UNIVERSITY POLITEHNICA OF BUCHAREST

DOCTORAL SCHOOL: Applied Chemistry and Materials Science

Summary

Doctoral Thesis

Biocompozite pe bază de polimeri naturali cu aplicații medicale
Biocomposites based on natural polymers for medical applications

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Introduction

This doctoral thesis entitled "Biocomposites based on natural polymers with medical applications" consists of 10 chapters and a last chapter dedicated to the bibliography. The main aim of this doctoral thesis was to develop biocomposites and biosensors based on natural polymers with medical applications in areas such as wound healing, bone tissue recovery or the study of the interaction between DNA and immunosuppressive drugs.

In the first chapter of the doctoral thesis entitled *„Literature data"* divided into 5 subchapters are presented introductory notions about the importance of biocomposites, their classification, main synthesis methods, natural polymers used in the thesis (bacterial cellulose, polyhydroxyalkanoates, silk fibroin) and also about biosensors, types of biosensors, DNA-based biosensors and their main applications. In this chapter you will find 2 tables and 4 figures.

Chapter two entitled *„Characterization techniques used for the study of biocomposites"* composed from three subchapters describes the characterization methods used to investigate the new biocomposites and DNA-based biosensors.

Chapter three entitled *„Thesis aims and original contributions"* is divided into three subchapters in which are presented the main purpose of the thesis, a case study and the original contributions that are found in this doctoral thesis.

The fourth chapter entitled *"Biocomposites based on bacterial cellulose and poly(3-hydroxybutyrate-co-3-hydroxyvalerate)"* divided into four subchapters describes the preparation and characterization of biocomposites based on bacterial cellulose and polyhydroxyalkanoates, biomaterials with potential applications in the field of wound healing. This chapter consists of 11 figures and a table.

Chapter five entitled *„Biocomposites based on polyhydroxyalkanoates and double layered hydroxides"* consisting of four subchapters and 8 figures describes the preparation and characterization of biocomposites based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and double-layered hydroxides modified sodium, materials that can be considered possible candidates for the field of wound healing taking into account the biocompatibility presented.

Chapter six entitled *„Biocomposites based on silk fibroin and magnetite"*, consisting of 4 subchapters and 9 figures, describes the preparation and characterization of new biocomposites with potential applications in wound healing.

In chapter seven entitled *„Biocomposites based on bacterial cellulose and magnetite"* divided into 4 subchapters is presented the obtaining of the biocomposites by *in situ* method, the resulting materials being characterized in terms of morpho-structural and biocompatibility, highlighting the fact that the samples with a higher magnetite content possess adequate characteristics for applications in the field of wound healing. This chapter contains 9 figures.

Chapter eight entitled *„Hydrogels based on fibroin, polyacrylamide and graphene oxide with potential medical applications"*, presents the preparation and characterization from a morpho-structural and biological point of view of new hydrogel biomaterials in order to obtain new materials with superior mechanical response to the mechanical properties of fibroin, but which show the excellent biocompatibility of the silk protein. Biostructures composed of osteoblast cells and hydrogels of different compositions were used to perform biological tests, because these biocomposites were designed for applications in the field of hard tissues. The chapter is divided into 4 subchapters and contains 11 figures.

The nine chapter entitled *„DNA biosensors and its interaction with azathioprine"* investigated the interaction between DNA and azathioprine using a DNA-based electrochemical biosensor, using complementary techniques such as mass spectrometry, UV-Vis spectroscopy and electron microscopy. sweep. This chapter consists of 3 subchapters in which there are 12 figures and 2 schemes.

The final chapter of the doctoral thesis entitled *"General conclusions and perspectives"* is **the ten chapter** in which the general conclusions related to each chapter and perspectives are presented.

This summary is a short form of the doctoral thesis, thus the numbers of the figures, references and pages from *„Contents"*are in the thesis format.

Key words: Biocomposites, bacterial cellulose, polyhydroxyalkanoates, silk fibroin, hydrogels, biosensors, azathioprine.

CHAPTER 1. Literature data

Biocomposites: definition, clasification, obtaining methods, aplications

Promoters of sustainable chemistry, biocomposites are considered the materials of the future because they come from renewable resources, are biodegradable and through them can be achieved the objectives of reducing environmental pollution and limiting the energy crisis caused by the depletion of oil resources. In addition to these properties, biocomposites based on natural polymers poses excellent biocompatibility, thus meeting the basic requirements for their use in the biomedical field. The development of biocomposites based on biodegradable natural polymers is of particular importance in the field of tissue engineering, recent approaches involving the use of three-dimensional supports as a backbone for cellular activities of healing, reconstruction or regeneration of damaged tissues.

