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COVER: Aspergillus sp. from a seat car. Photo: istock gallery.

#### **Bench Tests: Activities Report**

Arthur Vianna Dias da Silva Brim<sup>1\*</sup>, Gustavo Moura Costa<sup>2</sup> <sup>1</sup>EMBRAPII; <sup>2</sup>SENAI CIMATEC Universitary Center; Salvador, Bahia, Brazil

Laboring with electronics involves difficulties and sources of error, which sometimes causes the malfunction of the equipment. It is necessary to carry out inspection activities to locate points of uncertainty, isolate them and test the system without their interference to recognize a source of non-conformities and solve it. The article presents the activities of electronic systems enclosed in a prototype, describing the faults detected and the improvements implemented.

Keywords: Electronics. Tests. Inspection.

#### Introduction

Sensory deprivation tanks applied in restricted stimulus control therapies were conceived in 1954 by John C. Lilly, a physician, and neuroscientist engaged in the origin of consciousness [1]. The EMBRAPII Floatation Tank project designed the construction and testing of a modern flotation tank, applying modern communication and control systems for the elements inside and outside the capsule.

There are many steps to approve equipment. The bench tests are the ones of these steps. It consists of a simple activating of the components to assure that what the supplier points out corresponds to reality. Energizing the equipment and performing a visual inspection of its operation is usually sufficient for this type of activity, in the case of commercial items. Occasionally it may be necessary to use a bench source [2] or multimeter [3] to measure the electric current or voltage applied to the equipment.

For systems developed within the scope of research, the procedure acquires a more detailed aspect since it should be guaranteed all operating requirements contemplated. These requirements

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are usually but not limited to power dissipation, operating voltage, response time, maximum current [4,5].

This study aims to approach the inspection and validation of the chromotherapy circuits and prototype load activation of the Floatation Tank project.

#### **Material and Methods**

The analysis procedure begins by consulting the circuit diagram in question. It is possible to determine the scope of the circuit and the operation condition through the guidelines and the designer's comments. Once these points are delimited, a test bench is setting up using a voltage source to supply power to DC circuits or a safety relay to AC circuits.

Then, the circuit is activated to inspect it, following the guidelines. In case of failure, the first step is to ensure that the command signal reaches the destination terminal. Afterward, you can examine other sources: component power, solder quality, the material quality of the component. Another applicable measure is to review the datasheet or the report technique applied to the element to ensure that the model designed in the project matches reality.

If none of the above measures show results, the component must be replaced by a new one.

#### **Results and Discussion**

The RGB LEDs for chromotherapy are inspected first. Next, there is the schematic for

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activation [Figure 1]. Through it, a command must activate the MOSFET [6] to connect the GND signal to the LED, activating a green color. The same circuit is repeated for the red and blue colors. A MOSFET6 is an electronic switch controlled by voltage, controlled by a command. It allows the passage of current (without the command there is no passage) [4,5].

#### Figure 1. MOSFET key.



The bench tests proved the luminous intensity of the equipment, as well as the possibility of controlling which of the basic colors is activated (Red, Green, and Blue) and its intensity. A 1-hour floating session was simulated and the lamps did not show negative results.

The following circuit is for low-current AC load drives (Figure 2). During bench tests, improvement points were identified. The specified relay [7] presented tolerance for voltages up

Figura 3. High current activation.





to 110V in the first instance, which makes the system incompatible for bivolt installations. We observed that MOSFET [6] is crucial for the correct operation of the circuit. The system was tested with the coil activation command coming directly from the control unit [8]. However, this unit does not have the current capacity to guarantee the relay energization. Thus, MOSFET [6] acts by connecting the coil directly with the reference from a low current command coming from the controller [8].

The circuit for activating high currents uses a TRIAC as an electronic switch - component indicated in the literature for high current loads and heat dissipation [4,5]. During bench tests, we observed an error in the schematic representation of the MOC3032SM component. The connections



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presented in the schematic were not consistent with those indicated in the technical report. Moreover, during circuit stress tests, the Q605LH5TPcomponent got hot beyond the recommended limit despite the heat dissipation scaling. So, we proposed increasing the heatsink coupled to the electronic key to preventing damage to the component. However, we perceived a space limitation during the assembly procedure of the printed circuit board. Thus, the bench test pointed out that another electronic element is necessary to perform this function.

#### **Final Considerations**

We verified the precise operation of a proposed circuit and identified improvements in another one through bench tests. Therefore, the importance of the bench tests procedure to identify and correct failures without huge impacts on the project becomes evident.

#### Acknowledgements

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#### Extraction of Pectin from Epicarp and Mesocarp Fractions of Cocoa Shell

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Cocoa (*Theobroma cacao L.*) is a fruit widely cultivated in the world, mainly because it is the natural material used in the production of chocolate. Cocoa husk corresponds to approximately 80% of the fruit and is the main residue of production involving this fruit. Therefore, studies have been carried out to reuse the cocoa husk, including the extraction of pectin, which is a soluble dietary fiber present in the cell wall of many fruits. The study aimed to extract pectin from the cocoa husk, presenting parameters such as acid concentration, temperature, extraction from other sources. In the study, the yield of pectin extracted from cocoa husk using 0.086% citric acid at 90°C for 60 minutes was 10.75% for the epicarp and 5.75% for the mesocarp. Keywords: Cocoa Husk. Pectin. Soluble Fiber.

Abbreviations: ABIA: Brazilian Food Industry Association; GDP: Gross Domestic Product; C4: carbon 4; C5: carbon 5; IMC: Iquitos Mixed Calabacillo; SHMP: Sodium Hexametaphosphate; BPP: Banana peel pectin; HCl: Chloridric acid.

#### Introduction

The food industry has a great contribution to the national economy. According to data from the Brazilian Food Industry Association (ABIA) in the 2020 annual balance, the food industry was the sector that obtained 10.6% of the Brazilian Gross Domestic Product (GDP) with investments above 21.2 billion of reais and 64.4% contribution to the country's trade balance [1].

Over the years, the food sector has undergone several changes, one of which is the change in eating habits and the search for healthier and more natural foods [2]. In this scenario, fruits have been the target of several studies and researches to extract nutrients from fruit by-products.

Cocoa is one of the most cultivated fruits in the world. Brazil is one of the largest cocoa producers, occupying the seventh production in the global ranking [3]. In the cocoa industry,

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pulps and seeds are the most used part of the fruit and are destined for other related products. Thus, a large residue of the peel, which corresponds to 80% of the fruit, is discarded [4]. Among the possibilities for reusing the cocoa husk, there is the extraction of pectin, which is a dietary fiber present in the cell wall of many fruits and can be widely used in the food industry as an ingredient, acting as a flavor retention agent, as a hydrocolloid acting as a thickener and stabilizer and adding nutritional benefits to the food [5,6].

Cocoa has three important parts: the fruit itself, consisting mainly of pulp and seed. In addition, the fruit has a layer that covers it externally, called the epicarp, and the mesocarp, which is an intermediate layer considered the most developed part of the fruit (Figure 1) [7,8].

The extraction of pectin can be done through an acidic or basic aqueous medium, or by the action of enzymes. Among them, the most used is with the use of acid as an extracting agent, through acidic hydrolysis, where the breakdown of the glycosidic bond between carbons 4 and 5 (C4 and C5) occurs in which the hydrogen in C5, more acidic in the function of the methyl ester group is attacked by the hydroxide ion (Figure 2) [5, 9]. The extraction steps are summarized as obtaining the shell flour, extraction in an acidic medium, and isolation of pectin.

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#### Figure 1. Cocoa parts.



Adapted from Freepik [8].

Figure 2. Pectin acid hydrolysis reaction.



Adapted from Canteri [9].

As pectin presents itself as an alternative for adding value to solid waste generated by the cocoa industry, associated with minimizing the volume to be discarded, this work aimed to extract pectin from two different parts of the cocoa husk, epicarp, and mesocarp, through acid hydrolysis, comparing the results obtained.

