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Department of Science and Engineering of Oxide Materials and Nanomaterials

PhD Thesis

Therapeutic biomaterials for chronic wound healing

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Literature review – Introduction

The human body is covered by skin, which holds a very special role. It is the first layer of contact with the environment and represents around 16% of the total body weight [1,2]. Blood vascularization represents a very important human body component which has a lot of functions. In terms of skin, the vessels provide nutrients, deliver oxygen, control temperature, are involved in fluid homeostasis and regulate the immunologic series of action as well [3].

Any defect that occurs at the skin level which has a disrupting effect in the continuity of the cutaneous layers, is defined as a wound. A wound may appear because of many factors such as injuries, surgery, mechanical accidents – pressure, cuts, burns, or due to the host pathologic issues, the most common being diabetes or microcirculation. Following the appearance of a wound, it can be categorized as acute or chronic depending on certain specific criteria. By analyzing the sizes, depths, and degree of the wounds, the classification of the wounds can be done in the first phase. Conditional on these results, the wounds can heal in a normal and necessary time for an acute wound, or they can extend over long periods of time, becoming chronic. Chronic wounds fail to recover because of perpetual wound pathology, high-effect trauma, vascular disease, or because additional conditions arise due to the inefficiency of natural and anatomical healing action, such as infections or low levels of oxygen (ischemia) transported through the blood vessels [4-11].

Wound infection represents a host inflammatory response when the affected area interferes with microorganisms and impedes wound healing. Inflammation appears as the natural vascular response of the body when the tissue is physically, biologically, or chemically harmful and is the first attempt to avoid wound infection. Any modification of the integrity of the skin due to wounds, needle injection, surgery, lacerations, burns, and scratches is an “open door” for the normal microbiome found at the skin surface to

enter and develop a soft tissue infection. If infections can occur due to the normal microbiome found on the surface of the skin, then in the case of a chronic wound that fails to heal, much more serious complications are present. In chronic wounds, the affected area is exposed to numerous pathogens supporting microbial colonization. Also, the conditions of chronic wounds, such as low oxygenation, necrotic tissue and diminished host immune response, allow bacterial growth. The chronicity of the wound is influenced by the level of microbial colonization, which causes the infection to persist for certain periods [12-15].

The main role and principle of wound dressings are to offer assistance to the wound environment by supporting debridement, granulation tissue formation, regulating exudate level, to provide a protective layer, maintaining a moist environment, and offering sustenance for organism action against microorganisms and a shield opposed to those that could develop extra, as well. All these functions of the dressings facilitate the wound healing process in order to finally restore the original characteristics of the skin [16]. Just as in the case of wound type, these are classified as acute or chronic and respectively in several types depending on their characteristics, and the same is true in the case of dressings where there is a classification with large variations that depend on the needs of wound healing. Considering the fact that the wound healing mechanism is known in detail regardless of the type of wound and the fact that the variety of dressings is limitless, it is extremely important that the choice of dressing be appropriate after evaluating the characteristics of the wound. This discussion is particularly based on chronic wound healing due to the long time of the healing process that comes along with a multitude of other issues dependent on it. In the case of acute wounds, the body succeeds in managing this situation much more elegantly compared to chronic wounds, and choosing an optimal dressing is not exactly a challenge [17-20].

Nanotechnology includes the intensifying interest in the study of the synthesis, structure, and dominant imposing development and evolution of atomic and molecular

nanoparticles. By using nanoparticles in a specific context, nanoproducts are developed, which are considered nowadays, smart systems used in numerous medical applications due to their physicochemical features based on particle surfaces that increase exponentially while their volume decreases. However, silver nanoparticles were, are, and will be those that are loyal to their activity but deceptive for a varied range of microorganisms [1, 21-23]. Another characteristic of silver nanoparticles is their anti-inflammatory role when used in a wound dressing. The anti-inflammatory properties of silver nanoparticles make them effective in wound healing when applied topically as a dressing component. By reducing the release of cytokines and lymphocytes and inhibiting mast cell infiltration, silver nanoparticles contribute to wound healing with the slightest scarring. Additionally, in diabetic wounds, silver nanoparticles have been shown to accelerate the healing process by stimulating the proliferation and migration of keratinocytes while also promoting the differentiation of fibroblasts into myofibroblasts, which leads to wound contraction and faster healing of diabetic ulcers [24-27].

