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(Organizadores)

As Ciências Biológicas e da Saúde na Contemporaneidade 2

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APRESENTAÇÃO

A obra “As Ciências Biológicas e da Saúde na Contemporaneidade” consiste de uma série de livros de publicação da Atena Editora, em seus 22 capítulos do volume II, apresenta a importância do desenvolvimento de novas pesquisas nos âmbitos da saúde e da natureza e ainda a relevância da busca de novas terapias para o tratamento de variadas patologias.

O desenvolvimento de pesquisas no campo da saúde representa uma ferramenta importante para a busca de novas estratégias para o diagnóstico, acompanhamento do curso e tratamento de doenças. É na área da saúde que a biotecnologia encontra algumas de suas aplicações mais benéficas e abrangentes. Por meio de diferentes vertentes biotecnológicas, como a produção e atuação de organismos geneticamente modificados; a engenharia genética, que permite qualquer tipo de alteração em nível de DNA e experimentos empregando espécies vegetais e/ou compostos isolados para o desenvolvimento de terapias alternativas e aprimoramento das terapias convencionais.

Atualmente a busca por novos compostos com atividade terapêutica é feita majoritariamente através da experimentação de produtos naturais, uma vez que muitos destes têm comprovadas cientificamente suas propriedades antimicrobianas, antioxidantes, anti-inflamatórias, antineoplásicas, analgésicas, entre outras.

Desse modo, este volume II apresenta artigos que tratam: das propriedades antioxidantes de espécies vegetais como o alecrim e o chá verde; estudos microbiológicos e de toxicidade de espécies vegetais e animais; caracterização de ácidos nucleicos e proteínas; emprego da engenharia genética para elucidação de mecanismos de ação e desenvolvimento e experimentação de alimentos funcionais. Assim, esta obra é dedicada aos pesquisadores da área de saúde, que buscam reciclar seus conhecimentos por meio de pesquisas relevantes e se atualizar perante às novas tecnologias e descobertas científicas e biotecnológicas aplicadas às áreas da saúde.

Portanto, esperamos que este livro possa estimular outros estudantes e profissionais de saúde ao desenvolvimento de pesquisas e estudos a fim de incorporar à literatura referências atualizadas e possibilitar a aplicabilidade dos resultados dessas pesquisas às práticas profissionais diárias.

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SUMÁRIO

CAPÍTULO 18

NEW PROCESS FOR OBTAINING NANOCHITOSAN / BURITI OIL (*Mauritia flexuosa*) BIOCOMPOSITE: A BIOMATERIAL FOR REGENERATIVE MEDICINE AND TISSUE ENGINEERING

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ABSTRACT: For ages, wound healing is a big challenge for health area and scientific community and the major goal is to develop the most effective wound dressing to cover and protect the injuries from external infections and to accelerate healing process. Chitosan is the only biopolymer positively charged in nature, with antioxidant, antimicrobial and antifungal properties. In nanocrystalline chitosan, the crystallinity is reduced resulting in a nanomaterial with reactivity and adhesiveness. The complexation of nanochitosan with essential oils, such as *Mauritia flexuosa* (Buriti), provides a unique biomaterial with great potential for regenerative medicine. The objective of this work was to obtain a biocomposite with nanochitosan and Buriti oil through a new process, with great properties for the health area. The process is divided into two steps, starting with the dissolution of chitosan. The second step comprises in the coagulation of glucosamine molecules in a nanoparticulate matrix, performed in aqueous medium. In the Fourier-Transform Infrared Spectroscopy (FTIR-ATR) analysis, the characteristic groups of nanochitosan and Buriti oil were identified. The Scanning Electron Microscopy (SEM) analysis showed nanopolymer agglomerates, suggesting the encapsulation of the oil by nanochitosan. Particle Size analysis showed a slight increase in the biocomposite compared to nanochitosan, corroborating with the encapsulation, also a decrease in polydispersity that promotes a stable compound. Microbiological tests showed greater inhibition in the biocomposite compared to oil and polymer individually. The final biomaterial did not show phase separation, and intensified the expected characteristics, confirming the potential of the biomaterial.
KEYWORDS: *Regenerative Medicine, Chitosan, Essential Oil, Nanotechnology*

1 I INTRODUCTION

The skin is the largest and most exposed organ in our bodies, thus it is the most susceptible

to injuries (MERCURIO, 2015). The term wound refers to the damage and/or rupture (interruption of continuity) of the skin (FRANCO, 2014). The human body has the capacity of healing some injuries, being the wound healing one of the most commons.

1.1 Skin Wound: Development and Mechanisms

This process consists of 3 overlapping phases: inflammatory, proliferation, and remodeling (OLIVEIRA GONZALEZ, 2016).

- Inflammatory phase is described as the migration of leukocytes to the damaged area, aiming the destruction of microorganisms and dead cells. This phase is recognized by the erythema (redness of the surrounding tissue), occurring in the first 24 hours and lasting up to two days.
- Proliferation phase is the phase responsible for the closing of the wound, causing contraction, the formation of fibrous tissue, cell production for extracellular matrix creation, followed by reepithelization of the skin. This process begins in the first two days and can endure for about two weeks.
- Remodeling phase is the longest step in the whole process, taking from 2 or 3 weeks to over 1 year. Consists of the rearrangement of the extracellular matrix, with the purpose of achieving the maximum tensile strength. The rearrangement of the matrix is made by reorganization, degradation, and resynthesizes of it. The failed subsequent attempts of reobtaining previous tissue structure create the scar tissue.