#### **Material and Methods**

The raw material, object of study of this work, was cocoa (*Theobroma cacao L*.) supplied by the company Mais do Cacau, located in Ilhéus, in the south of Bahia (Brazil). For better pectin extraction efficiency, the cocoa husks were submitted to pre-processing to be transformed into a powder. For this, the fruits were received, selected, sanitized, manually cut to separate the skin from the pulp, the skins were dried in an oven at 50°C and crushed in a mill until the powder was obtained (Figure 3). For the extraction of pectin, the methodology of Canteri (2010) was followed with some adaptations [10]. An extracting agent, citric acid, at a concentration of 0.086% in a 1:50 ratio (solute/solvent) was used. Initially, the shell powder was hydrated with distilled water for 10 minutes with stirring. Then citric acid was added and the solution was heated on a hot plate with stirring until reaching a temperature of 90°C. After reaching the extraction time of 60 minutes, the solution was cooled to 30°C in an ice bath and then filtered using synthetic fabric.

The supernatant was filtered and the wet solid was discarded. Then, pectin was precipitated by adding 96% ethanol (1:2 v/v) to the supernatant for 30 minutes at rest under refrigeration temperature.

After precipitation, the pectin was oven-dried at 40°C for approximately 12 hours for yield calculations. Figure 4 [10] contains the flowchart with the pectin extraction steps.









Adapted from Canteri [10].

#### **Results and Discussion**

After the pectin precipitation step (Figure 5), a sample of pectin extracted from the epicarp flour (Figure 6) and cocoa mesocarp, after drying and crushing (Figure 7), was used to calculate the yield (Equation 1). To calculate the pectin yield obtained during the extraction, the following calculation was used:

$$Yield = \frac{\text{extracted pectin}}{\text{flour dough (dry)}} x \ 100 \ (1)$$

The yield of pectin extracted from the cocoa husk in this work, under the conditions mentioned, was 10.75% for the epicarp and 5.75% for the mesocarp. According to Vriesmann (2012) [11], among the polysaccharides present in cocoa husks, 60% are pectins. The study by Barazarte and colleagues (2008) [12], obtained yields between 2.64 and 4.69% of pectin from the cocoa husk, variety Forastero clone IMC 67, coming from the Caucagua zone in Venezuela, using acid extraction. Partially purified pectins extracted from cocoa husks, according to Arlorio and colleagues (2001), [13] had a yield of  $1.29\pm0.08\%$ , through the use of sodium hexametaphosphate (SHMP) solution at 75 °C for 60 minutes. In the study by Mollea and colleagues (2008) [14], the authors realized that the amount of pectins is influenced by the extraction time in which when the time goes from 1 to 2 hours, the extracted amount is doubled (2.0% and 4.0%, respectively.

Commercial pectins are generally obtained from citrus fruit peels and pomace or apple pomace, byproducts obtained after juice extraction by industries. Apple pomace contains 10% to 15% pectins and citrus peels contain 20% to 30% pectins [15]. Other sources of pectins have been studied, such as banana, passion fruit, and mango peels. In the research by Maneerat and colleagues (2017) [16]. Banana peel pectin (BPP) was obtained through an aqueous acid extraction with HCl and water for 30 to 12

Figure 5. Precipitation of pectin with 96% ethanol.



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Figure 6. Pectin obtained from cocoa epicarp flour.

Figure 7. Dry pectin after grinding.



minutes at 90±5 °C and obtained a yield between 7 and 11% of dry basis. In a study with mango, Koubala and colleagues (2008) [17] considered three extraction conditions: HCl, deionized water, and ammonium oxalate and found higher yields with ammonium oxalate and HCl and lower yields with water. According to Canteri (2010) [10], pectins extracted from passion fruit using nitric acid as an extracting agent, the mesocarp fraction

obtained a higher yield with 13.6% of dry base. However, due to the yield and characteristics of the pectins obtained, further studies are needed to improve the properties and yields.

#### Conclusion

The yield of pectin extracted from cocoa husk in this work showed a satisfactory result when compared to pectin obtained from other sources, thus, the work showed to be very promising. Furthermore, this study contributes to the further characterization of pectin extracted through analysis of the physicochemical properties of soluble dietary fiber.

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#### Characterization of Bioactive Compounds in Fruit and Vegetable Bagasse

Fernanda dos Santos Cardoso<sup>1\*</sup>, Ingrid Lessa Leal<sup>1</sup>, Tatiana Barreto Rocha Nery<sup>1</sup> <sup>1</sup>Senai Cimatec University Center; Salvador, Bahia, Brazil

The use of agro-industrial residues is presented in food waste. The processing of them is an opportunity for the development of by-products, as well as the aggregation of lost value, and the sustainable use of these residues. The study aimed to characterize the bioactive compounds in grape, carrot, cocoa, and banana skins from the processing of juice, banana chips, and chocolate. The analyzed samples showed good moisture, satisfactory water activity value, with the highest flavonoid content in the grape sample (1.679 mg EQ/g) and the highest phenolic content in the cocoa epicarp sample (1.367 mg EAG/g). In this way, we verified the viability of using food peels in the food industry, enabling the use of waste generated. Keywords: Food Waste. By-products. Bioactive Compounds.

#### Introduction

The Food and Agriculture Organization of the United Nations – FAO, estimates that the world production of agro-industrial residues reaches 1.3 billion tons per year, since 1/3 of the food potentially destined for human consumption is wasted, either as residues, from processing or as a loss in the production chain [1].

The residues generated by the juice industry, fruit peels, and seeds can represent a loss of biomass and nutrients, in addition to the inadequate disposal causing the pollution of soils and water bodies, causing potential public health problems [2].

Studies show that these residues contain few calories, are a source of micronutrients, fiber and are rich in phenolic compounds and flavonoids in large amounts. These compounds can reduce the risk of cardiovascular disease and some cancers [3]. The use of agro-industrial residues, especially fruit peels and seeds, is an opportunity for the development of by-products, aggregation of lost value, and sustainable use of these residues [4].

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Thus, investigating as a way to minimize this disposal, aiming to know the nutritional quality of by-products generated by food processing industries located in Bahia, such as grape and carrot bagasse from the company PUDJA, mesocarp and cocoa epicarp from the company Mais do Cocoa, and banana peel from Bioalimentos land, this work aimed to analyze the proximate composition and quantify phenolic compounds and flavonoids from fruit residues from different processes.

#### **Material and Methods**

The systematic review selected keywords such as bioactive compounds, phenolics, flavonoids, and related terms. The research focused on the period from 2020 to 2021.

The techniques used to perform the characterization of bioactive compounds were following the literature [5, 6].

We received the bioactive compounds in fruit and vegetable peels (grape, carrot, cocoa, and banana peel), by the Bionutrition Laboratory of SENAI CIMATEC. They went through the milling process and stored in vacuum packaging until the moment of analysis. We collected the moisture and water activity of these products. The physical-chemical assessment was carried out concerning moisture content, using an infrared moisture balance (MOC-120H; Shimadzu), and concerning water activity, we used a decagon (Novasina, Lab Master aw).

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For the analysis of bioactive compounds, extraction was performed by diluting the samples (flour) in 80% cereal alcohol. Afterward, filtration was performed in previously tared vials and the samples were dried in a concentrator (Genevac, DUC 22060 – N00). To prepare the mother solution, the dry material was dissolved with grain alcohol and then the solutions were stored under refrigeration for 24 hours.

The content of total phenolic compounds was determined based on the methodology proposed in the literature by using the sample's mother solution, aqueous folin solution, and sodium carbonate [5]. The absorbance evaluation was performed in a spectrophotometer (Femto, 600 plus) with a wavelength of 765 nm.

The quantifying of total flavonoids was performed according to the methodology described by Meda A. [6]. The origin solution of each sample and the methanolic aluminum chloride solution were used. Absorbance reading was performed using a spectrophotometer (Femto, 600 plus), with a wavelength of 415 nm.

#### **Results and Discussion**

We observed that the carrot peel had the highest moisture value, while the banana peel had the lowest value. Besides, the results are within the standards required by RDC 263/2005 and establish a maximum moisture content of 15% for flour obtained from fruits and seeds [7].

The results also showed low water activity for all analyzed bark samples, indicating values ranging between 0.389 and 0.426. So, we inferred that the flours used have satisfactory water activity since for the development of bacteria pathogens the value found must exceed 0.854 [8].

The phenolic compounds in a plant contribute to the growth and reproduction of vegetables. In food, they are responsible for the color and aroma, whereas, for humans, the consumption of foods rich in phenolic compounds reduces the risk of developing pathologies such as arteriosclerosis and cancer [9].

