstitution occurs, which can be subdivided into five main stages: coagulation, inflammation, proliferation, contraction and wound remodeling. According to ARANTES et al. (2018), electrical microcurrents are those that lie in the range of 0.5 to 1000 µA, while for GUIRRO (2002), this value does not exceed 900 microamperes. Despite this conceptual uncertainty, according to BORGES (2006), electrical stimulation through microcurrents can trigger biochemical effects in biological tissues, being able to reestablish tissue bioelectricity, increase the permeability of cell membranes and the transport of amino acids, in addition to assisting in the synthesis protein. As a result, the five stages of healing are affected by the electrical stimulus driven by the application of microcurrents, since the chain reaction caused by them allows for increased interaction between the nervous system and the epithelial and connective tissues present in the skin. For this analysis, a protocol for this treatment was developed, based on in vitro studies and the energy availability of the Microbial Fuel Cell.

However, the research decided to innovate in the use of microcurrents. The application plan with microcurrents promoted the use of very low intensity and electrical voltage, with the aim of maintaining the painless nature of the tests, as well as gradually evaluating the electrical effects of lower intensity currents on human beings, which had not been done before. analyzed with measurements from 2 to 10 microamps, nor did they take advantage of the original Microbial Fuel Cell model developed by the team.

Next, the ideal patient profile was stipulated: all those who had at least two chronic or difficult-to-heal wounds, in a necrotic state and/or bullous appearance, such as diabetic injuries, generalized venous wounds and burns of second and third degree. The presence of multiple lesions is essential to guarantee the effectiveness of the tests, since, in these conditions, it is possible to apply electrotherapy to just one wound and observe its evolution in comparison with the other untreated wound. It is also essential that the lesions are from the same patient so that it is possible to eliminate interference from other factors in the results, such as genetic differences. Furthermore, the study analysis modality chosen was a non-randomized controlled case report, which allows a better evaluation of the results for each patient to be captured. According to the plan developed, the treatment of patients will take place from one to four months, with weekly sessions of 45 minutes of application of microcurrents within the stipulated intensity and voltage range.

The advancement of research aroused the interest of the Salvador Army General Hospital (HGeS) in starting a clinical test project, contributing to the infrastructure and eligible patients. The research was submitted to two ethics and safety panels at the institution itself to ensure the scientific study was carried out. The panels examined and evaluated the following aspects of the research: the biological safety of the prototype, the theoretical basis used in the studies and the feasibility of the research according to the profile of HGeS patients.

After the hearing and approval of the project by the ethics panel, the program presented by the hospital compatible with the research was the Home Care Program (PAD), in which the majority of patient cases fit the stipulated profile. While the testing period has not started, SENAI-CIMATEC Bahia (National Industry Learning Service) showed interest in evaluating the effects of microcurrents in an in vitro analysis, proposing a partnership to monitor patient treatment, analyzing cellular evolution of each one. This will be the next stage of the methodology, which will effectively conclude the research studies, together with the support of HGeS.

RESULTS

After the effective construction of an accessible CCM model, it was concluded that different modalities of Microbial Fuel Cell can be realized with accessible materials, generating low intensity energy. The long exposure to the substrate and the addition of coconut water allow the Cell's productivity to increase, which can be adapted for different types of use, from home to hospital environments, from simple to more sophisticated materials. Its peak productivity, reaching 10 microamperes, was fundamental for its use in the electrotherapy field.

This way, the prototypes built were able to successfully convert the chemical energy of the fermentation carried out by the yeast Saccharomyces cerevisiae into electrical energy, within the range of intensity and electrical voltage stipulated as ideal based on the compilation of studies carried out by KLOTH (2005): 0.14V to 0.22V and 2μA to 5μA. Thus, satisfactory results were observed that allow the expansion of bioelectricity to the most diverse areas of activity, as well as the replication of the constructed model.

Furthermore, regarding the microcurrent application plan, its approval by both HGeS and SENAI/CIMATEC reveals a positive result that reflects its viability. Finally, it can be stated that the objectives of generating bioelectricity in a sustainable and accessible manner were achieved, as well as presenting an innovative solution to the difficulty of healing in patients, especially diabetics, which also contributes to the solution of SDGs 3 and 7.

FINAL CONSIDERATIONS

The high visibility of this project raised interest in the partnership with SENAI-Cimatec Bahia, seeking to analyze the effects of microcurrents on patients with diabetes at the cellular level. With the current relevance of the work, a partnership proposal was presented between the authors of the project and the medical wing of SENAI, responsible for the development of in vitro techniques, which will be the final part of the project: the cellular analysis of how microcurrents would act in the human body, simulating the conditions of stress and metabolic weakness of the cell.

Furthermore, it is expected, with the approval of the research by the Research Ethics Board, that patients will be recruited for the study, through the partnership with HGeS. Thus, the effects of electrical microcurrents on living beings will be evaluated, and with the partnership with Senai-Cimatec, the in vitro results of microcurrents will be monitored. Furthermore, the aim is to disseminate the new method demonstrated through the Microbial Fuel Cell in the production of electrical microcurrents. This way, the union of energy generation by CCM and the healing properties of microcurrents has a high chance of being increasingly disseminated in the scientific community.

It is expected that, through this project, it will be possible to expand this type of treatment, as well as more in-depth analyzes of this electrotherapy model, with the aim of safeguarding the environmental balance, generated by the non-polluting nature of the Microbial Fuel Cell, the balance social, generated by the treatment of wounds carried out with electrical microcurrents, which improves the quality of life of the population, in addition to the economic balance, generated by the accessibility of the developed prototype, thus intensifying the process of transition of energy matrices and providing progress in the medicinal field.

As a final analysis of this project, it allows, in the future, to achieve the objectives of implementing electrotherapeutic treatment in human tissue, analyzing the effects of microcurrents on tissue recovery from diabetic wounds, in addition to proving the effectiveness of producing electrical energy through Microbial Fuel Cell.

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