Being a recurrent issue that all the world population is susceptible to has caused the WHO (WORLD HEALTH ORGANIZATION, 2017) to recognize the skin wound as a hidden epidemy. There is a huge concern about the efficiency and price of existing treatments, causing the necessity of research on new materials and methods.

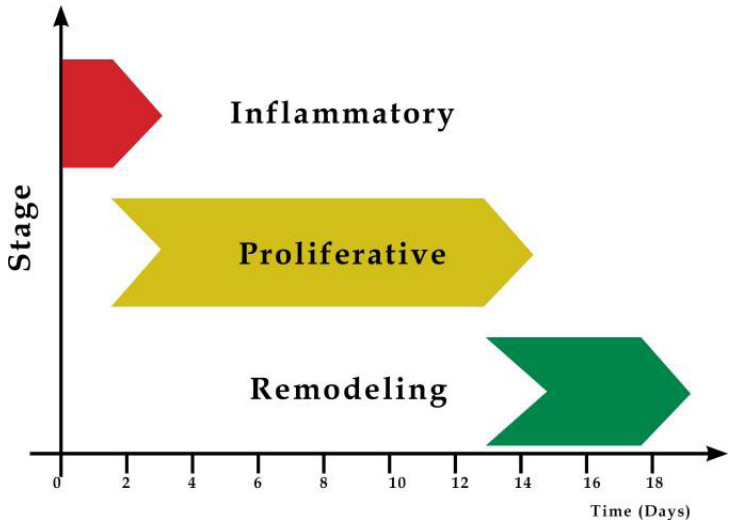


Figure 1: Stages of wound healing. Source: OLIVEIRA GONZALEZ, 2016.

1.2 Existing Treatments

The mainly employed treatment consists in the seaming (if needed) and appliance of dressings such as cotton gauze, preferentially with a nonadherent and antimicrobial

material applied, to prevent the removal of newly formed epithelial tissue and infections by external agents (ARAMWIT, 2016). It is common for it has low cost, although it's not the most efficient.

Another used method is the autografting, which consists of grafting skin tissue removed from another area of the same individual. This method has high efficiency but has a limit on how much skin can be grafted, plus the shortness on skin donations. This issue contributes the research and development of artificial tissue for grafting, although these researches face many obstacles, such as instability and risk of infection. The focus on material development is now in the biomaterials since they possess many properties that make them eligible in the health area. There are various biomaterials already present in research and healthcare market, from both synthetical or natural sources.

As representatives of the synthetical biopolymers in wound healing, we can find in literature: Polyurethanes (and derivatives), polytetrafluoroethylene (Teflon®), silicone, among others.

The natural biopolymers have furtherly more desirable properties, making them suitable for potential substitution of ECM (extracellular matrix). The shared properties they carry are: relieving effect, astringent, antimicrobial, anti-inflammatory, etc. These polymers are used as a coating, serving as protection from external agents, as well as stimulating the angiogenesis (cell proliferation). Another characteristic most biopolymers share is that they are negatively charged ions, except for one: chitosan. Chitosan is the deacetylated version of chitin, a biopolymer obtained throughout demineralization and deproteination of fishing industries waste such as shrimp and crab shells.

1.3 Chitin and Chitosan

Chitin (N-Acetyl β-D-Glucosamine) is the second most abundant structural biopolymer on Earth. In nature, it possesses two types of structural configuration: alpha and beta, being the first the one with higher mechanical resistance and the second the one with higher elasticity. A third allomorph can be created in a laboratory, mixing the previous configurations: gamma.

The worldwide fishing industries can sum about 10^{11} tons of waste and from that paradigm grew the interest of scientists to study and apply the biopolymer obtained from it in other sectors of interest, such as healthcare and cosmetics. Chitin is obtained from that waste, then suffers a process of deacetylation, which consists in a strong attack on acetyl groups, giving way to the amino groups to remain on the molecule surface, improving the biopolymer reactiveness. It is precisely that amino group the one responsible for the positive ionic charge of chitosan. This trait brings us an enormous amount of materials that can be complexed with chitosan, furtherly enhancing its properties and even giving it new ones (BROQUÁ, 2018).

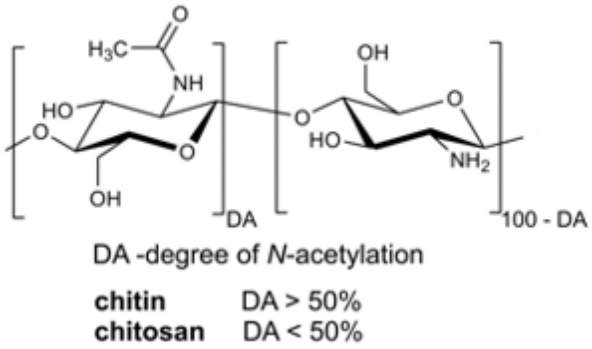


Figure 2: Representation of chitin and chitosan molecules. Source: KUMIRSKA et al, 2010.