We found that the Epicarp of cocoa (1.367 mg EAG/g) and the Mesocarp of cocoa (1.088 mg EAG/g) were the husks that presented the highest levels of phenolic compounds. The carrot husks (0.310 mg EAG/g) g) and banana (0.638 mg EAG/g) had both the lowest levels, but still within the average established in the gallic acid behavior graph, which is 0.124 to 2.122 mg EAG/g (Table 1 and Figure 1) [7].

Flavonoids are bioactive compounds that can be found in many foods. In plants, it helps to protect against microorganisms and defend against the incidence of ultraviolet rays. In humans, the consumption of foods rich in flavonoids favors antioxidant reactions which act positively to delay aging and prevent inflammatory reactions, in addition to other benefits [10].

The samples showed that the highest levels of flavonoids found were from grape skins (1.679 mg EQ/g) and banana (0.640 mg EQ/g), while the

[] µ/mL (EAG)	ABS	ABS	ABS	Average	Standard deviation
10	0.124	0.123	0.124	0.124	0.000
25	0.316	0.321	0.320	0.319	0.002
50	0.631	0.625	0.65	0.635	0.011
100	1.143	1.161	1.155	1.153	0.007
150	1.640	1.636	1.636	1.637	0.002
200	2.092	2.125	2.149	2.122	0.023

Table 1. Collected data in the phenolic test.

Carbonate: Anhydrol Folin: Exodus



lowest levels were from carrot skins (0.253 mg EQ/g) and mesocarp cocoa (0.320 mg EQ/g). They are within the mean variation established in the quercetin graph, which ranges from 0.037 to 1.859 mg EQ/g (Table 2 and Figure 2)[9].

Table 3 presents the values of the physicochemical characterization of the samples of grape and carrot bagasse, cocoa mesocarp and epicarp, and banana peel.

#### Conclusion

The samples analyzed presented good moisture, satisfactory water activity, and phenolic compounds and flavonoids within the average. Bananas stand out as having lower moisture and water activity, carrots with higher moisture but fewer phenolics and flavonoids, cocoa mesocarp with higher water activity, cocoa epicarp with higher phenolic content, and grape with the highest flavonoid content. Thus, the feasibility of using food peels in the food industry is reported in the study through enabling a waste reduction, helping to strengthen the immune system, and using the waste generated by food processing companies.

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[] µg/mL (EQ)	Abs 01	Abs 02	Abs 03	Average	Standard deviation
1	0.034	0.043	0.034	0.037	0.004
15	0.499	0.498	0.491	0.496	0.004
30	0.963	0.979	0.991	0.978	0.011
45	1.461	1.452	1.451	1.455	0.004
60	1.863	1.854	1.86	1.859	0.004
75	2.179	2.16	2.166	2.168	0.008

 Table 2. Collected data in the flavonoid test.

Chloride: Vetec Methanol: Anhydrol

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#### Figure 2. Quercetin behavior graph.



Table 3. Physicochemical characterization, bioactive compounds from fruit and vegetable bagasse.

Tests	Grape	Carrot	Cocoa mesocarp	Cocoa epicarp	Banana
Moisture (%)	10.42±0.14	12.96±0.24	9.17±1.02	10.07±0.41	3.33±0.96
Water activity	0.390±0.01	0.403±0.01	0.426±0.03	0.396±0.01	0.389±0.01
Total phenolics (mg EAG/g)	0.679±0.68	0.310±0.03	1.088±0.01	1.367±0.05	0.638±0.04
Flavonoids (mg EQ/g)	1.679±0.06	0.253±0.03	0.320±0.11	0.521±0.03	0.640±0.03

EAG: equivalent in gallic acid; EQ: Quercetin equivalent.

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#### **Fundamentals of 3D Bioprinting Technology**

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3D bioprinting consists in the printing of synthetic 3D structures used as biomaterials, along with cells, growth factors, and other components necessary to create a new functional organ. This technology can be applied to regenerative medicine and tissue engineering to treat diseases, test pharmaceuticals, and study the mechanisms underlying diseases. Currently, there are three basic types of 3D bioprinting technologies: laser, droplet, and extrusion. Laser-based bioprinters (LBP) use laser energy to induce the bioink transfer. Dropletbased bioprinters (DBP) expel the bioink dropwise throughout a nozzle. Inkjet-based bioprinters are the DBP commonly used for biological proposes, it is also a non-contact approach that releases controlled volumes of bioink drops in a continuous (CIJ) or under demand way (DOD). The extrusion-based bioprinters (EBB) also use pressure to force out the bioink, but consists of a syringe containing the material with a pneumatic or mechanical mechanism as dispensing system. Comparing to the other bioprinting technologies, extrusion printing is the most versatile and is indicated for bioprinting of scaffold prosthetic implants. The bioinks used in 3D bioprinting are composed of a solution with a biomaterial mixture, usually encapsulating cells. Biomaterials are essential components of 3D bioprinting technologies because they provide scaffolds as supporting physical structures for cells to attach, grow, differentiate, and develop into tissues. Numerous cell types have been used in 3D bioprinting to build cardiovascular, musculoskeletal, neural, hepatic, adipose and skin tissues. Bioprinting is an emerging technology that has the ability to revolutionize the way we address many health issues. Keywords: Laser. Droplet. Extrusion. Bioprinters. Bioinks.

Abbreviations: CAD/CAM: computer-aided design or manufacturing; LBP: Laser-based bioprinter; DBP: droplet-based bioprinter; EBB: extrusion-based; CIJ: continuous inkjet; DOD: drop-on-demand; iPSC: induced pluripotent stem cell.

#### Introduction

The advance of the printing technology from 2D to 3D has been continuously changing research in many fields, including health sciences. The advent of 3D bioprinting brought a new universe of possibilities. 3D bioprinting consists in the printing of synthetic 3D structures used as biomaterials, along with cells, growth factors, and other components necessary, at least in theory, to create a new functional organ [1].

The first 3D printing was performed in 1986 by creating thin layers of materials in rheological

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conditions of appropriate viscosity that were solidified by a process of cure with ultraviolet light [2]. This former method was not yet appropriate for use in biological creations since it used solvents and reagents that did not grant cell viability. After some decades, the process was improved, and biomaterials were created and used in solvent-free processes, generating structures for biomedical applications, until bioprinting was created [1, 3].

3D bioprinting has been used to fill the gaps in the current techniques, in which the models of interest have complex geometries, in order to achieve a faster and more scalable production [4]. This apparatus uses the so-called bioinks, components composed of biomaterials with certain properties, biochemical factors, and cells printed in a layer-by-layer process. They go through a final curing step, in which the structure turns solid with the support properties of interest [1, 4].

The first bioprinting methods were limited to scaffolds only with support properties. However, the development of the method, along with the

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increasing interest in tissue engineering and regenerative medicine, led to new models and proposals, aiming the creation of tissues and organs for drug screening, cancer models, regeneration of damaged tissue, organ and tissue transplantation, among other conditions [5]. Research advanced to improve the printing technique by building scaffolds with increasingly anatomical fitness and properties similar to the original tissues, enabling a certain biomimetic degree to the native tissues in heterocellular environments [6].

Those tissue engineering approaches can be performed in many ways, varying in printing method, matrix material, cell type, and other factors that can constitute the bioink, which come with advantages, disadvantages, and challenges. As summarized in Figure 1, in this review we aim to establish the 3D bioprinting technology.

Although bioprinting is generally performed by controlled depositions of layers of bioink, the principle used by the printing technology can vary. Currently, there are three basic types of bioink deposition: laser, droplet, and extrusion. These techniques vary among themselves, not only by the rinciple used to release the ink, but also in the resolution of the results obtained. Therefore, it is important to review their limitations before selecting the proper technology [4]. Regardless of the method used, they all need a computer model to be followed during printing, usually generated

**Figure 1.** Overview of the 3D bioprinting technology. The bioink includes cells and biomaterials under certain parameters and is used by the bioprinter technology to construct 3D scaffold structures by layer-by-layer deposition, following a design made in the computer. After a curing step, the engineered organ or tissue have several useful biomedical applications.



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through tools such as computer-aided design or manufacturing (CAD/CAM) [7].

#### **Laser-based Bioprinters**

As the name says, the laser-based bioprinter (LBP) uses laser energy to induce the bioink transfer (Figure 2). It is the most unusual technology, but it has been gaining ground in tissue engineering [4]. The main components are a laser radiation highly focused with a monochromatic base, which can be pulsed or continuous, a laser transparent print ribbon carrying the bioink, and a collecting plate on a controlled movement platform [7]. The ribbon has a laser-absorbing layer that receives the irradiation, forming a high-pressure environment where bubbles of the bioink are generated and expelled to the collector [4].