Definition

Biocomposites can be defined as follows: i) composite materials containing one or more elements of biological origin; ii) composite materials containing only elements of biological origin or iii) composite materials biocompatible and/or environmentally friendly for medical applications [1–3]. In the literature, biocomposites are also known as biohybrid or green composites.

Biocomposites combine the properties of the component materials to provide the best possible mechanical and physiological compatibility with the host tissues. The major advantage of biocomposites is the possibility of obtaining a wide range of materials with controlled properties with applications in various fields. Similar to classical composites, biocomposites have superior properties to those specific to each component.

From a compositional point of view, biocomposites are made up of a basic material (matrix) and a complementary material (reinforcement material).

The matrix is the continuous phase from biocomposites, which can be organic (natural polymers - polysaccharides, proteins, lignin, etc. or synthetic polymers - polyvinyl alcohol, polyacrylamide, polylactic acid, polypyrrole, etc.) or inorganic (hydroxyapatite (Hap))[3].

The reinforcing material is the discontinuous phase from biocomposites, which can be organic in nature (natural fibers, polymers, carbon nanostructures) or inorganic in nature (metals, double-layered hydroxides, magnetic nanoparticles, ceramic materials, etc.) and have an important influence on the final properties of the synthesized material.

Clasification

Biocomposites have applications in medicine, bioengineering, automobiles, construction, etc. In the case of biocomposites used in the medical field, a classification may be made according to the origin of the components [3]:

- **organic/organic**;
- **organic/inorganic**;
- **inorganic/inorganic**.

Biocompozitele organic/organic sunt compozite de tip polimer natural/polimer natural, de exemplu alginat de sodiu/fibroină din mătase naturală, amidon/lignină, chitosan/celuloză sau polimer natural/polimer sintetic, de exemplu acid polilactic/fibre de celuloză, amidon/alcool polivinilic/celuloză. Acest tip de biocompozite au numeroase aplicații deoarece prezintă o biocompatibilitate ridicată și o flexibilitate excelentă[3,40].

Organic/organic biocomposites could be natural polymer/natural polymer composites, for example sodium alginate/silk fibroin, starch/lignin, chitosan/cellulose or natural polymer/synthetic polymer, for example polylactic acid/cellulose fibers, starch/polyvinyl alcohol/cellulose. This type of biocomposites have many applications due to theirs high biocompatibility and excellent flexibility[3,40].

Organic/inorganic biocomposites have many applications in the medical field due to their advantages such as flexibility, biocompatibility or biodegradability from the organic matrix and improved mechanical and/or antimicrobial properties obtained due to the presence of the inorganic component. One of the most important advantages of this type of composites is represented by the fact that hard tissues are generally natural biocomposites of organic/inorganic type, this facilitating the obtaining of biocomposites with properties similar to those characteristic of human tissues. The best examples are bone or teeth, which are made of an organic matrix of collagen fibers type I, reinforced with hydroxyapatite crystals, and bioactive natural substances or cements (proteins, polysaccharides, mucopolysaccharides) and water [40,41].

Inorganic/inorganic biocomposites include materials based on hydroxyapatite/alumina/zirconium,hydroxyapatite/carbon/silvernanotubes,hydroxyapatite/silver nanoparticles, with applications in hard tissue engineering [3,40].

The biocomposites with applications in the biomedical field takes into account the degree of their degradation in the human body, from this point of view biocomposites can be divided as follows:

- **non-resorbable biocomposites** - both the matrix and the reinforcing agent cannot be resorbed in the human body. This type of composite materials have applications in the field of orthopedic medicine such as knee prostheses, bone plates, external fixators [42];
- **partially biodegradable biocomposites** - the matrix is composed of a biodegradable polymer, and the reinforcing agent is made of a non-absorbable material. The materials have applications in areas such as cartilage reconstruction [43], biosensors [44];
- **resorbable biocomposites** - both the matrix and the reinforcing agent come from biodegradable materials in the human body [45]. This type of material is used in fixing fractures and arthrodesis of various anatomical parts.

Methods of obtaining biocomposites based on natural polymers with medical applications

Methods of obtaining can be systematized into three categories: conventional, solution and solid state methods, in **Figure 1.1.** being presented techniques belonging to each class [46]. Depending on the characteristics required for the application concerned, the type of materials used and the method of preparation used, biocomposites can be obtained in the form of fibers, films, membranes, tubes, coatings, foams, sponges or hydrogels.