As shown in this previous figure, chitosan is nothing more than the version of chitin with less than 50% of Degree of Acetylation (DA). The reduction of acetyl groups reduces the biopolymer's strength, but on the other hand, provides some more properties, which we can compare below.

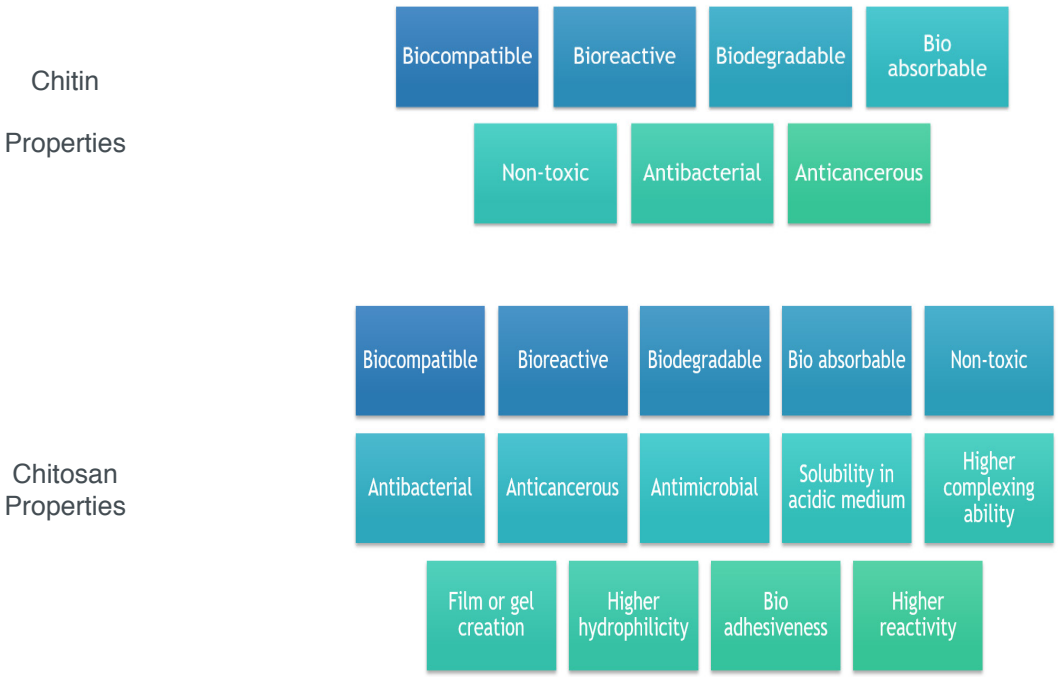


Figure 3: Board presenting chitin and chitosan properties. Source: FIGUEIREDO et al, 2018

These properties have been studied along many decades, yet the optimum chitosan is still to be developed since there are various conditions of extraction and production that affect the properties, thus being advised to assemble a process for each different necessity, using a different production method. The industrial production employs chemical agents in demineralization, deproteination, and deacetylation, which is a cheap and practical method but results in the production of effluents that will require treatment. There are studies regarding biotechnological means of production, that are not yet usable in mass production for they require higher control and time. The optimum

response could be the combination of both methods, using practicality and less aggressive materials to obtain the best both processes have to offer.

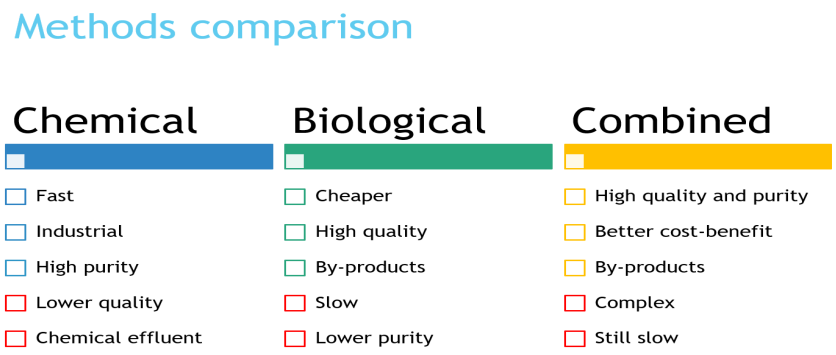
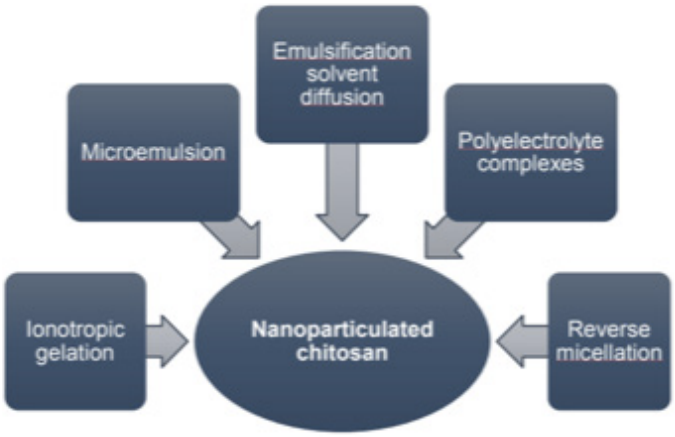


Figure 4: Board with a comparison between chitin production methods. Source: FIGUEIREDO et al, 2018.