Chronic wounds, which fail to heal within the typical timeframe, often exhibit a debilitating condition known as hypoxia. This occurs when wounds do not receive sufficient oxygen, hindering the body's natural healing process. A range of underlying factors can contribute to the development of chronic wounds, including: Impaired blood flow, which reduces the delivery of oxygen and nutrients to the wound tissue; Inflammation which can lead to increased permeability of blood vessels, causing fluid leakage and reduced blood flow to the wound area; Large or deep wounds can have limited access to oxygen, making it difficult for the wound tissue to heal; Tissue itself is unable to extract oxygen from the blood due to damage to the capillaries or microvasculature; Hormonal changes, such as those seen in diabetes, can impair blood flow and reduce oxygen delivery to the wound; Nerve damage can disrupt blood flow and reduce sensation, making it difficult to detect signs of hypoxia. Moreover, there are harmful consequences of hypoxia in chronic wounds including delayed healing,

increased risk of infection, tissue damage, increased risk of amputation, pain and discomfort, mental health impact [28-32]. Therefore, the need to incorporate different systems that can facilitate the healing of wounds by releasing oxygen to overcome hypoxia, is in the attention of researchers in the field. Different methods can be approached in this sense, but in terms of the field of nanotechnology, calcium peroxide nanoparticles are recognized for their activity. When exposed to moisture, represented by the wound fluid, calcium peroxide nanoparticles release oxygen which has multiple benefits in wound healing process. Moreover, these nanoparticles have an increased activity in acidic conditions which is extremely suitable for chronic wound healing regarding their acidic environment [33-36].

Personal contributions

Objectives and originality of the doctoral thesis

The current state of research on dressing-type materials for treating chronic wounds is characterized by ease of access, accessibility, and browsability. This means that relevant information on developing novel wound dressings is readily available, allowing for a seamless exploration of existing research in the field. This awareness brings a dual advantage to the researchers: (1) The recognition that complete information is unattainable in the field of wound dressing research, acknowledging inherent limitations. From (1) being correlated to the second fact, namely that: (2) This realization can spark mental stimulation, encouraging researchers to think creatively and develop innovative solutions to overcome the challenges that remain unsolved.

Following the intensive review of the specialized literature regarding the biological processes that occur when a defect appears on the skin and cannot be treated by the body's automatic self-healing due to chronicity, two primary approaches that have been shown to have the ability to greatly enhance the wound healing process. These solutions are rooted in the body's natural physiological processes that occur during wound healing, but in severe chronic cases, attempting to restore the skin's functions poses a significant risk of failure, leading to undesirable outcomes for patients. The proposed solutions focus on providing two essential elements: delivering additional

oxygen to the wound site and providing specialized protection against pathogenic microorganisms. In this regard, three fundamental objectives were pursued:

1. Testing the capacity of a hydrogel dressing to release oxygen in the context of a chronic wound that does not have the ability to heal. An alginate-based hydrogel was conceptualized, synthesized, and tested, containing PLA microspheres encapsulating hydrogen peroxide that were uniformly distributed within the polymeric matrix. This was the first attempt to perform a controlled release of oxygen due to PLA oxygen permeability.
2. Testing the ability of a hydrogel type dressing to protect against bacteria as well as the capacity to lessen infection that has previously been experienced in chronic wound cases. Using a cross-shaped chip and a microfluidic technique, a sequence of silver nanoparticles were produced, having control over their final properties due to the modern synthesis method. The antibacterial efficacy of silver was evaluated in the context of a composite hydrogel comprising alginate and hyaluronic acid to assess its performance in this specific formulation.
3. Investigating the capacity of a hydrogel dressing to deliver sustained oxygen release and prevent the formation of bacterial biofilms, ensuring optimal wound healing and infection control. A novel alginate-based hydrogel featuring dual functionality was designed and evaluated. The incorporation of calcium peroxide nanoparticles, synthesized via the precipitation method, enabled the controlled release of oxygen. Additionally, the incorporation of silver nanoparticles, synthesized using microfluidic technology, provided antimicrobial properties to control infection.