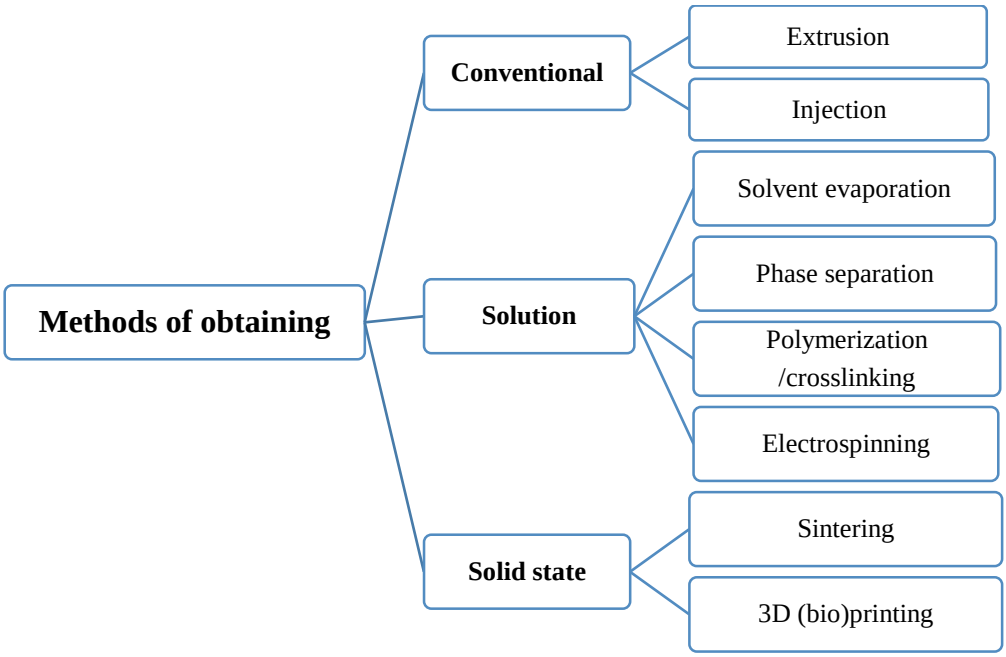


Figura 1.1. Representative methods of obtaining of biocomposite materials with applications in the medical field.

Biosensors

Definition

A biosensor is a device consisting of two elements: a bioreceptor represented by an immobilized biological element that interacts with the analyte and a transducer used to convert the (bio) chemical effect, resulting from the interaction of the analyte with the bioreceptor, into an electronic one[217,218].

The bioreceptor can be represented by tissue[219], cells[220,221], enzymes[222,223], antibodies[223], nucleic acids (such as deoxyribonucleic acid (DNA))[224], while the transducer has the role of transforming the energy in a useful analytical signal that may be electrochemical[224–227], optical[228] or piezoelectric[229].

The use of the first biosensor was reported in 1962 to monitor the level of the blood gases during an surgery 230]. Currently one of the best known biosensors is the glucose biosensor[231], but the field of biosensors is intensively studied with the aim of developing biosensors with applications in many fields such as medicine, agriculture, security, food safety, etc.[218,232–234] . Moreover, due to the discoveries in the field of electronic instrumentation, biosensors can be miniaturized leading to obtaining lab-on-chip devices for real time monitoring or as portable devices[218,222,235].

CHAPTER 2. Characterization techniques used for the study of biocomposites

Biocomposites for medical applications are materials that require interdisciplinary characterization. Thus, in this doctoral thesis, the following techniques were used to investigate the structural, morphological, thermal, wettability, swelling capacity, mineralization capacity, biocompatibility and cytotoxicity of prepared biocomposites: Fourier transform infrared spectroscopy (FTIR), RAMAN Spectroscopy, Mass Spectrometry (MS), UV-VIS Spectrometry, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM) , contact angle; Thermogravimetric analysis (TGA), differential scanning calorimetry (DSC). Another type of assays used to characterize biosensors are electrochemical ones such as cyclic voltammetry (CV) and differential pulse (DPV). The biological tests consisted of biocompatibility assessment (Live/Dead by fluorescence microscopy), viability assessment and cell proliferation (MTT) and biomaterial cytotoxicity assessment (LDH).