Another tendency in researches regards the use of nanoparticulated materials. In the case of chitosan, there are some methods of nanoparticulated chitosan obtention. These methods work by amplifying the biopolymer's reactivity, enhancing previous properties even further and allowing new possibilities such as encapsulating other materials, which improves its capacity of complexation. Some of the methods found in the literature (DIVYA, 2018) are:



Nanomaterials are usually hard to obtain, the methods above do not run from that statement. One of the most accessible and operative methods in literature is the coagulation method (PIGHINELLI, 2016), the one we chose to apply in our studies of skin wound healing.

1.3.1 Chitosan Complexed Biomaterials

As a multi-property biopolymer that has the ability to link with other materials, chitosan is widely studied around the world, hence being the protagonist of various

projects involving complexed materials.

From literature, we can resume some of its applications regarding biotechnology, especially in the medical and pharmaceutical areas, in the following figure:

Biocatalysts	Scaffolds as a support for metals in order to produce catalysts.
Biomedical	Enzyme immobilization and purification chelator; an emulsifier; flocculent; blood cholesterol control; Lectin affinity chromatography; biosensor; immobilization of antibody in the presence of alginate; hemostatic agents.
Medical	Fibers; membranes; artificial organs and skin; surgical sutures; bone and cartilage regeneration; wound healing and dressings; cancer diagnosis; aid in cataract surgery; periodontal disease treatment; collagen synthesis; contact lenses; tumor therapy; stem cell technology.
Pharmaceutical	Manganese supplement complex; drug release; gene delivery.

Figure 6: a board with main chitosan applications in healthcare. Source: BROQUÁ et al, 2018.

According to Dhandayuthapani et al (2011), chitosan is mainly used as a scaffold, a 3D structure that serves as support to the complexed materials. There are many types of scaffold: Nano/microsphere encapsulation (bone and tissue regeneration), injectable gel scaffolds (cartilage and bone regeneration, drug delivery), hydrogel scaffolds (biosensors, matrix components and cell-cell interactions), fibrous scaffolds (vascular grafts, tissue scaffolds, etc.) and a group named functional scaffolds (includes membranes, foams, hydrogels, microspheres, and particles; used for angiogenesis stimuli, bone regeneration and wound healing).

The applications, as seen before, are nearly unlimited. Having such biopolymer with such vast applications, the next step in the development of a new biocomposite is to choose another material with the properties that are desirable for the product to be developed that is compatible with the positive electrochemical charge of the chitosan, thus being required a negative charge from the added component.

The Brazilian biodiversity is an example of a natural biopolymer source, especially in the Amazonian area (ROMERO, 2017). Among the supplies available due to the climate conditions of that area, the ones with high applicability in the healthcare area are the essential oils. These oils have been used by society for a long while, based on some sort of popular belief of it's healing properties.

The Buriti (*Mauritia Flexuosa*) essential oil has been used for wound healing in Brazil by its Amazonian population, and those healing properties have been confirmed in studies (SARAIVA, 2009). According to Freitas (2018), the Buriti essential oil can be encapsulated by isolated soy protein positively charged, under the PH of 4.6, corroborating the fact that the essential oil in question has a negative electrical charge due to its fatty acids.

2 I EXPERIMENTAL METHOD

The research and method were developed in Biomatter P&D and Innovation on Biomaterials Lab, Lutheran University of Brazil. The SENAI Institute of Innovation in Polymer Engineering and Advanced Microscopy Multiuser Laboratory (LMMA) in Piauí Federal University realized the analyses. The method of obtaining the Nanocrystalline Chitosan / Buriti Oil biocomposite (NCCH/B) occurred according to the patents: INBI BR 102017022292-6 and INBI BR 102017022720-0. The process can be divided into two steps, starting with the dissolution into a chitosan salt and then coagulation with Buriti oil.

2.1 Chitosan Dissolution

The commercial chitosan was purchased from Polymar (Fortaleza, CE) with a 95% degree of deacetylation, 12,4% moisture content, 0,31 g/mL density with a yellowish color. Firstly, 1% of the polymer powder was mixed with 500mL of deionized water. Acetic acid solution 0,4% (w/v) was prepared in half of the quantity of water and then added to the chitosan solution (2h, 600-800 RPM). The dissolution ends with 1L of a transparent solution. Afterward, the chitosan acetate (CA) was filtered to removing insoluble particles. The CA was stored at 5°C for 24h.