To achieve the desired outcomes, detailed plans and visions for the synthesis processes were developed to optimize the properties of the dressing materials. Accurate control over the final characteristics of the materials was made possible by the use of laboratory apparatus such as microfluidic platforms, baths, peristaltic pumps, and ultrasonic probes. These advanced techniques are characteristic of modern materials development, particularly in the field of nanomaterials. Crucial factors such as particle size uniformity, morphology, charge, stability, and multiple properties were carefully considered to ensure high-performance and efficient materials for wound therapy applications. Even if a material has a long history of use in a particular field, it's not

necessarily a guarantee that it has exhausted its potential for innovation. The constant emergence of new synthesis methods and techniques can lead to modifying existing materials, allowing them to be improved or optimized. This ongoing evolution ensures that even seemingly well-established materials can continue to be innovative and original. Incorporating calcium peroxide into hydrogels is a relatively new development, and using microfluidic synthesis methods is a cutting-edge technique in modern engineering. To our knowledge, however, no published record of combining calcium peroxide nanoparticles with silver nanoparticles in the same formulation for a dressing-type material makes this a novel and unprecedented approach.

General Conclusions

The engineering field of developing new dressing-type materials with optimized and advanced properties is still a prominent area of scientific inquiry, driven by the need to address the complex challenges associated with the wound-healing process. Thanks to the availability of advanced technology and vast amounts of information, it was possible to unravel the intricate processes and factors that occur when a skin wound is produced and the subsequent body's healing response from the beginning, progression, and ends. As a result, it has been determined that ideal dressings must possess specific properties that support and enhance the body's natural wound-healing abilities, thereby facilitating the implicit healing mechanism. The following qualities are essential for the perfect dressing: biocompatibility, maintenance of a moist environment, absorption of excess fluids, antimicrobial protection, biodegradability, promotion of tissue healing, oxygen permeability and supply, diminished side effects, less scarring, pain reduction, minimal frequency of dressing change, ease of application and removal, comfort, cost-effectiveness.

Regarding these specific demands, the objective of the doctoral thesis was to design and create novel improvement dressings that closely meet the requirements for optimal and successful wound healing.

The first scientifically engineered study focused on the development and evaluation of a novel experimental dressing, comprising an alginate-based hydrogel complexed with Matrigel and polymeric microspheres that encapsulate hydrogen peroxide. These microspheres are designed to release oxygen at the site of ulceration, fostering a healing environment.

The microspheres were synthesized by preparing a microemulsion based on polylactic acid (PLA) - the hydrophobic component, and polyvinyl alcohol (PVA) - the hydrophilic part with the addition of hydrogen peroxide (H_2O_2). The microemulsion containing H_2O_2 was subjected to the ultrasound probe for microspheres creation. A washing procedure and a centrifugation process were applied to the sonicated solution, obtaining a precipitate referred to as OMs. The hydrogel formulations are based on alginate as the main matrix, consisting of a 5 mg/mL solution. This formulation serves as the control and is referred to as HG. The control hydrogel (HG) is embedded with OMs, resulting in the formation of the HG_OMs hydrogel. A similar protocol was employed to produce the HG_OMs_MG hydrogel, with the sole distinction being the inclusion of Matrigel in this sample. All hydrogel formulations were cross-linked with calcium chloride solution. The following investigation techniques were used to identify and establish the physicochemical properties of the dressings that were obtained: FTIR, SEM, FT-ICR, and swelling and degradation rate. The biological evaluation involved in vivo testing of the dressings on murine animal models. Diabetic animal models were created by administering streptozotocin, allowing for the evaluation of the efficacy of hydrogels in treating wounds with impaired healing, such as diabetic ulcers, which are notorious for their poor healing outcomes.

FTIR analysis was performed on HG, OMs, and HG_OMs as well, in order to include or exclude the presence of the functional group corresponding to the specific vibrational bands. Due to the high hydrophilicity of the samples, an abundant OH group is identified between 3100 and 3400 cm^{-1}. Moreover, the C=O chemical group characteristics for PLA and COO^{-} moieties from alginate are identified at the absorption bands at 1500 and 1750 cm^{-1}, respectively. The integration of OMs through the HG matrix was confirmed by the FTIR spectra results. SEM investigation results confirmed that the OMs formation was successful, highlighting clear round spheres with dimensions between 1 and 3 μm. The micrographs recorded for the hydrogels establish their macroporous structure with pore sizes that vary between 79 (for HG control sample) and 110 (for HG_OMs sample) μm. Distinct differences are observed between the HG and HG_OMs formulations because of the existence of the OMs on the surface and through the entire alginate matrix showing uniform distribution. In order to quantify the amount of H_2O_2, which, by releasing the healing process, is improved due to the influence on the cellular function and proliferation support, FT-ICR analysis was used. By reference to the calibration curve, a value of approximately 1.97 ppm was determined. The amount of