CHAPTER 3. Thesis aims and original contributions

Thesis aims

The aim of this doctoral thesis is to development new biocomposite materials based on biopolymers with medical applications in areas such as dressings for wound healing or supports for bone tissue recovery and to study the interaction between DNA and azathioprine using DNA-based electrochemical biosensors.

For this purpose the following objectives have been set:

O_1. Identification of suitable biopolymers for the targeted applications. This step consisted of choosing biocompatible natural polymers and reinforcing agents to improve the properties of the polymer matrix according to the requirements of the chosen applications.

O_2. Obtaining new biocomposites using the identified materials in order to prepare biocompatible systems with appropriate characteristics for the mentioned applications.

O_3. Characterization of the obtained biocomposites in terms of structural, morphological, thermal, watering properties, swelling capacity, mineralization capacity, biocompatibility and/or cytotoxicity. Thus, the interaction mode and the optimal ratios between the two components of biocomposites were evaluated in the newly synthesized materials by FTIR, Raman, XRD, SEM, TEM, AFM, TGA, DSC techniques, contact angle, the biocompatibility and/or cytotoxicity of the materials being evaluated on human adipocyte-derived stem cell lines (hASCs) for biocomposites with potential applications in wound healing and on MC3T3-E1 preosteoblast cells from mice (ATCC® CRL-2593 ™) for biocomposite hydrogels with possible engineering applications hard tissues.

O_4. Determination of the electrochemical behavior and electronic transfer mechanism of azathioprine using voltammetric techniques such as cyclic voltammetry and differential pulse voltammetry at glass carbon electrode. It is also important to study the interaction process between this immunosuppressive drug and deoxyribonucleic acid (DNA) using DNA-based electrochemical biosensors.

Original contributions.

In relation to the current state of research, the original elements of this doctoral thesis will be presented below.

The novelty elements in the study of biocomposites based on cellulose and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (BC/PHBHV) with potential applications in the

field of wound healing are represented both by the use of dry bacterial cellulose membrane in the process of obtaining of materials as well as the use of PHBHV.

In the case of biocomposites based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and double-layered hydroxides with potential applications in the wound healing field, the novelty is the designed of this materials for medical applications, because until the publication of this study this type of materials was mainly investigated for industrial applications in the field of water purification.

Regarding the development of biocomposites based on bacterial cellulose and magnetite, an original approach consisted in the dispersion of magnetic nanoparticles in the culture medium of the producing bacteria leading to the *in situ* obtaining of nanocomposite materials. The subsequent characterization of the obtained materials revealed that these biocomposites have properties that recommend them as potential candidates for the field of wound healing.

In the case of biocomposites based on fibroins and magnetic nanoparticles, the synthesis of this type of materials was reported for the first time in the literature, the materials obtained having properties suitable for biomedical applications.

The use of hydrogels based on fibroin, acrylamide and graphene oxide for applications in the field of bone reconstruction, is a novelty in the literature, the obtaining results highlighting appropriate morpho-structural properties for the medical field and a good mineralization capacity obtained by a synergistic effect of the properties of the components used, qualities that make them eligible for the field of hard tissue engineering.

În cazul studiului legat de mecanismul redox al azatioprinei și interacției acesteia cu ADN-ul, elementul de noutate constă în propunerea unui mecanism de intereacție între azatioprină și ADN pe baza datelor electrochimice și de spectrometrie de masă obținute.

Regarding the study related to the redox mechanism of azathioprine and its interaction with DNA, the novelty consists in proposing a mechanism of interaction between azathioprine and DNA based on the electrochemical and mass spectrometry data obtained.

CHAPTER 4. Biocomposites based on bacterial cellulose and poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

This chapter presents the development of organic-organic biocomposites based on bacterial cellulose and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with potential applications in the field of wound healing. The field of wound healing is a constantly expanding field due to rising care costs and an aging population, but also due to the increasing incidence of diabetes and obesity. One of the most studied polymers for applications in the field of wound healing is bacterial cellulose because of its characteristics such as a high degree of purity, good absorption and retention capacity of fluids and mechanical stability.

However, in order to obtain biocomposites with properties suitable for wound healing, it is necessary to improve the biocompatibility, the adhesion of the dressing to the dermal tissue, the absorption and release of fluids, the barrier and antimicrobial properties, the main purpose of using polyhydroxyalkanoates being to improve properties such as biocompatibility such as biocompatibility, biodegradability and barrier properties.