**Figure 2.** Laser-based bioprinter primary construction. Demonstration of basic items and bioink deposition by the laser irradiation pressure.



LBP makes a direct impression on the collecting surface, without the need for a needle to intermediate it, decreasing the impacts on the cells, since the passage of cells immersed in a substrate of a certain viscosity is one of the main causes of reduction in cell viability [8]. In addition to bypassing the problems resulting from shear stress, this contactless approach abolishes the chances of undue clotting, and allows the cells to

be individually printed in droplets, increasing cell densities, and consequently increasing resolution [9].

Although the cells do not suffer damages by passing the needle, the interaction with the laser and the substrate can alter its integrity, reinforcing the importance of a suitable cell density to create a viable scaffold [10]. Cell density is one of the factors that alters the bionic viscosity, a parameter that is decisive for cell viability in printers that use needles. Since LBP does not use one, it can work with different ranges of viscosity, increasing the ability to carry more cells per mL [11].

The resolution of 3D bioprinting using LPB can be adjusted by several factors. Altering parameters such as surface tension, wettability of the substrate, bioink viscosity, among others, interfere with the resolution [9]. By adjusting these factors, the technique allows the creation of high-resolution models in micrometric rates with different cell type environments [12]. However, in order to mimic a complex model, the bioink needs to solidify rapidly in the desired shape, limiting the viscosity [4].

LBP has some disadvantages beyond the laser-cell interaction and high cost. Some LBP bioprinters use metal layers to absorb laser energy, generating nanoparticles in the process, which may be cytotoxic. To use multiple cell types, different ribbons must be prepared since cellular position is not so accurate, and scaling is still a problem [4]. The alternatives to overcome these issues are time-consuming, onerous, and still not practical; therefore, the technique is recommended for simpler and smaller scaffolds, even for diseases and drug studies [7].

#### **Droplet-based Bioprinters**

Droplet-based bioprinters (DBP) expel the bioink dropwise throughout a nozzle. It is possible to classify them according to the mechanism used to dispense the material. These printers can use pressure, thermal, piezoelectric, electrostatic, electrohydrodynamic, or even acoustic forces as motors. Regardless the method, they all have similar structures. They are composed of a nozzle submitted to the push where goes the bioink, and a collecting plate [7].

Inkjet-based bioprinters, among DBP, are the most used for regular 3D printing and bioprinting. It is also a non-contact approach that releases controlled volumes of bioink drops in a continuous (CIJ) or under demand way (DOD). The CIJ method uses a pressure instability to release the material, hampering the control of the printing process, and is unadvised for bioprinting [11].

The inkjet bioprinters can print scaffolds with different cell gradients, also allowing the input customization of biochemical factors in the scaffold structure, which better mimicks the cellular environment of interest, in addition to being a high-speed method [13,14]. Nonetheless, it is a versatile method that can be used in combination with more than one technique, such as electrospinning [15]

The drop-on-demand method only releases the material when requested. The trigger can be thermal, piezoelectric or electrostatic (Figure 3). Thermal DODs use the temperature rise to create different pressure points, forming a vapor blister that compells out the droplet when it bursts [16]. Piezoelectric crystals can be placed into the nozzle head, where they are submitted to a voltage pulse, causing a crystal contraction or dilation, resulting in a pressure difference inside the nozzle that releases the droplet onto the substrate. Electrostatic approaches use the change in the volume of the bioink container, created by the application of high voltage in the system, which is composed of electrodes and deflection plates to eject the ink [17].

Every motive force applied has its ups and downs that need to be taken into consideration when applied in DBP [16]. The thermal method is the most simple and cost-effective but creates uneven drops. Because this technique uses temperatures to create the perturbation, the cells are exposed to some adverse conditions. Even if the increase in temperature does not directly affect the cells, the shear stress does [17].

The piezoelectrical generates more uniform droplets released in a more controlled manner, without variation in temperature, allowing the use of a system with a wider nozzle and decreasing the risks of clotting and shear stress [4]. The major issue with this technique lies in the possible cell damage caused by the frequency used to print and in the necessary refinement of the matrix [18].

Electrostatic DOD has in its nozzle an electrode and a high voltage deflection plate in place of the piezoelectrical crystal. A voltage pulse flows





through the structure, altering the local pressure, dislocating the ink and causing the droplet deposition in the substrate [7]. Regardless of the principle, inkjet bioprinters function only for bioinks with limited viscosity, thus influencing the final cell concentration printed per mL, to prevent clogging and reduce shear stress [19].

In bioinks with low viscosity, the use of some post-print gelation is a key point in which a widely used option is the cross-linkers. Although necessary, must be used carefully because some chemical changes can occur in the material in order to gelatinize the final scaffold, altering its structure and properties beyond possible cytotoxicity [4].

#### **Extrusion-based Bioprinters**

The extrusion-based bioprinters (EBB) are printers that also use pressure to force out the bioink, but in this case, the system consists of a syringe containing the material to be printed, which can use a pneumatic or mechanical mechanism as dispensing system (Figure 4) [12]. It is the most common bioprinter due to its ease to scale up the production, allowing the construction of larger scaffolds, and is available both in simpler models

**Figure 4.** Types of extrusion-based bioprinters. Mechanical or pneumatic dispensing systems use pressure to release the bioink.



for research applications, or more robust models for industrial production [7, 20].

Beyond the multiple plastic syringe system for liquid output, the newest EBB bioprinters contain at least extruders for pellet deposition, to enable the use of a wider range of materials, a mobile collecting table, and photocuring systems. It is also possible to combine with other deposition techniques, such as electrospinning, in the same printer, favoring the synthesis of hybrid scaffolds [21].

Differently from the other methods, extrusion has a continuous deposition while in contact with the collector substrate. This approach makes it possible to control the temperature (both in the container and on the table), the pressure applied, the extrusion speed, and other parameters such as table type and position [12].

Mechanically controlled extrusion offers a better direct and spatial control of the dispensing system of the material; therefore, they are recommended for more viscous fluids [22]. Although the pneumatic approach has a delay between the gas compression and the material release, it could also be used to print viscous materials by adjusting the pressure and pressure time, which is limited only by the amount of compressed air endured by the system [23].

Thanks to the possibility to use more viscous fluids, extrusion bioprinters can use bioinks with higher cell density, generating tissues with greater similarity to the native tissue. Moreover, some studies have used EBB with more than one cell type printed simultaneously in the same scaffold, and biochemical compounds, including even DNA and RNA molecules [15, 24–27].

Extrusion bioprinting, however, has disadvantages. This technique does not withstand a bioink with high cell densities. The cell survival rate and the pressure that the cells are subjected to pass the syringe are lower than in the other methods and tends to decrease even more with increasing flow viscosity due to the magnification of the shear stress [28]. Another frequent issue to be address is the nozzle clogging, a consequence of this contact technique and its high viscous fluids, leading to misprinted scaffolds and equipment problems [29].

To bypass clogging and cell damage issues, it is possible to use a large nozzle, but it would result in a drop of resolution. In order to overcome the inferior resolution, some parameters must be adjusted, resulting again in a higher pressure and shear stress. Therefore, the optimization of printing parameters are crucial steps in this method [5, 7, 29]. Comparing to the other bioprinting technologies, extrusion printing is the most versatile and is indicated for bioprinting of scaffold prosthetic implants [12].

#### Bioinks

The bioinks used in 3D bioprinting are composed of a solution with a biomaterial mixture, usually encapsulating cells. During or immediately after bioprinting, the bioinks can be cross-linked or stabilized to create the tissue constructs. While synthetic or natural biomaterials can be combined to make hybrid scaffolds, aggregating cells can also be used as bioinks without addition of biomaterials. To achieve the correct functionalities of the target tissues, the bioinks must have proper mechanical, rheological, and biological properties [30].

Before, during, and after gelation, the following properties of the bioinks are essential for their printability: structural resolution, shape fidelity, and cell survival. The cross-linking extent and charge densities determine the swelling behavior of the biomaterials, which eventually influences the shape and size of the bioprinted tissue. Cell viability can be enhanced while maintaining printability by combining biomaterials optimized for cell survival with biomaterials that have mechanical stability and provides shape fidelity [31].