2.2 Nanocrystalline Chitosan / Buriti Oil Process of Obtaining

A new methodology was developed based on the agglutination method to synthesize the nanoparticulate chitosan (INBI BR 102017022292-6). A solution of NaOH was prepared according to to the same ratio of the acetic acid solution. Using the CA solution obtained, as previously described, the NaOH solution was gradually added with constant stirring (1000 RPM) and pH control. This is the crucial step of the process because both complexation and the nanostructured chitosan are obtained; therefore, all the parameters are extremally controlled for the correct formation of nanocrystals. In the pH 5, started the slow addition of Buriti oil in the same ratio of polymer content. This ratio was chosen to preserve the integrity of the oil, which has pH 4,5. After all the oil was added, at the same polymer content, the acidic solution was neutralized to pH 7,4 (3h, 700 RPM). The final solution (NCCH/B) was stored at 5°C for 24h. The sodium hydroxide has the function of agglomerating the glucosamine monomers in the chitosan acetate in a nanostructured matrix, neutralizing the solution at the same time. The free oil in the solution is encapsulated while the coagulation occurs. The NCCH/B solution was filtered and washed until the discarded water becomes neutral, in order to eliminate the residual sodium acetate salt that was formed in the neutralization phase. It was no observed residual oil in the washing process. Thereafter, 10 samples with 25mL of NCCH/B biocomposite was added in Petri plates to dry for the posterior analyses and a high concentration solution was stored at 5°C.

3 I RESULTS AND DISCUSSION

3.1 Nanocrystalline Chitosan / Buriti Oil Biocomposite

This methodology was performed using three different concentrations of oil to obtain the biocomposite: 0,5%, 1%, and 2%. The 1% solution was chosen to continue the studies because it was homogeneous, with no phase segregation or migration of the oil to the film surface and with the complete encapsulation of the oil by the nano-chitosan. The NCCH/B films had a yellowish and orange color, according to the Buriti oil ratio, and the solution with a gel texture (Figure 7).

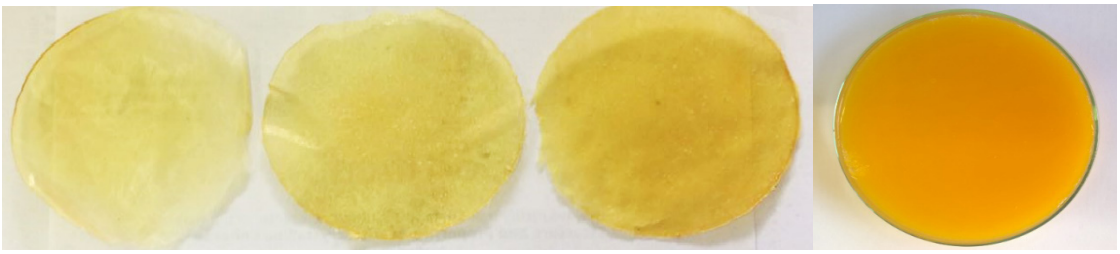


Figure 7: NCCH/B films with 1:0,5, 1:1 and 1:2 of Buriti oil and polymer content, and NCCH/B gel-like solution, respectively. Source: FIGUEIREDO et al, 2018.The synthesis of NCCH/B biocomposite is not characterized as an ordinary mixture of biopolymer and oil, but a new method of obtaining the material studied. Since this composite has been obtained in an aqueous medium and the difference of polarity, composition of the substances, different densities and structures support the idea of complexation. Buriti oil presents in its composition many fatty acids containing ions compatible with the amine group from chitosan, which justifies the complexation with one or more of these fatty acids, still not identified.

3.2 Fourier-Transform Infrared Spectroscopy (FTIR-ATR)

Figure 9 represents the spectra of nanocrystalline chitosan (NCCH). The presence of the functional groups NH_2 (1590 cm^{-1}), amide I (1746 cm^{-1}), amide II (1419 cm^{-1}) from the NCCH is observed. The most intense energy peaks (1026 and 938 cm^{-1}) identified the COC (glucose-β-1-4) stretch, corresponding to the vibrations of the skeletal stretch of the polysaccharide structure. N-H and O-H energy peaks are also observed at 3357 cm^{-1} and 3287 cm^{-1}.

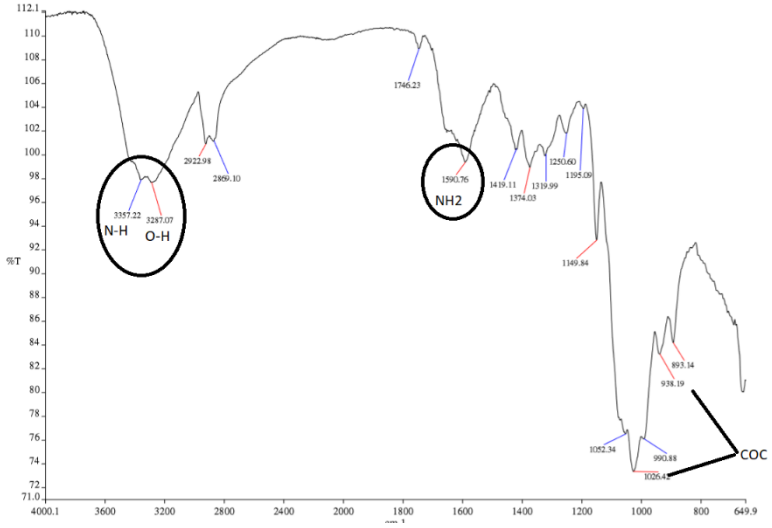


Figure 9: FTIR spectra of NCCH. Source: PIGHINELLI, 2016.