In this study, BC was modified by an *ex situ* method, respectively by impregnation with PHBHV solutions in chloroform using different ratios between the two biopolymers, the obtaining process of the biocomposites being shown in **Figure 4.1**.

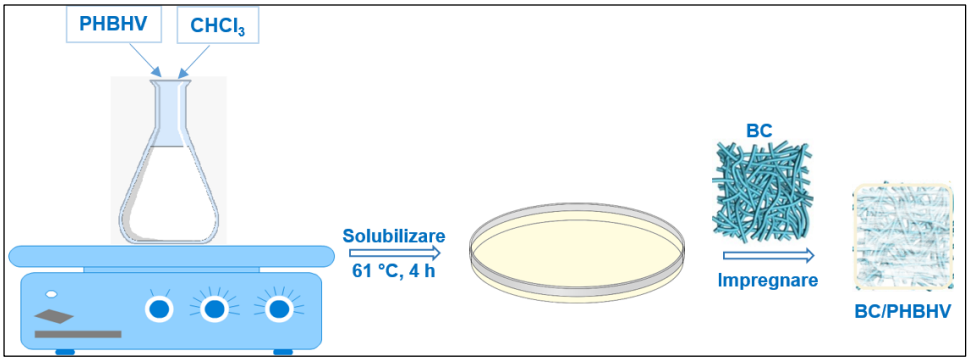


Figure 4.1. Schematic representation of the obtaining of BC/PHBHV biocomposites (1/1, 1/2 and 1/5).

The XRD analysis investigated the evolution of the crystallization mode of BC with the increase of the polyhydroxyalkanoate concentration, observing the increase of the crystallite size with the increase of the natural polyester concentration.

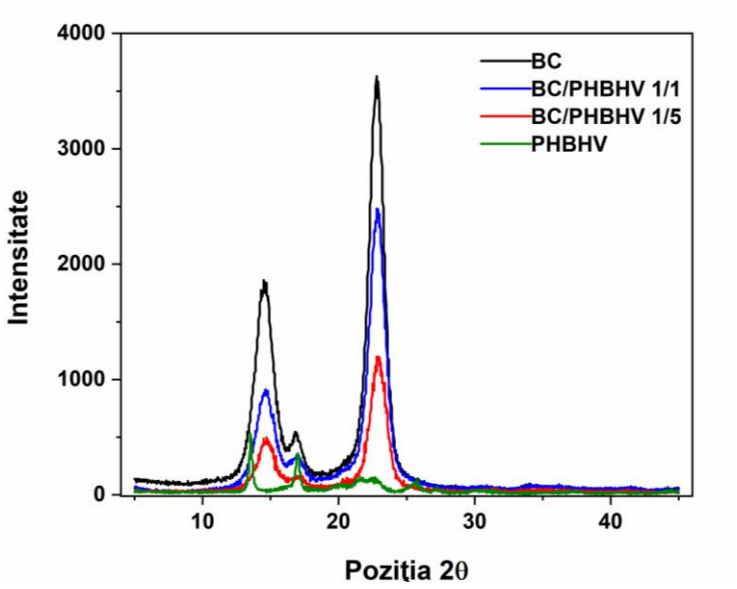


Figure 4.5. X-ray diffractograms of BC, PHBHV and BC/PHBHV biocomposites with different mass ratio 1/1 and 1/5.

Next, the morphology of BC/PHBHV biocomposites was analyzed, the SEM images presented in **Figure 4.6** revealing the architecture of the BC membrane and the random dispersion of the natural polyester film among the BC nanofibers.