Biomaterials are essential components of 3D bioprinting technologies because they provide scaffolds as supporting physical structures for cells to attach, grow, differentiate, and develop into tissues [32]. Biocompatibility significantly limits the number of appropriate materials. They must suit both encapsulated cells and host's body without inducing inflammation or rejection. Since hydrogels

have an elevated water content and low toxicity, they are common biomaterials used in tissue engineering to construct tissues that better mimic their extracellular matrix microenvironments [33].

The bioink parameters of viscosity, cell density, resolution, fabrication speed, and cell viability vary for each bioprinting technology. While DBP supports low viscosity and cell density but has a fast fabrication speed, LBP supports a higher viscosity but medium cell density and fabrication speed. EBP has the higher viscosity and cell density support but has the lowest fabrication speed. Moreover, EBP can achieve the highest resolution among all current bioprinters [31, 32].

Ranging from differentiated somatic cells to stem and progenitor cells, numerous cell types have been used in 3D bioprinting to build cardiovascular, musculoskeletal, neural, hepatic, adipose, and skin tissues [34]. However, bioinks that are both printable and able to convey the tissue architecture to restore an organ's function are scarce. In addition, vascularization of the bioprinted constructs with integration to the host's vasculature is a major challenge [35].

While embryonic stem cells are pluripotent and derived from the inner mass cells of the blastocyst stage of an embryo, mesenchymal stem cells are multipotent adult cells present in various tissues. Induced pluripotent stem cells (iPSCs) have promising applications in regenerative medicine. iPSCs are somatic cells genetically reprogrammed back to a stem cell phenotype [36]. They represent an unlimited source of patient-specific cells that do not trigger host rejection, and they can be applied in disease modeling with phenotype variability. The 3D bioprinting technologies can employ stem cells, which have the ability to progress into different cell lineages, progenitor cells (with limited capacity to follow other cell lines), or somatic cells that have already achieved their last differentiation stage [34].

Shear stress during the printing process is detrimental to stem cells, especially to iPSCs, and

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it can even affect their gene expression profile. Therefore, the bioprinting procedures need to be tested beforehand in order to effectively produce tissues and organoids for drug testing and disease models as well as for the regeneration of damaged tissues and treatment of diseases [35].

#### Conclusions

Bioprinting is an emerging technology that has the ability to revolutionize the way we address many health issues. It allows the opening of a new door to regenerative medicine, where the main goal is not simply to replace non-functional or damaged tissues by temporary implants with side effects. Bioprinting is about creating a system in which the body can rebuild itself properly, producing new healthy, functional, and longlasting tissues [37]. In addition to the applications in regenerative medicine, bioprinting has also been used in the research of drug mechanisms and disease models, pursuing to validate more humanlike models instead of the animal models currently used [12].

Although bioprinting has many advantages, there are some drawbacks that need to be overcome. The main one involves the bioink preparation and deposition. However, there is also the necessity to find proper materials and cell combinations that generate suitable bioinks with viscosity ranges that allow a printing process with less cellular damages and with a final scaffold able to maintain the structure's properties without collisions. Because it is a new technology, it has yet to be improved to increase post-print scaffold viability and functionality and, therefore, it is suitable for clinical uses. Finally, long-term tests, in all steps, need to be done wherefore new advances can be achieved with this promising technique [7, 12].

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#### Presence of Microorganisms and Use of Antimicrobial Agents in Car Seat Fabrics: A Brief Review

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The present paper discusses the presence of microorganisms in textile materials, especially in car seats, and the use of antimicrobial agents. We observed that the main agent between the user and the car's microbial system are the seats, due to the direct contact with the users. In addition, the environment in which the car is inserted, the type of car, and the seat fabric-type influence directly the reproduction of the microbial system. Microorganisms found in different parts of cars, especially in seats, could be potential threats to human health. Thus, to combat these microorganisms, it is necessary to study antimicrobial agents aimed at eliminating or inhibiting their reproduction and, consequently, promoting hygiene, ensuring the health and well-being of car users.

Keywords: Bacteria. Fungi. Textile. Automotive Seats. Antimicrobial Agents.

Abbreviations: Ag-NPs: silver nanoparticles; Cu-NPs: copper nanoparticles; NOX: oxidation number.

#### Introduction

In a globalized world, the need to constantly move has contributed to individuals spending more time inside the cars. The seat covering is the part of the car that the user has the most contact with, and this can have consequences on human microbial environment. The microbial systems present inside a car vary according to the circumstances in which it is inserted, type of car, the internal composition, and principally with the structural organization of the fabric, which is pointed out as good substrates for the development of microorganisms [1,2].

The most used fabrics for the manufacture of automotive seats are leather, synthetic leather, looms, and knitwear. The structural organization of the fabric is related to the absorption of water, oxygen, and nutrients, which makes the fabrics of natural fibers and vegetables more susceptible to the proliferation of microorganisms [1,3].

Moreover, there is a growing expectation around fabrics with antibacterial properties. These fabrics have the insertion of antimicrobial agents in the fabric structure, allowing the ability to prevent or inhibit the spread of microorganisms, being considered, respectively, as biocide or biostatic. The incorporation of the antimicrobial agents into fabrics varies according to its performance, type of fiber in the textile material, and the most susceptible microorganism to the fabric. The main antimicrobials that have been used in textile materials are the quaternary ammonium compounds, pyrithione of zinc, nitrogen compounds, silver nanoparticles, and copper nanoparticles. [4] However, there are still few papers that demonstrate the use and effectiveness of the use of antimicrobial agents in fabrics used in automotive seats.

Therefore, to provide greater safety to the health of car users, this paper aimed to carry out a brief literature review on aspects related to the presence of microorganisms inside vehicles, with an emphasis on automotive seats, as well as the use of antimicrobial agents to minimize the microbiological load on the seats.

The literature research was limited to a maximum period of 7 years and carried out in databases on sites such as Scielo and Science Direct. For the study, it was considered automotive fabrics, microorganisms present inside cars, and their

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respective impacts on human health. In addition, the application and action of antimicrobial agents in textile materials were considered. Thus, the keywords "fabric", microorganism" and "car" were used to identify papers related to the topic.

#### **Literature Search Results**

We observed that the most frequent fungi found in the internal air of a vehicle are *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp. and *Alternariaem* sp. [1]. *Staphylococcus* sp. and *Propionibacterium acnes* sp. have a considerable population in common areas of the car, showing up in large numbers on steering wheels, gear knob and area near the cupholder (Table 1). These microorganisms are capable of colonizing inanimate objects commonly touched. Thus, the car inside becomes a favorable environment as a reservoir of these microorganisms [1,3]. Species of the genus *Staphylococcus* sp. are more pathogenic. These microorganisms can promote a variety of diseases, from skin infections to respiratory diseases [5,6]. In addition to this genus, there are *Pseudomonas* sp. bacteria, capable of causing lung diseases, in the bloodstream, in the heart valves, as well as ear diseases [7].

As for fungi, despite having about 250,000 species, only 200 of them are recognized as pathogenic. In general, the genera *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp. e *Alternariaem* sp. could be encountered on the vehicle inside

$\mathbf{c}$													
Comus		Car 1		Ca	r 2	Ca	r 3		Car 4			Car 5	
Genus	Α	В	С	В	С	В	С	Α	В	С	Α	В	С
Staphylococcus sp.	77.1	74.1	10.2	19.1	3.32	14	63.7	12.5	10.5	1.05	19.7	29.2	28.2
Propionibacterium acnes sp.	9.72	17.5	3.5	7.44	0.5	6.04	2.75	22.6	8.53	0.06	21.3	17.4	0.08
Acidovorax sp.	1.23	0.17	6.55	3.41	0	9.22	0.02	7.55	3.75	0	1.5	2.96	23.5
Streptococcus sp.	1.02	0.07	4.17	7.3	0	12.5	5.49	5.8	0	0.06	9.93	13.2	5.15
Clostridium sp.	0.15	1.16	0	14.3	0	3	0.11	0.23	13.1	0.03	1.79	5.67	1.93
<i>Mycobacterium</i> sp.	0.22	0.01	0.39	0	0	0.09	0.12	0	38.0	0.05	0	0	0.13
Acinetobacter sp.	0.64	1.32	0.57	1	0	2.61	0	1.66	0.21	0.01	9.06	1.48	3.22
Pseudomonas sp.	0	1.02	0.03	3.2	0.08	2.18	0.68	1.79	0.02	0	0.05	0	5.6

Source: Adapted from Stephenson and colleagues (2014) [3].