H_2O_2 in the healing process should be low but sufficient for restoring the angiogenesis process, which is stopped by the chronic hypoxia observed, especially in diabetic ulcers. The moisture absorption capacity of the hydrogels was assessed under similar ionic concentrations of blood plasma using SBF prepared by the Kokubo's protocol. HG and HG_OMs samples demonstrate proper ability to hold onto fluids from the initial minutes of contact with the SBF solution, and after 24 hours of immersion, a maximal behavior was recorded at around 200% for HG_OMs and 160% for HG. Following this, a decline in the rate of swelling was noted due to the onset of the degradation process, which was evaluated after 24 and 48 hours. A reduced degradation behavior was observed for the HG_OMs sample due to the stability offered by the OMs presence through the alginate matrix. However, the dressings present enhanced fluid retention capacity and moderate degradation rate due to the materials used and porous structure. The healing property of the obtained dressings was assessed on full-thickness dermal wounds on murine models with diabetic-induced disorder. The time-dependent action treatment was established for 3 and 7 days, followed by histopathological analysis. The histopathological results and discussion were made by comparing the dressing-free wound with the other treated wounds with proposed formulations. Within a span of 3 days, the application of HG dressing led to a significant decrease in localized swelling and the presence of cells associated with inflammation. Additionally, after 7 days, new outer skin layer was formed and a greater number of blood vessels were seen. After applying the HG_OMs hydrogel dressing, the wound showed no signs of edema and had a notable decrease in inflammation over a span of 3 days. After a much longer contact (7 days) of the wound with the hydrogel embedding H_2O_2-PLA microspheres, the histopathological result indicates that capillaries are formed and uniformly distributed, as well as the presence of collagen fibers. The superior outcomes achieved in this group (wounds treated with HG_OMs) suggest that the oxygen released from the microspheres played a significant role in promoting these beneficial effects, considering the beneficial effects of oxygen in promoting angiogenesis and collagen synthesis. In addition, the incorporation of Matrigel into the HG_OMs dressing facilitates the formation of distinct granulation tissue and new capillaries over a span of 3 days. This is due to the structural reinforcement and introduction of biomolecules supplied by Matrigel. After 7 days of treatment, a normal and complete epidermal layer and a denser collagen network were observed. In addition, the process of keratinization occurred exclusively in the epidermis that was connected with the wound treated with HG_OMs_MG.

The second methodology employed in the PhD thesis involves creating wound dressings utilizing a microfluidic technique using a cross-shaped chip to produce silver nanoparticles incorporated into alginate-hyaluronic acid materials. These types of dressings are used especially on infected chronic wounds due to the significant efficacy of silver nanoparticles. However, the impact of silver nanoparticles on the healing process is enhanced by their physicochemical features, including size, shape, surface charge, and hydrodynamic diameter. This improvement occurs without any added complications. In this sense, a new and facile method to synthesize silver nanoparticles is represented by the microfluidic platform, which offers controllable parameters and, subsequently controllable characteristics of the particles.