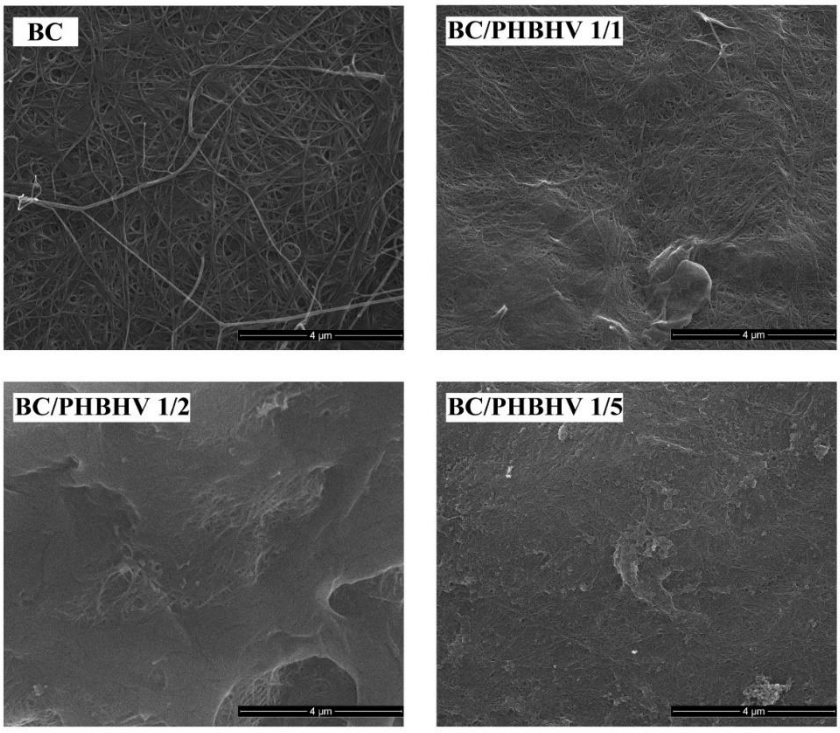


Figure 4.6. SEM images of BC membrane and BC/PHBHV biocomposites with different mass ratios: 1/1, 1/2 and 1/5.

The next step was to evaluate the presence/absence of rezidual solvent (chloroform) from the biocomposites using TGA analysis, the obtained results highlighting the presence of traces of residual solvent (**Figure 4.7.**).

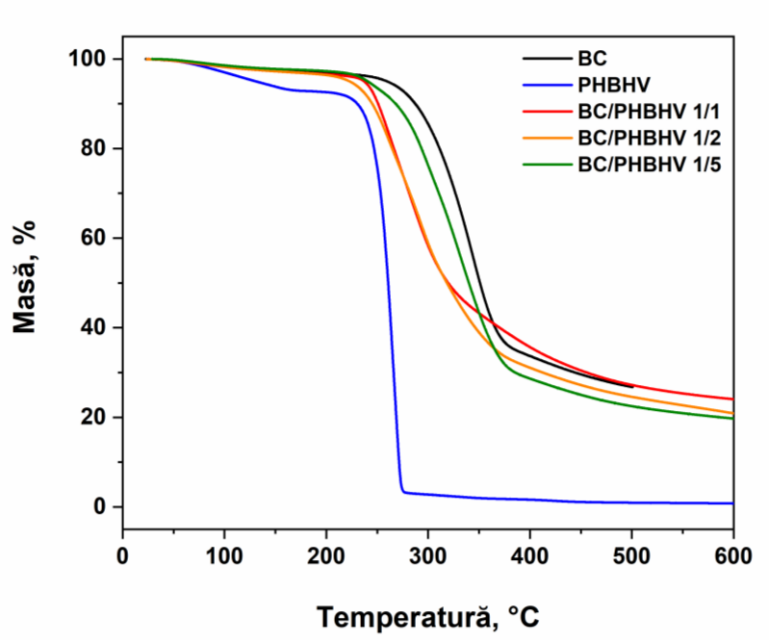


Figure 4.7. TGA curves of BC, PHBHV-film and BC/PHBHV biocomposite (1/1, 1/2, 1/5).

The wetting properties of the biocomposites were tested by measuring the contact angle which showed an increase of the contact angle values of biocomposites compared to the value obtained for BC, highlighting the tendency of hydrophobicization of the BC surface after impregnation with PHBHV. In Figure 4.9. the optical images obtained are revealed.

Figure 4.9. Optical photographs of the water droplet placed on the surface of the BC, BC/PHBHV 1/5 biocomposite and PHBHV samples.

The final step of this study was to perform biocompatibility tests on BC/PHBHV bicomposites on human adipocyte-derived stem cell lines (hASC) which showed that the synthesized materials present a relatively good biocompatibility.