Figure 10 represents the spectra of Buriti oil, with a band formed in the region near 1750 cm^{-1}, where the C=O bond is observed. This band is a hallmark of the oils spectra. Figure 11 represents the spectra of NCCH/B biocomposite, where it is observed that the band near 1750 cm^{-1}, characteristic of the bond C=O of Buriti oil, remained with some important peaks of nano-chitosan, as the functional group NH_2 (near 1600 cm^{-1}). Other important peaks of NCCH were observed with small displacements such as (1100 cm^{-1} and 1050 cm^{-1}) that identified COC (glucose-β-1-4) stretch corresponding to the stretching vibrations of the polysaccharide backbone structure. N-H and O-H near 3500 cm^{-1} are also observed.

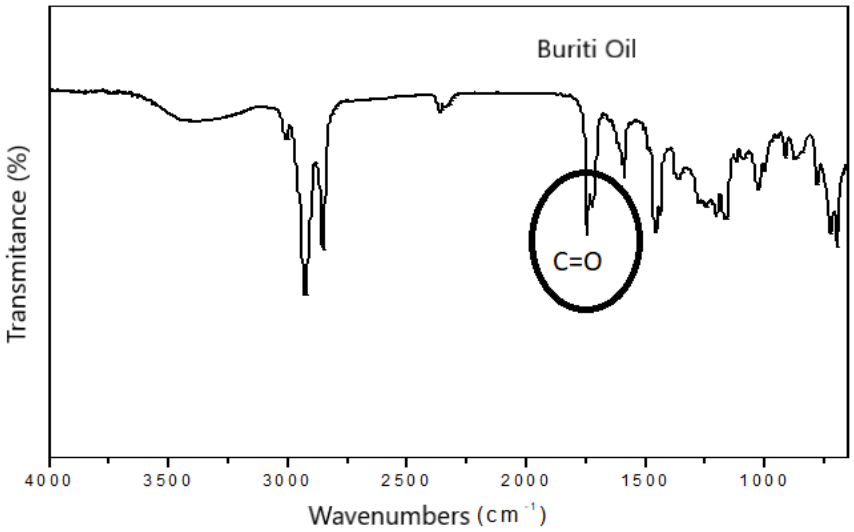


Figure 10: FTIR spectra of Buriti oil. Source: FIGUEIREDO et al, 2018.

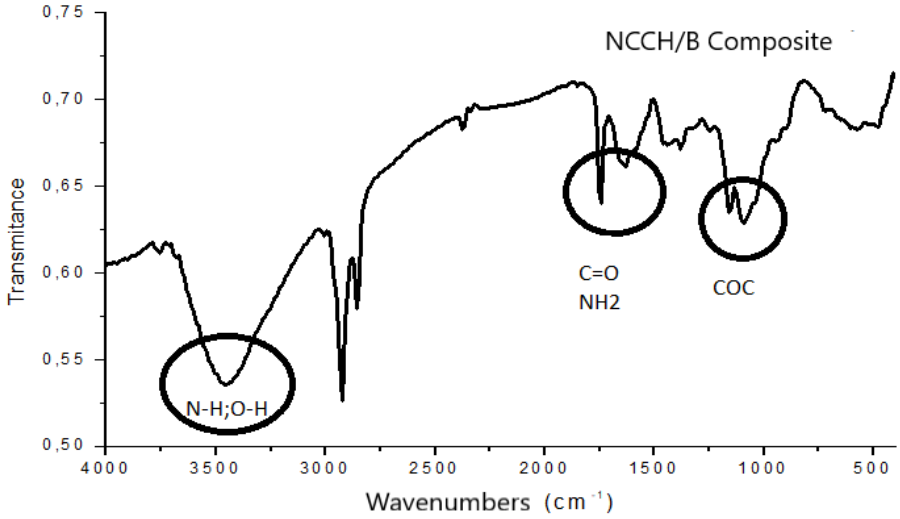


Figure 11: FTIR spectra of NCCH/B biocomposite. Source: FIGUEIREDO et al, 2018.

3.3 Scanning Electron Spectroscopy (SEM) Analysis

The SEM analysis of nanoparticulate chitosan (Figure 12-a) are homogeneous, with roughness and formation of agglomerates due to their amorphous nature, whereas the SEM analysis of NCCH/B biocomposite shows a velvety area related to the oil (Figure 12-b), with many agglomerates of NCCH due to high polydispersity (Figure 12-c).

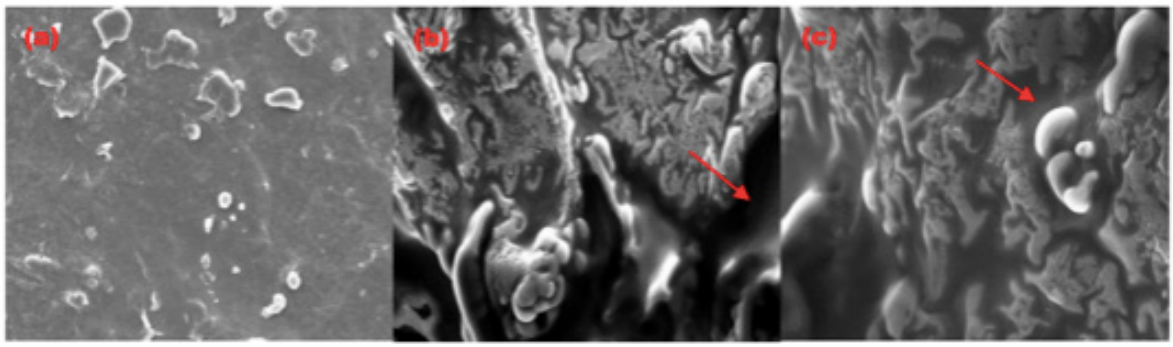


Figure 12: (a) The SEM analysis of NCCH in x500 magnitude, while (b) and (c) are the SEM analysis of NCCH / B biocomposite in x500 and x1000, respectively. Source: FIGUEIREDO et al, 2018.