The synthesis process involves the utilization of a peristaltic pump, which enables the accurate manipulation of input variables, such as the volume of solutions required for nanoparticles formation, by setting the rotations per minute (RPM) values; a microfluidic platform made by PMMA, designed and obtained by a CNC cutting laser equipment, which represents the core of the silver nanoparticle synthesis; circulation hoses connected to the pomp and chip that supply the metallic precursor solution and the organic reduction solution. Four RPM inputs were set for silver nanoparticles synthesis expressed for $AgNO_3$ solution and reducing solution as well (15 – 15, 15 – 30, 30 – 15, 10 – 15; $AgNO_3$ – 2 x organic solution). The side inputs (x2 because of the cross-shape) were intended for the organic reducing solution, and the central input was designed for the $AgNO_3$ solution. Two distinct batches of silver nanoparticles were synthesized, differing only in the concentration of the silver precursor – $AgNO_3$. The initial series was acquired utilizing a solution having a concentration of 0. 5 weight percent $AgNO_3$, however the subsequent series was obtained utilizing a solution having a concentration of 0.25 weight percent $AgNO_3$. The physicochemical properties of all silver samples were analyzed (XRD, SEM, DLS, TEM), and subsequent evaluation of the results led to the identification of the most optimal sample, which was deemed to possess the most desirable properties. This sample (Ag(0.25_15)) was subsequently incorporated into an alginate-hyaluronic acid-based dressing and underwent further evaluation, both physico-chemically and biologically (FTIR, SEM, TG-DSC, swelling and degradation rate, antimicrobial assessment, as well as biological assessment). For comparison between the properties of the dressings, three samples were obtained: Alg – the control alginate hydrogel; Alg_HA – the composite hydrogel of alginate and hyaluronic acid; Alg_HA_S

– the similar constituents with the second sample, but with the addition of the silver nanoparticles selected sample.

The XRD data obtained for the 8 silver nanoparticles samples clearly indicate the silver as a single phase with a cubic crystallographic system and well-crystalized properties. The crystallite size was calculated, and sample differences were identified, concluding that the utilization of a lower concentration of $AgNO_3$ induced a smaller crystallite size. SEM micrographs obtained for all silver samples show differences between the morphology and size uniformity depending on the precursor concentration solution. Series 2 (0.25% $AgNO_3$) presents a decrease in the size of the nanoparticles, a more spherical appearance of the morphology, and uniformity in terms of size. Furthermore, TEM micrographs corroborated the data on how the concentration of AgNO3 affects the shape and dimensions of the silver nanoparticles, as well as the crystal structure of the samples (as shown by the SAED pattern), as observed in the XRD results. The results acquired using Dynamic Light Scattering (DLS) indicated the presence of a negative charge and the stability of the nanoparticle solution based on the zeta potential measurements. The hydrodynamic radius values were in the range of 103 – 184 nanometers for series 1, and between 58 – 148 nanometers for series 2, highlighting again the influence of the precursor concentration solution and the correlation with the physical sizes measured from the electron microscopy images. According to the results, the sample with the most favorable physicochemical properties is part of series 2, where a lower concentration of the silver precursor solution was used. The subsequent step involved incorporating the selected sample into an alginate-hyaluronic acid-based hydrogel and conducting a thorough evaluation to assess its potential as a treatment solution for combating infections and promoting wound healing. The hydrophilic character and the intention to form a composite material is confirmed with the results of the FTIR analysis in which the functional groups attributed to OH are observed in all samples around 3200 cm^{-1} , and C – H stretching at 2907 $cm^{-1,}$ respectively, as a confirmation for the hyaluronic alginate acid. SEM micrographs support the porous properties of the dressings that were obtained and indicate differences between the Alg and Alg_HA samples in terms of pore size and appearance. The addition of hyaluronic acid offers an intensified presence of pores and more elongated shapes. Furthermore, the Alg_S and Alg_HA_S samples exhibit discernible differences in the evenness of silver nanoparticle dispersion due to the presence of hyaluronic acid. The inclusion of hyaluronic acid has a beneficial effect on both the skin and the healing process, as well as on the overall physical and chemical

characteristics of the dressings. The TG-DSC analysis results highlight the decomposition process appearing at a specific temperature, and finally, the residual mass was used to indicate the silver content in each sample (1.27% for Alg_S, 0.36% for Alg_HA_S). The swelling capacity of the hydrogels was evaluated, and it was reported that from the first minutes of immersion, a significant rate of absorption was registered, and these values were maintained for approximately 72 hours when they began to decrease as an effect of the degradation process. The antibacterial characteristics of the synthesized dressings were assessed based on their ability to inhibit the formation of biofilms. The antibacterial activity of silver nanoparticles was evaluated by exposing two bacterial strains, S. aureus and E. coli, to the dressings for 24, 48, and 72 hours. Alg_S and Alg_HA_S dressings induce a diminishing biofilm formation, especially on the Gram-positive strain, and are maintained at all testing times. Moreover, the implications of hyaluronic acid were also observed, due to its bacteriostatic effect, the Alg_HA_S sample shows much lower colony-forming units compared to the Alg_S sample on both types of bacteria. The biocompatibility of the samples was evaluated both qualitatively and quantitatively using HaCaT keratinocytes, which were incubated with the materials for 24 hours. Fluorescence microscopy revealed that the keratinocytes in contact with the materials maintained healthy actin cytoskeleton morphology, indicating biocompatibility. The synthesized materials demonstrated the ability to stimulate cell proliferation, and lactate dehydrogenase (LDH) leakage was minimal, indicating the integrity of cell membranes. This indicates that these materials exhibit several favorable characteristics of an optimal dressing, rendering them a prospective remedy for treating wounds with slow healing rates and bacterial infections.