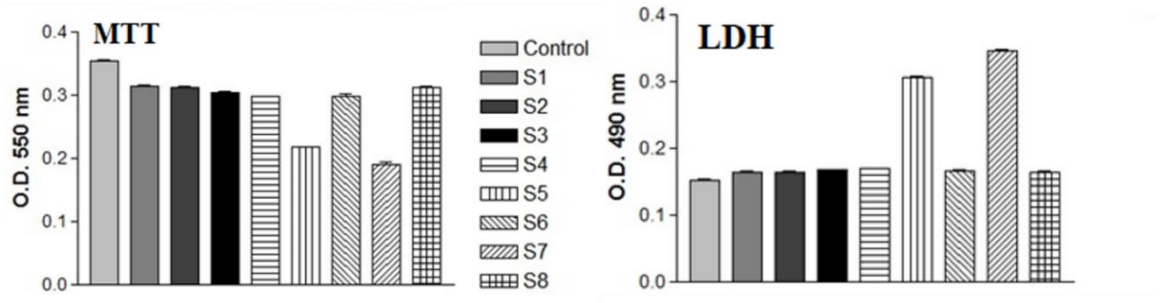


Figure 4.11. MTT and LDH tests for samples of BC/PHBHV biocomposites with different compositions (S1-4), BC membrane (S5), BC/PHBHV obtained directly from the culture medium (S6); PHBHV film (S7) and BC/PHBHV obtained directly from the culture medium, lyophilized membrane (S8).

In order to improve the biocompatibility of these materials, a more efficient purification of the synthesized materials would be necessary in order to remove as efficiently as possible the residual solvent or the use of other obtaining methods of such as those from melting.

CHAPTER 5. Biocomposites based polyhydroxyalckanoates and double layered hydroxides

Optimizing materials for development of dressings used in the field of open wound healing is a challenge for researchers in medicine, biology or materials science because it is necessary to obtain materials that ensure rapid wound healing, are biocompatible and maintain an antimicrobial environment to prevent infections. Biocomposites are suitable for this type of application because they have low toxicity, biodegradability and high biocompatibility.

In this context, this chapter describes the synthesis and characterization of film-forming biocomposites based on polyhydroxyalkanoates and double-layered organophilized hydroxides prepared by ultrasonic dispersion of anionic clay in the polymer solution. Biocomposite films were obtained by the solvent evaporation technique (**Figure 5.1**).

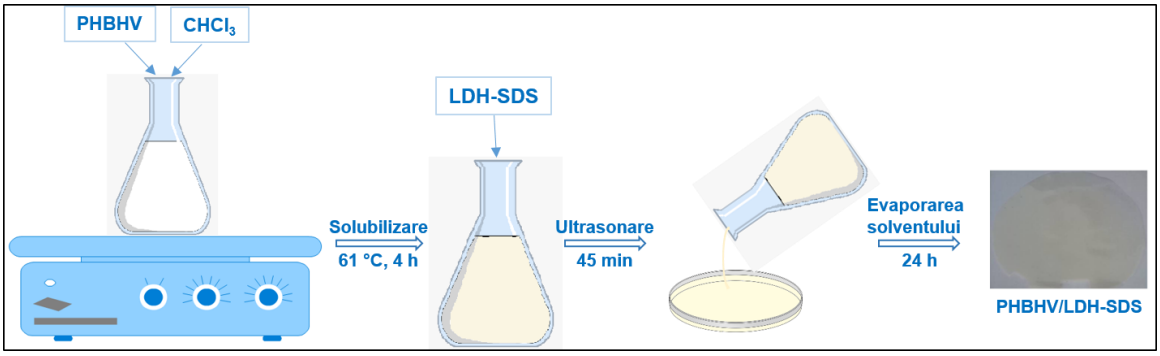


Figure 5.1. Schematic representation of the obtaining process of PHBHV/LDH-SDS biocomposite (1%, 2% and 3%)

The investigation of the morpho-structural properties represents the first step in the characterization of the organic-inorganic biocomposites and were performed by FTIR, XRD and SEM techniques.

FTIR spectra of biocomposites based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and double-layered hydroxides modified with sodium dodecyl sulphate shown in **Figure 5.2.** revealed that the presence of anionic clay does not influence the organic structure of the polymer matrix.

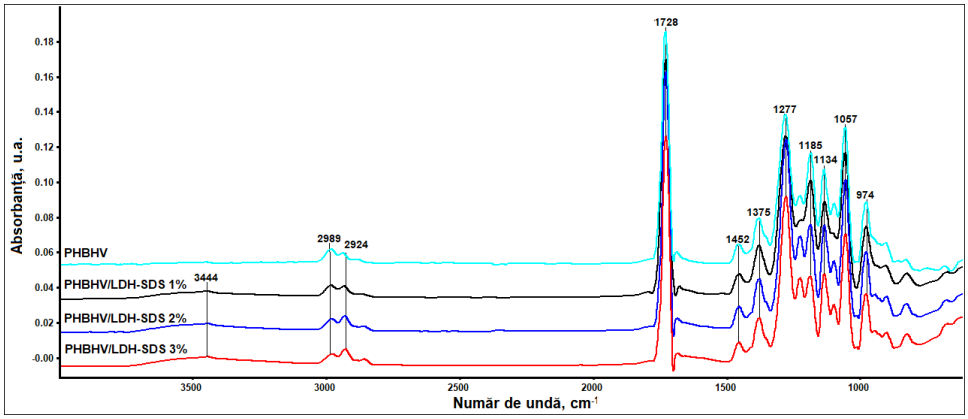


Figure 5.2. FTIR spectra of the PHBHV film PHBHV/LDH-SDS biocomposites with 1%, 2% and 3% LDH-SDS