\* A - Steering wheel. B - gearshift button. C - Area next to the cupholder.

and are considered opportunistic-infectious, as they only play the role of mild allergy sufferers. However, when in large quantities and with the individual's immune system weakened can cause serious respiratory problems [8,9].

Due to the possibility of these microorganisms being found inside cars, it is of great importance to assess their presence in automotive seat fabric, which is the part of the vehicle in greater contact with users of this means of transport.

A car seat has different parts, such as a metal frame, plastic parts, and a textile covering. The textile coating is the part that the user has contact with for a longer time and it can be made up of different textile materials in its composition, such as fabrics, carpets, leather, and vinyl material. Among these materials, the fabric is the material that has the greatest superficial use in automotive seats and, in the automotive industry, synthetic fibers are mostly used. Synthetic fibers have a technological advantage over natural fibers as they are more resistant to the proliferation of microorganisms due to their hydrophobic nature. However, they have the disadvantage of retaining more moisture from perspiration, which can promote the development of microbial cultures on the surface of the automotive seat covering. Thus, car seat fabric can present unpleasant odors and a possibility of contamination of human skin in contact with these microorganisms [10].

So, the emergence of diseases from unwanted microorganisms on surfaces made it necessary the study the antimicrobial agents for the promotion of personal and collective hygiene. The forms of action of these agents can be through the establishment of new intermolecular interactions or, even, through the oxidation of the organic matter present in these organisms [4,11]. Among these products, there are ethyl alcohol, isopropyl alcohol, and quaternary ammonium salts that act in the denaturation of proteins of the microorganisms; phenols that act on the disruption of the outer membrane, inactivation of enzymatic systems, among others; chlorine derivatives and peroxides that are capable of oxidizing the microorganism's organic matter [11]. In addition, there are silver nanoparticles (Ag-NPs) and copper nanoparticles (Cu-NPs) [12,13].

Some studies indicate that the antimicrobial activity of silver nanoparticles is related to the release of  $Ag^+$  ions in the medium. Accordingly, an increase in its effect is observed when used in smaller volumes (particles with smaller sizes, in this case, nanoparticles), due to the increase in the

contact surface. When used in low concentrations, silver does not have significant effects on the human body, but Ag-NPs have an antibacterial effect for Gram-positive and Gram-negative bacteria. A priori, the interactions between the negative charges present on the surface of the bacteria and the silver cations are fundamental for the bonds between these structures to occur; these ions promote the deactivation of cellular enzymes and, consequently, cell lysis (Figure 1) [12].

Copper nanoparticles work similarly to Ag-NPs. It also presents a relationship between the velocity and the contact surface of the nanoparticles in which smaller sizes are favored. Copper nanoparticles can change NOX (oxidation number) in a continuous process, which can lead to penetration into the cell membrane, causing dysfunction in this region, as well as in the cytoplasm (Figure 2) [13].

However, for the impregnation of these agents in textile materials, it is necessary to know how they work in the fabric. [4] This application is often done by the method of immersing the fabric in a solution containing the antimicrobial agent. The padding method (foulardage) is one of the most used in the textile industry where the immersion

Figure 1. Mechanism and toxicity of the silver nanoparticles in microorganisms.



Source: Adapted from Benedito (2017) [14].



Figure 2. Mechanism and toxicity of the copper nanoparticles (Cu-NPs) in microorganisms.

Source: Adapted from Ribeiro (2019) [13].

technique is used, however, a greater pressure of the agent is added to the fabric [15]. In chemical terms, antimicrobial agents can be inserted into the fabric by incorporation into the polymer matrix as well as by application to the fiber surface [4]. Table 2 shows different forms of interaction, for impregnation, between antimicrobial agents and fabric structure. It is noteworthy that there is still little information in the literature about the nature of the microorganisms present in the car seat, as well as the use of antimicrobial agents to reduce the population of microbes in automotive fabrics.

Therefore, it is important to study the genera of the microorganisms present and the impregnation efficiency of antimicrobial agents

Table 2. Processes and connections between antimicrobial agents and a textile structure.

Most common processes	Link between antimicrobial agent and textile structure				
<ul><li>a) Addition of active compounds to the polymer matrix</li><li>b) Microencapsulation of the active ingredient and</li><li>addition to the polymer matrix</li></ul>	Adsorbed on substrate				
<ul><li>c) Impregnation of active agents and fixation with resins</li><li>d) Coating application</li><li>e) Layer-by-layer application</li></ul>	Adsorbed to the surface				
<ul><li>f) Chemical modification of fiber by introduction of reactive groups and reaction with active agents</li><li>g) Chemical fiber modification by graft copolymerization</li><li>h) Active ingredient microencapsulation and fixation to the fiber</li></ul>	Chemically bonded				

Source: Adapted from Scacchetti (2017) [4].

in fabrics, especially those used in automotive seat coverings. The reduction of the microbial load in the fabrics used in car seats can enable a reduction in the spread of diseases caused by different types of microorganisms, including bacteria, fungi, or viruses. It is noteworthy that more and more frequent car users are seeking greater health safety during the period they are traveling, especially in this period of the pandemic that the world is facing. Thus, this work showed different technologies that can be used in fabrics used in automotive seats to enable the use of antimicrobial agents, which have an action against different types of microorganisms that may be present inside vehicles.

#### Conclusion

It is possible to consider that automotive seats are significant sources for the spread of microorganisms, which can negatively influence the health of car users. Technological and scientific advances related to the study of antimicrobial agents have significantly contributed to the promotion of a less aggressive environment to human health.

Due to the limited research available on microorganisms in each type of fabric used in automotive seats, as well as evidence on the mechanism of action of some antimicrobial agents, this work shows the need for more detailed studies on the use and effectiveness of agents antimicrobials in fabrics used in car seat coverings.

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#### Biomaterials Development for Application in Tissue Engineering: Literature Review

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The search for tissue regeneration has attracted a lot of attention due to its wide applicability, and great potential to solve several problems in advanced therapies. Currently, functional systems have been widely used as therapeutic solutions in the treatment of some skin diseases, whether chronic, acquired, or as a consequence of accidents and burns. It is possible due to the feasibility of releasing and/or acting in loco of some active substances with properties and characteristics very similar to human skin. In this sense, this work aimed to carry out a literature review research regarding the development of new biomaterials, including hydrated membranes composed of biopolymers such as keratin and bacterial cellulose. Keywords: Hydrated Membranes. Biopolymers. Properties.

#### Introduction

In recent decades, the biopolymer market has been a growing interdisciplinary area due to its countless alternatives of wound recovery and disease treatments. In the field of tissue engineering, biomaterials are used to recover tissue from an injured area.

Injuries caused by burns are the field of application of hydrated membranes. Their function is to promote cell proliferation for tissue reconstitution, keeping the wound moist, therefore, less painful. The moisture also facilitates the dressing exchange process, or even makes it dismissable, since a fully degradable membrane is one of the explored possibilities by tissue engineering.

Therefore, the objective of this work is to carry out a literature review on the development of hydrated membranes composed of chitosan and bacterial cellulose biopolymers. They are widely explored in this area for their great potential to achieve the key features for a biomaterial.

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#### **Material and Methods**

The present study is a literature review realized between December 2020 to February 2021, by consulting articles previously indicated and archives of periodicals such as Scielo, PubMed, and ScienceDirect.

At first, a search for biomaterials was carried out, followed by the most common forms of hydrated membrane production for dressing wounds, as well as their desired characteristics and the materials used. Finally, it was made a final search about articles related to the synthesis of these membranes and the analysis of their properties. All the analyzed research dates from the 2000s until now.

## Essential Characteristics and Properties of a Biomaterial

For a useful biomaterial, it must present indispensable basic characteristics and properties.

#### **Biocompatibility**

Biocompatibility is a property related to the ability of adhesion and interaction between the biomaterial and the cells [1]. A biocompatible material enables the exchange of cellular materials to occur normally and prevents the body from rejecting the inserted material [1].

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#### Non-Toxic Biodegradability

Since one of the main goals is to allow the growth of new cells, the biomaterial must be able to degrade [2]. This property refers to the ability of a biomaterial to degrade in the body, releasing components that won't cause cell death in the surrounding cells [1,2]. Therefore, it is extremely important to know the byproducts generated from the degradation of a material.

#### **Compatible Mechanical Properties**

Mechanical properties of a biomaterial must be compatible with the area where it will be inserted, allowing the component to be able to support the mechanical solicitations without breaking or overloading other organs or tissues, and allowing the patient to move normally [1].