The last resolution of the doctoral research involves the creation of a novel dressing that combines two essential features: antimicrobial properties to prevent or erase infection and controlled oxygen release, which collectively provide the most favorable environment for promoting healing in chronic wounds.

The dressing's antibacterial qualities are accomplished by using silver nanoparticles, which were synthesized using a microfluidic method with a cross-shaped chip featuring three input channels. Building on the previous study's findings, the same synthesis setup was employed, with the same pump parameters maintained in rotations per minute (RPM). The difference in this study was substituting ascorbic acid and adding polyvinylpyrrolidone (PVP) to improve the nanoparticles' stability and performance. Four silver nanoparticles samples were obtained and analyzed through the following

techniques: XRD, SEM, TEM, DLS. Similarly to the previous study, a single sample (S(15_15)) of silver nanoparticles was selected from the four available options, demonstrating optimal characteristics for the specific application in question. The oxygen release function of the dressing is associated with calcium peroxide nanoparticles (CaO_2) coated with tannic acid (TA). In this sense, a precipitation synthesis method was performed to obtain CaO_2@TA nanoparticles starting from $CaCl_2$ x $2H_2O$ and TA, followed by the addition of NH_3 and the dropping procedure of H_2O_2 solution (35%). A sonication process was applied at the final practice, and then the complete reaction was allowed to happen during the night at ambient temperature. Additionally, the material underwent ethanol washing by consecutive centrifugation, and then the nanoparticles were dried at 60°C and characterized by XRD, FTIR, SEM, TEM, DLS analysis. The next step was to obtain the hydrogel formulations based on alginate as the polymeric matrix and the control reference (Alg) in order to compare each individual characteristic of the dressings. The nanoparticles were incorporated separately into the alginate matrix, resulting in two distinctive dressings: one containing silver nanoparticles, designed as Alg_S(15_15), and another containing tannylated calcium peroxide nanoparticles, denoted as Alg_CaO_2@TA. The ultimate dressing formulation, which goes beyond the basic functions, integrates silver and tannylated calcium peroxide nanoparticles into the alginate matrix, providing a dual-functional dressing that combines both nanoparticle types' benefits. The hydrogel formulations were comprehensively analyzed using various techniques, including SEM, FTIR, UV-Vis spectroscopy, and investigation of swelling and degradation rates. Additionally, microbiological and biological assessments were conducted to evaluate key aspects of the hydrogels' properties and behavior.

The X-ray diffraction data results for silver nanoparticles samples reveal the silver as the single phase obtained, according to the PDF-ICDD database, and the well-crystalline appearance of the diffractograms peaks. The determined average crystallite size was around 21 for each of the four samples of silver nanoparticles. The scanning electron microscope (SEM) images reveal a clear clustering of tiny spherical particles, ranging in size from 27 to 49 nanometers. These particles form larger spheres with diameters between 168 and 235 nanometers. The morphology of the nanoparticles is influenced by ascorbic acid and PVP during the reduction process of silver. TEM microscopy supports the information obtained through SEM analysis, highlighting the arrangement of small nanoparticles in individual micron-sized spheres. Zeta potential was measured by DLS technique, and thus, the negative charge of the nanoparticles,