The nanostructured chitosan is more reactive, with high absorption potential up to 600% (PIGHINELLI, 2017)proliferation (fibroblast phase. Due to the process, the glucosamine macromolecule breakage occurs in dissolution, and then reconstructed in the coagulation process, with a high reduction of the crystalline area and improvement of the amorphous area. This crystalline modification allows the improvement of some characteristics, such as reactivity and adhesion potential.

In NCCH/B biocomposite images, it can be noticed that the chitosan nanoparticles remain intact, wrapped by Buriti oil outside the capsules. It is also observed a swelling effect in the nanoparticles, suggesting the encapsulation of the oil by the polymer,

based on literature review.

3.4 Particle Size

Particle Size analyses were performed in NCCH 1% and NCCH/B 1:1 for comparison. A Mesh 0,450 filter was used in order to reduce the high polydispersity of the nanoparticles and standardize the particle size. Furthermore, were performed six measures for each, and calculated the average value of the results. The particle size of nanoparticulate chitosan originated from agglomeration process ranges from 20 nm for 120 nm, with an average of 55,4 nm (PIGHINELLI, 2017) proliferation (fibroblast phase. The results are illustrated in Table x. The NCCH has an average of 85 nm, while the NCCH/B biocomposite has an average of 134 nm.

SAMPLES	MEASURES	PARTICLE SIZE (nm)	POLYDISPERSITY
NCCH	1	80,93	0,370
NCCH	2	96,06	0,766
NCCH	3	57,91	0,923
NCCH	4	123,91	0,338
NCCH	5	65,63	0,353
NCCH	6	85,56	0,550
NCCH	**AVERAGE**	**85,00**	**0,550**
NCCH/B	1	171,57	0,312
NCCH/B	2	197,56	0,278
NCCH/B	3	95,43	0,215
NCCH/B	4	112,65	0,271
NCCH/B	5	98,71	0,284
NCCH/B	6	126,51	0,248
NCCH/B	**AVERAGE**	**134,00**	**0,268**

Table 1: Particle Size results of NCCH and NCCH/B biocomposite. Source: FIGUEIREDO et al, 2018.

It is noted an increase in particle sizes of NCCH/B biocomposite (134 nm) when compared to NCCH (85 nm). This phenomenon suggests the encapsulation of the oil, already studied and performed in literature, with Souza *et al.* (2014) and Hosseini *et al.* (2013) works. Freitas (2016) had a similar average of particle size using Zein, chitosan, and essential oils particles, with the same increase phenomenon, compared to the single chitosan. It's still possible to observe a reduction of polydispersity in NCCH/B, from 0,550 to 0,268, suggesting more stability in NCCH/B biocomposite solution.

3.5 Microbiological Test

The microbiological tests were carried out in the ULBRA Canoas, Microbiology Lab, using the macrodilution technique. Increasing amounts of each of the samples

analyzed were used to determine the Minimum Bactericidal Concentration (MBC). The macrodilution method was applied by the Microbiology Laboratory in a way it could be applied to the biopolymers potential properties appointed by the literature. The selected bacteria were: *Escherichia coli* (as a Gram-negative) and *Staphylococcus aureus* (as a Gram-positive). For this test, three different analyses were performed: nanocrystalline chitosan and Buriti oil alone, and NCCH/B biocomposite. All these samples for the assays were pre-sterilized in an autoclave at 121° C for 15 minutes, as well as all materials used and culture media, to avoid interference of other bacteria from the environment. The technical procedures were performed according to the Quality Criteria for Microbiological Analysis (OPLUSTIL, 2010).

3.5.1 Inoculum preparation

The bacterial strains references used were Staphylococcus aureus (ATCC 25922) and Escherichia coli (ATCC 25923). Lyophilized bacteria were prepared according to the manufacturer's recommendation. Afterward, they were sown in a petri dish containing TSA Agar and incubated at 35ºC for 24h (OPLUSTIL, 2010).