These properties are affected by the geometry of the hydrated membrane scaffold, the material, and the strength-enhancing materials of which it is constituted [3].

## Essential Characteristics and Properties of a Hydrated Membrane

The most important role of a hydrated membrane is to promote cell proliferation of injured tissue, so it can reconstitute itself. However, one of the main difficulties in the treatment of injuries caused by burns is to change the dressings causing minimum pain for the patient. Keeping the lesion moist is important both to facilitate this exchange, to avoid infection, and to reduce fluid loss. Achieving those requirements are the standard protocol for burn management healing [4]. Therefore, it is necessary to keep these needs in mind so that the properties related to them are properly covered.

#### **Biomimetics**

Biomimetic is a property related to the ability to stimulate cell proliferation [5]. As previously mentioned, since the main goal of a hydrated membrane is to allow the damaged tissue to grow again, the biomaterial must be biomimetic.

#### **Wettability**

Since moisture is extremely important for a hydrated membrane, the biomaterial must be hydrophilic, so the interaction between the membrane and the tissue increases. This property is evaluated through the measurement of wettability, which is determined by the tangent angle between a drop of a liquid and the surface where it relies on [6]. If this angle is less than 90°, then the analyzed material is hydrophilic, if it is greater than 90°, the material is considered non-hydrophilic [6].

#### Geometry and Porosity

The geometry of a scaffold affects many attributes besides the mechanical properties. To function properly, tissue must be able to exchange biological nutrients and waste. Therefore, to reconstitute a damaged tissue the scaffold of the hydrated membrane must possess large pores and high pore interconnectivity so that exchange can occur normally [1,3]. Besides that, permeability to water vapor and oxygen is also required to keep the cells alive [7].

Therefore, it is extremely important to study how geometry is affected, so it can be adapted accordingly to the application. Scaffold fabrication methods significantly affect the geometry of the membrane. Casting, electrospinning, and freezedrying methods are the ones that offer more control over pore diameter size and the porosity (usually greater than 90%) of the membrane [3].

#### Antimicrobial Action

Infections may occur in a wound, aggravating its condition slightly or even severely, causing the patient's death [8]. Therefore, to prevent it, microbial action must be eliminated. This attribute can be achieved by incorporating a material with antimicrobial properties into the membrane when developing a biomaterial.

#### **Biopolymers as Materials for Wound Dressing**

Biopolymers have been a major target of study for the development of biomaterials since they are biodegradable and non-toxic, biocompatible, in addition to having low cost, easy processing, and promoting excellent adhesion and cell growth [1]. However, one of the biggest challenges faced in tissue engineering is to find a method of production that can be applicable on large scale. Some of the most studied biopolymers are chitosan and bacterial cellulose, which will be discussed in this review.

#### Bacterial Cellulose

Bacterial cellulose is produced through the biosynthesis of bacteria. This polysaccharide has many interesting characteristics for tissue engineering. In addition to being biocompatible, non-allergenic, and non-toxic, bacterial cellulose also has high porosity due to the formation of nanofibers (3nm to 8nm), high crystallinity (60% to 80%), high hydrophilicity, high water holding capacity, and great mechanical properties, such as high strength tension and elasticity [7]. However, its production still lacks an efficient large-scale fermentation system (Figure 1) [7].

Figure 1. Bacterial cellulose membrane.



#### <u>Chitosan</u>

Chitosan is a biopolymer resulting from the deacetylation of chitin, a polysaccharide present in the shell of crustaceans, some insects, mollusks, and in the cell wall of fungi (Figure 2)[8]. This biopolymer has great potential for study in tissue engineering due to its antimicrobial and healing

Figure 2. Pure chitosan membrane.



properties, in addition to being fully absorbable by the body [8]. To improve its properties, it is often used with additives and physical or chemical modifications [6].

#### **Recent Studies on Hydrated Membranes Characterization**

#### Wettability

Polymer blends and treatments with other components are some of the used methods, which usuallygenerateresultswhosecontactangleisreduced. In the study realized by Silva and colleagues [6], chitosan membranes treated with glycerol, plasma, or glycerol and plasma, presented good results (Graph 1). **Graph 1.** The contact angle for pure chitosan membrane (MQ), plasma-treated chitosan membrane (MQ-P), glyceride treated chitosan membrane (MQ-G), and plasma glycerin treated chitosan membrane (MQ-GP).



Pure chitosan membranes already show contact angles that qualify the material as hydrophilic but when treated with plasma, glycerin, or both, the contact angle can be strongly reduced.

Another study, in which corn starch was added to the bacterial cellulose membrane, also showed reduced contact angles over time for both membranes but especially when the addition was performed (Table 1) [9].

**Table 1.** Contact angle over time found in the analysis of the pure bacterial cellulose membrane and the sample treated with corn starch, glycerin, and water.

C	В	Sample				
Time (s)	Angle (°)	Time (s)	Angle (°)			
0	32.55	0	10.7			
5	30.45	5	6.6			
30	26.70					

#### Geometry and Porosity

Geometry is mostly affected by the chosen form of membrane production and by the concentration

of the materials involved [3]. In a study performed by Alavarse and colleagues [10], the influence of different chitosan concentrations in a polymer blend with PVA was evaluated. The membranes were produced through electrospinning, which is one of the methods that best contributes to high porosity (Table 2) [3].

**Table 2.** Porosity percentage of the Polymerscaffold according to chitosan concentration.

Samples	Porosity (%)
PVA/Qui. 100/0	50.71±4.38
PVA/Qui. 90/10	41.52±1.8
PVA/Qui. 80/20	47.34±2.85
PVA/Qui. 70/30	49.44±3.79

The membrane with PVA/Qui. 70/30 presented the smallest nanofiber diameters (Graph 2), which is relevant since, the smaller the nanofiber is, the better it acts as a barrier against bacteria, allows oxygen diffusion, and has mechanical stability to act as a scaffold [3].

**Graph 2.** Mean and standard deviation of the nanofiber diameter and the behavior of the samples (with stars at the top of the standard deviation limit: samples with statistically equal mean diameters).



#### Antimicrobial Action

Chitosan itself already has antimicrobial properties, however, it depends on the concentration

of chitosan. Therefore, for this reason, and since bacterial cellulose does not have those properties, micro, and nanoparticles of metals with such properties are used to enhance or add antimicrobial properties.

In a study performed by Fischer and colleagues [5], silver nanoparticles were added to a bacterial nanocellulose membrane (BNC) and the antimicrobial action for *S.aureus* and *P. Aeruginosa* bacteria was evaluated (Table 3).

**Table 3.** Data obtained from the antimicrobial assay with samples of pure BNC (bacterial nanocellulose) and BNC incorporated with NPAg.

Sample	Colonies (n) of <i>S. aureus</i> in 24 hours	Colonies (n) of <i>P. aeruginosa</i> in 24 hours
pure BNC	3.1 x 10 <sup>5</sup>	1.8 x 10 <sup>3</sup>
incorporated BNC	160	23
bacterial reduction (%)	98	89

#### Conclusion

This study presented the potential dimension of hydrated membranes made of biopolymers. The materials and a few methods recently studied have great potential for exploration due to their satisfactory results in contemplating the necessary attributes in the membrane. However, the challenge is an economically viable way to produce the materials to be developed.

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#### Review on the Use of Recyclable and Biodegradable Materials as Geosynthetics

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The emergence of geosynthetics changed many aspects of the project and construction of civil and environmental works. Due to the existence of a wide variety of products and the constant advancement in the development and dissemination of new technologies. These materials are applied in different engineering solutions, highlighting the possibility of using recyclable and biodegradable materials and executing geotechnical and environmental works ranging from the control of erosion to the protection of groundwater. In this context, this work presents a literature review on the use of recyclable and biodegradable materials as geosynthetic products, as well as their association with traditional geosynthetics for solutions in engineering works. The review was carried out through the platforms Google Scholar and Portal of Journals of CAPES, using keywords and Boolean connectors as descriptors. It is observed that there is a potential for the use of recyclable and biodegradable materials as geosynthetics, limitations, and impacts caused by the use of these wastes.

Keywords: Geosynthetics. Recyclable Materials. Biodegradable Materials. Applications.