together with their moderate stability, was established. The hydrodynamic diameter expressed in nanometers was in concordance with the agglomeration tendency of the nanoparticles and the electronic microscopy results. A comparison of the results from four samples of silver nanoparticles revealed that sample S(15_15) stood out due to its optimal physical-chemical properties, making it the most suitable choice for further development and incorporation into dressings with antimicrobial properties. The following data results are for CaO_2@TA nanoparticles, starting with identifying the phase by XRD analysis and the functional groups by the FTIR method. Both analysis results demonstrated the successful obtaining process through the diffractogram associated with calcium peroxide, according to PDF-ICDD database and the vibrational band characteristic for O-Ca-O at ~517 cm^{-1}. The SEM data indicates that the particles have a spherical shape with an average size of 153.11 ± 3.36 nanometers and elemental identification of calcium and oxygen by EDS module. Furthermore, the polycrystalline structure and spherical form of the CaO2@TA nanoparticles are confirmed by TEM microscopy data. The hydrodynamic diameter and zeta potential measurements of the particles show that they have a negative charge and a mean zeta potential value of around -16.48 millivolts. The mean size of the particles is roughly 393.2 nanometers. For five days, the oxygenation activity of the CaO2@TA nanoparticles in PBS at two distinct pH values—6 and 7.4—was visually observed. Oxygen bubbles were identified in both pH conditions from the first 10 minutes, suggesting the oxygen-releasing capability. The FTIR method was used to examine all hydrogel formulations with alginate serving as the primary matrix and being implanted with silver and tannylated calcium peroxide nanoparticles. The resulting spectra present a mostly similar appearance due to the alginate-based composition. The SEM examination brought attention to the hydrogels' porous structure and showed that the individual nanoparticles (S(15_15) and CaO_2@TA) were distributed uniformly. This finding was also consistently seen in the Alg_S_O sample. Using the EDS module, the elemental composition was examined, and the successful completion of the synthesis process was noted by the confirmation of the presence of silver, calcium, oxygen, and sodium. After measuring the hydrogels' swelling capacity in interaction with SBF solution, it was determined that all samples exhibited successful swelling, with values above 100% during the testing phase. The Fenton method was applied to evaluate the amount of the H_2O_2 release in acidic conditions (pH 6) using a layered architecture of PMMA discs coated with Alg_S_O hydrogel. UV-Vis data results were obtained under kinetics from minutes to minutes for 8.5 hours, with an initial

concentration of around 3.5 mg/L seen in the first minute. After two hours, the concentration remained stable within the range of 5 – 5.5 mg/L, with no significant variations throughout the entire duration. Based on the hydrogels' antibacterial activity features, which included their ability to suppress the formation of monospecific bacterial biofilms, additional evaluations were conducted. The results clearly indicate a drastic diminishing of the bacterial biofilm offered by the Alg_S_O hydrogel, compared with the control alginate and with each nanoparticle type as well. After 48 hours, a total eradication of the staphylococcal biofilm was registered, while significative inhibition of biofilm development was maintained on the *Ps. aeruginosa* strain. The findings clearly indicate that combining both types of nanoparticles yields a significant enhancement in antimicrobial activity, highlighting the synergistic benefits of using these nanoparticles together. The hydrogels' biocompatibility and cell proliferation feature were assayed in contact with HaCaT keratinocytes after 24 and 48 hours. After 24 hours of incubation, the keratinocyte viability in the nanoparticle-containing samples was significantly higher than that of the alginate-free nanoparticle sample. The hydrogels' capacity to sustain proliferation was clearly demonstrated by the keratinocytes' ability to remain viable after 72 hours of interaction with the Alg_S_O sample. Additionally, the LDH levels were assessed throughout the same time frame, indicating agreement with the MTT results that revealed the cells treated with Alg_S_O hydrogel to have the best cytocompatibility pattern. The minimal quantities of NO production suggest that the tested materials are non-cytotoxic, indicating that they do not cause harm to human keratinocyte cells. Specifically, the Alg_S_O formulation shows no signs of inducing cellular stress in human keratinocyte cell cultures, demonstrating its safe and non-toxic nature.

In conclusion, the research findings of this doctoral thesis have validated the initial concept, demonstrating that by combining common materials, it is possible to create new advanced and smart dressings with enhanced properties. The results show that the synergy between these materials is crucial along with the synthesis method, as it allows for the balance and effectiveness of the final product to be achieved.