3.5.2 Macrodilution method

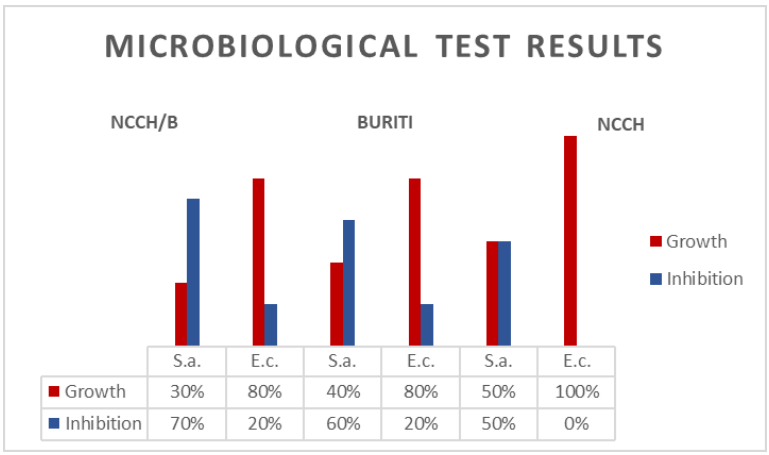
The dilution method used two series of 10 Erlenmeyer flasks with BHI (Brain and heart Infusion) culture medium for each of the 3 samples (Buriti, NCCH, and NCCH/B) containing 1.0; 2.0; 3.0; 4.0; 5.0 ... to 10 ml of samples in each of the bottles of the respective sequence. In the first Erlenmeyer, 1 ml of sample and 18 ml of BHI broth was pipetted. In the second Erlenmeyer, 2 ml of sample and 17 ml of BHI were pipetted, successively, always increasing 1 ml of sample to each new flask and reducing 1 ml in the broth. Afterward, 1ml of the bacterial suspension was added to the first flask. The flask n°1 was thoroughly homogenized and 1 ml was collected to be transferred to flask n°2. After homogenizing the flask n°2 as well, 1 ml was transferred to flask n°3 and so on until flask n°10. This procedure was carried out for each of the samples, in two series, one of which was inoculated with 1 ml of reference *Escherichia coli* and the other with *Staphylococcus aureus*.

All flasks were incubated at 35 °C for 24h. Visual observation of growth after incubation was impaired by the turbidity of some samples, and all samples were seeded on a PCA plate. The plates were incubated in a bacteriological oven at 35 °C for 24 hours and then, analysis occurred with the presence or absence of bacterial growth.

3.5.3 Microbiological Test Results

Following the described methodology, the results are presented in Figure 13 containing the NCCH/B biocomposite and both standards NCCH and Buriti oil. Reduction

of growth from n°7 vial plate onward was observed for all positive samples, regardless of the bacterium tested. This decrease occurred around 20 to 30% of the growth for all samples tested. In these plates, it was not possible to count because the number of colonies was much more than 300 CFU (Colony Forming Unit)/ml. No growth was observed from vial n°4 to *S. aureus* of NCCH/B, from vial n°5 of Buriti oil and vial n°6 of NCCH samples. However, the absence of growth for *E. coli* occurred from n°9 for the NCCH/B and Buriti oil samples. In this study, for the NCCH sample, it was not possible to determine the MBC (Minimum Bactericidal Count) for *Escherichia coli,* probably due to the distinct mechanism of action, since it's a Gram-negative bacterium with a complex cell wall structure different from Gram-positive. As approached in literature, chitosan has a more antibacterial effect on Gram-negative bacteria, due to the positive surface provided from free amino groups and nanoparticulate chitosan has more bacteriostatic behavior (YOUNES et al, 2014). Due to the personalized microbiological test that was required to attend the potential of the biocomposite, the bacteriostatic behavior of nanochitosan couldn't achieve. The best results were obtained from NCCH/B biocomposite when compared to the standards NCCH and oil, as observed in Figure 13, with bacteriostatic effect identified in flasks with smaller amounts of the compound.



	S.a.	E.c.	S.a.	E.c.	S.a.	E.c.
Growth	30%	80%	40%	80%	50%	100%
Inhibition	70%	20%	60%	20%	50%	0%

Figure 13: a graphical comparison of microbiological activity. Source: FIGUEIREDO et al, 2018.

4 I CONCLUSION

Nanoparticulate chitosan has high polydispersity, reactivity and absorption potential due to its low crystallinity. This property allows the creation of new materials, such as the Buriti oil complexed with the biopolymer. The main of this work was to develop a new method of obtaining a biocomposite material through a new process, for application in regenerative medicine as a wound healing.

In the FTIR analyses, it was noticed that the characteristic peaks of both chitosan and Buriti oil are present in the NCCH/B, with preservation of the original structures, without a new different formation.

The SEM analyses showed characteristic agglomerations of chitosan,

an expected phenomenon due to the amorphous structure of nanoparticulate chitosan, and a velvety area related to the remaining oil was observed. The "swelling" effect of nanocrystalline chitosan suggests the encapsulation of the oil by the polymer, and the reduction of polydispersity is considerable. in Particle Size analyses, nanocrystalline chitosan showed a lower ratio compared to the biocomposite, still in nanoscale. This increase is interpreted as the encapsulation again. The reduction of polydispersity in NCCH/B biocomposite indicates more stability for the material.

It is still necessary to determine which specific group of the oil is bounding with the free amino groups of chitosan to assert as a complex indeed.

The Microbiological Tests indicated more inhibition of bacterial growth in the biocomposite than the standard chitosan and Buriti oil for *Staphylococcus aureus*. The low effect in *Escherichia coli*, as mentioned before, is a behavior of chitosan. In nanocrystalline chitosan, we have a bacteriostatic effect on Gram-negative bacteria. This observation corroborates with the complexation that this research approaches.

More analyses are being provided to characterize the complexation. *In vivo* tests are scheduled to confirm the healing potential and move a step forward as a new product.

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