Abbreviations: ABNT: Associação Brasileira de Normas Técnicas (Brazilian Association of Technical Standards); PET: Polyethylene Terephthalate; PETG: Polyethylene Terephthalate Glycol; PE: Polyethylene; PLA: Polylactic Acid; BPVDs: Biodegradable Prefabricated Vertical Drains.

#### Introduction

In recent years, the generation of solid waste in urban centers has not been accompanied by an offer of the required infrastructure to deal with all this waste. The production of these wastes is increasing in Brazil, but their proper destination, recycling, and recovery do not follow this generation's growth. In an attempt to reduce the impacts caused by these residues, the recycling of materials that are difficult to degrade has been one of the biggest concerns of our time. According to ABNT, geosynthetics are products composed of synthetic or natural polymers, in the form of a blanket, strip, or three-dimensional structure, in contact with soil or other materials, used in geotechnical and civil engineering [1]. Recyclable materials can be used as material raw in geosynthetics, filler materials for drainage, as

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J Bioeng. Tech. Appl. Health 2021;4(2):81-84. © 2021 by SENAI CIMATEC. All rights reserved. a core for geocomposites as well. They can also be used with the function of reinforcement, for the manufacture of geocells and geofibers that will act to improve the behavior of the soil [2]. This research aimed to carry out a literature review of works related to the use of recyclable and biodegradable materials as geosynthetics to better understand the possibilities and variety of products and applications in geotechnical works.

#### **Materials and Methods**

Aiming at identifying works related to the use of recyclable and biodegradable materials as geosynthetic products, as well as their association with traditional geosynthetics for solutions in engineering works, a survey of works published in the last 30 years was carried out through platforms such as Google Scholar, Portal of Journals of CAPES and the websites of renowned companies in the geosynthetics sector, such as Maccaferri and Huesker. The search descriptors adopted for the data collection were: Geosynthetics Environmental AND Biodegradable AND AND Geotechnical AND Engineering AND "Recyclable Materials". This was done to direct the results more towards the object of study.

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The logical operator AND provides results that necessarily contain these descriptors in the same document, regardless of the number of words that may exist between them. Quotation marks (" ") were used to search for words exactly as they are written ("Recyclable Materials"), without there being any other words between them in any field. After reading the titles and abstracts, the works that had a greater focus on the research topic were identified, downloading, and reading them in full.

#### **Results and Discussion**

The number of articles found with the descriptors informed was 29. Of these articles, the 8 most relevant articles were presented (Table after excluding those that had nothing to do with the purpose of the work.

We observed that there is a potential for the use of biodegradable geosynthetics composed of natural fibers or biopolymers and recyclable materials, especially PET bottles, in the manufacture of geosynthetics and associated with products made with conventional materials. We also verified the increase of interest in Geotechnical Engineering with these materials due to their resistance, flexibility, and low environmental impact. So there is a necessity to develop a system for evaluating the performance of their use as an alternative to conventional materials.

#### Conclusion

We concluded that the best use of waste in engineering works is certainly important for reducing the exploitation of finite natural resources, and for preserving the environment. On the other hand, geosynthetics with their product variations and their versatility have great potential for application in geotechnical works, as well they are in constant development with new technologies, incorporation of new materials, and application methods. It is necessary to develop more careful studies to assess the performance, benefits, limitations, and impacts caused by the use of materials discarded and biodegradable materials in the manufacture of geosynthetic products, or their use associated with traditional geosynthetics in solutions in geotechnical works.

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Types of analyzed geosynthetics	Materials studied or analyzed	Main Results	*Ref.
Geogrids	Biomaterials and biopolymers made from biodegradable Polyethylene Terephthalate Glycol (PETG) and polylactic acid (PLA)	Among a wide variety of biodegradable materials available on the market, 6 different PLA-based materials and 2 petroleum derivatives were selected as the best candidates for practical geoenvironmental engineering applications. PLA-based materials are the most promising for a wide range of applications in different fields.	[3]
Biodegradable Prefabricated Vertical Drains (BPVDs)	Jute vegetable textile fiber	Biodegradable drains have characteristics similar to conventional ones, enabling their use in similar circumstances. BPVDs did not influence soil consolidation in the period analyzed, but there was a small reduction in filter tension and a slight lateral displacement due to its greater flexibility compared to conventional drains.	[4]
Geotextiles	Natural sisal, coconut and jute fibers	Geotextiles made from natural fibers had satisfactory results in the tests carried out. In relation to tensile strength, they had results similar to a conventional geotextile, but they presented better results in terms of shear strength and durability.	[5]
Nonwoven Geotextiles	Recycled materials (PET and PE), biodegradable polymers (polylactic acid cellulose and chitosan) and natural fibers.	The use of sustainable materials in disposable non-woven applications is seen as a positive factor and a way to balance human needs with a finite resource reality. Verifying that such materials have characteristics similar to commonly used synthetic nonwovens.	[6]
Geosynthetics (geocells and geofibers)	PET bottles	The combinations of geosynthetics with PET bottles reduce construction costs and reduce the volume of waste that would end up in landfills. PET bottles can be used as a raw material for geotextiles, as a filling material for drainage in buildings and to form drainage mats under a geotextile filter. They can also be used with the function of reinforcement, for the manufacture of geocells and geofibers that will act to improve the behavior of the soil.	[2]
Geotextiles	Natural fibers and biopolymers	Considering the containment of the accumulation of additives and microplastics in the environment, as well as the need to recover the materials after the growth of natural vegetation, biodegradable geotextiles made from jute and coconut mats are inexpensive and remain intact for about 1–3 years. They show improvement in the sowing process and growth of planted vegetation and represent a suitable alternative to melt-spun thermoplastic fibers for slopes, which are not very steep (<45 °). However, natural fibers absorb large amounts of water, which can lead to significant weight gain and premature degradation of the geotextile making the application of natural fibers in specific environments, such as riverbanks or steep slopes, unfeasible.	[7]
Geotextiles	Natural fibers and biopolymers	Biotextiles have great potential to become commonly used materials due to the growing interest in the market, their competitive properties and the need to use them not only in innovative solutions, but also in widely known environmental works. There are several examples of plant and animal source fibers that provide a competitive alternative option to synthetic products commonly used in short term solutions, they include jute, coconut fiber or wool. These fibers are characterized by having good mechanical properties and low production costs. Furthermore, they break down into elements that pose no risk to the environment and can even be used as a fertilizer for plants.	[8]
Geocell	PET bottles	PET bottles have adequate mechanical strength, which is an indication of their viability for use in the construction industry. A PET bottle geocell has properties that make it able to respond very well to the necessary stresses, as the alternative structure is very similar: hollow, so the opening mesh allows for greater interaction and anchoring in the inserted soil.	[9]

#### Table 1. Selected articles on the use of recyclable and biodegradable materials as geosynthetics.

- Daria M, Krzysztof L, Jakub M. Characteristics of biodegradable textiles used in environmental engineering: A comprehensive review. Journal of Cleaner Production 2020:122-129. Available in: https://www.sciencedirect.com/science/article/pii/ S0959652620321764. Accessed in: 29 mar. 2021.
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Number of Words - Title	120	90	95	85	70	60	120	90
Font Size/Space- Title	12; double space	12; double space	12; double space	12; double space				
Font Size/Space- Abstracts/Key Words and Abbreviations	10; single space	10; single space	10; single space	10; single space	-	-	10; single space	10; single space
Number of Words – Abstracts/Key Words	300/5	300/5	200/5	250/5	-	-	300/5	300/5
Font Size/Space- Text	12; Double space	12; Double space	12; Double space	12; Double space				
Number of Words – Text	5,000 including spaces	5,500 including spaces	2,500 including spaces	1,000 including spaces	1,000 including spaces	550 including spaces	5,000 including spaces	5,500 including spaces
Number of Figures	8 (title font size 12, double space)	3 (title font size 12, double space)	2 (title font size 12, double space)	2 (title font size 12, double space)	-	2 (title font size 12, double space)	8 (title font size 12, double space)	8 (title font size 12, double space)
Number of Tables/Graphic	7 title font size 12, double space	2 title font size 12, double space	2(title font size 12, double space)	1(title font size 12, double space)	-	-	7 title font size 12, double space	4 title font size 12, double space
Number of Authors and Co- authors*	15	10	5	10	3	3	15	10
References	20 (font size 10, single space	30(font size 10,single space	15 (font size 10,single space)	10 (font size 10,single space)	10 (font size 10,single space	5(font size 10,single space	20 (font size 10, single space	20

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