Research frameworks established to facilitate further investigations – Perspectives

A unified perspective on advancing research in the field is to embrace a holistic approach that draws on the strengths of interdisciplinary methodologies, including

chemical engineering, biology, mathematics, and electronics. Chemical engineering expertise is essential for understanding the underlying properties of materials, including their physical and chemical characteristics, and for developing innovative ideas that exceed current standards. By integrating insights from biology and molecular biology, researchers can develop a deeper understanding of the skin cells' interactions and mechanism, enabling the identification of key triggers that can be exploited to induce changes in response to severe conditions experienced at the skin level. The precision of mathematics, similar to that of chemistry, enables researchers to derive accurate and reliable results, even if minor errors are encountered. Scientists may obtain a thorough grasp of the synthesis process and the chemical mechanisms involved by using a mathematical framework, which will eventually provide them with a comprehensive image of the experiment's conclusion. Through the intersection of electronics, chemistry, and biology, researchers can create sensors that can be inserted into artificial skin, allowing it to mimic the sensory functions of human skin. This includes the ability to detect temperature changes, touch sensations, and pain signals, enabling the development of more sophisticated prosthetics and medical devices.

The research conducted for the doctoral thesis provided a solid foundation for conceptualizing achievable future directions, allowing for a more informed and strategic approach to future development. Our research aims to capitalize on the oxygen-releasing properties of hydrogels by developing and testing dressings containing MnO, MgO, and CeO_2 nanoparticles. Additionally, we will investigate two eco-friendly alternatives that utilize seaweed with inherent oxygen-releasing capabilities and red blood cells, which have a proven ability to release oxygen and are already used in medical settings. Considering the importance of antimicrobial activity in encouraging chronic wound healing, silver nanoparticles remain the most promising choice from a multidisciplinary perspective. We will employ microfluidic platforms with adjustable flow parameters, varying solution compositions, and different reducing agents and methods. Also, explore the effects of different particle sizes, reducing agents, and methods on the antimicrobial activity of silver nanoparticles.

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Co-author publications related to the thesis theme:

1. Stoica (Oprea), A.E.; **Bîrcă, A.C.**; Pițigoi (Pandel) M.L.; Grumezescu A.M.; Vasile, B.S.; Iordache, F.; Ficai, A.; Andronescu, E.; Nanostructured zinc oxide for wound dressings. U.P.B. Scientific Buletin – Series B: Chemistry and Materials Sciences 2024, 86, 2.
2. Puiu, R.A., **Birca, A.C.**, Grumezescu, V., Duta, L., Oprea, O.C., Holban, A.M., Hudiță, A., Gălățeanu, B., Balaure, P.C., Grumezescu, A.M., Andronescu, E.;

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Conference participation during the PhD stage:

1. Cross-shape Microfluidic Platform for Silver Nanoparticles: Synthesis Focused on Alginate Wound Dressings; **Alexandra Cătălina Bîrcă**, Adelina Gabriela Niculescu, Alexandru Mihai Grumezescu, Bogdan Stefan Vasile, Ecaterina Andronescu; The 5th Conference of the Romanian Electron Microscopy Society – C.R.E.M.S (2023)
2. Solid Lipid Nanoparticles for acne treatment; **Alexandra Cătălina Bîrcă**, Luca Huștiuc, Alexandru Mihai Grumezescu, Cristina Chircov, Bogdan Ștefan Vasile, Alina Maria Holban, Ecaterina Andronescu; 22nd Romanian International Conference on Chemistry and Chemical Engineering – RICCCE 22. (2022)
3. Collagen Wound Dressings Loaded with CuO- and ZnO-based Microspheres; **Alexandra Cătălina Bîrcă**, Mihai Adrian Minculescu, Alexandru Mihai Grumezescu, Cristina Chircov, Ecaterina Andronescu; Applications of Chemistry in Nanosciences and Biomaterials Engineering – NanoBioMat 2022 – Summer Edition. (2022)
4. Synthesis of Nanoparticles through Lab-on-Chip Devices; **Alexandra Cătălina Bîrcă**, Alexandru Mihai Grumezescu, Ecaterina Andronescu; Applications of Chemistry in Nanosciences and Biomaterials Engineering – NanoBioMat 2021 – Winter Edition. (2021)
5. Regenerative medicine testing models; **Alexandra Cătălina Bîrcă**, Ecaterina Andronescu, Alexandru Mihai Grumezescu; Applications of Chemistry in Nanosciences and Biomaterials Engineering – NanoBioMat 2020. (2020)

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