The XRD analysis showed that the double-layered hydroxides keep their ordered structure within the biocomposites, resulting in obtaining of the intercalated type biocomposites (**Figure 5.3.).**

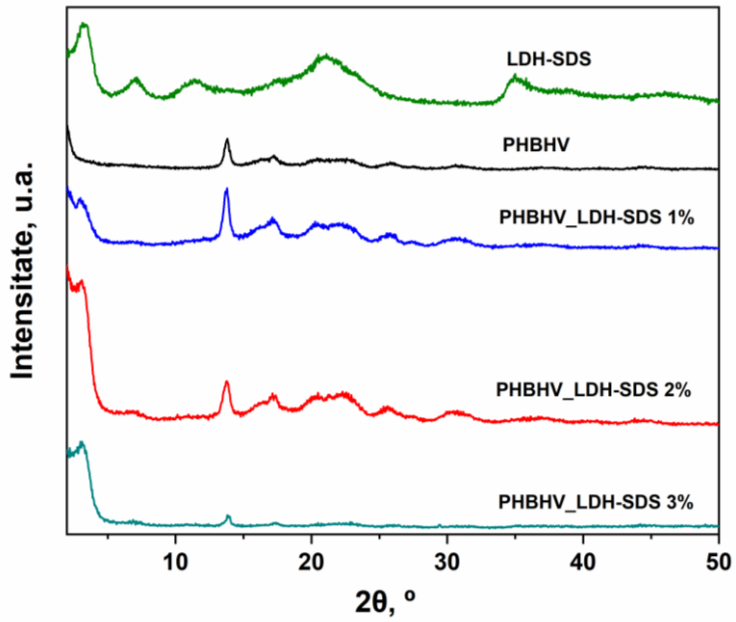


Figure 5.3. X-ray diffractograms of LDH-SDS, PHBHV film and PHBHV/LDH-SDS biocomposites with 1%, 2% and 3% LDH-SDS.

SEM images revealed the obtaining of biocomposites with rough surfaces due to the dispersion of anionic clay particles in the polymer matrix.

TGA analyzes showed that the presence of double layered hydroxide leads to a decrease in the degradation temperature of the polymer matrix, noting that as the clay

concentration increases, the degradation temperature of the biocomposites presents a slow increase (**Figure 5.4**.)

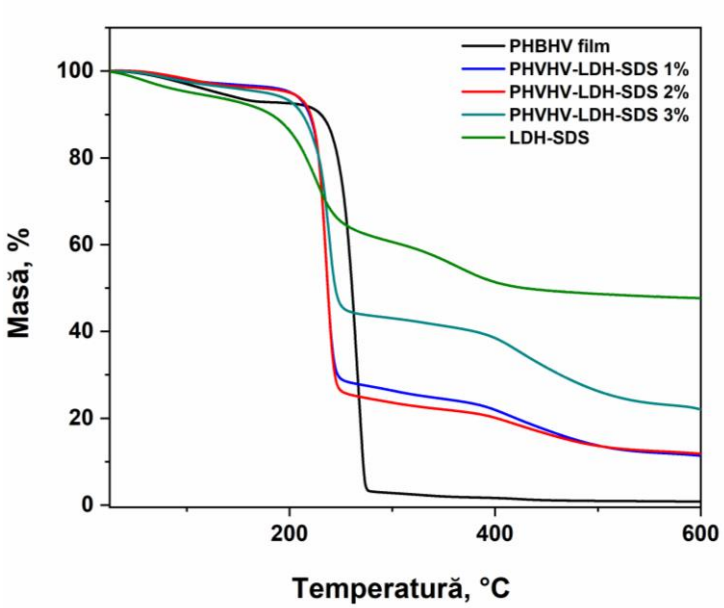


Figure 5.4. Thermogravimetric curves for: PHBHV film, and LDH-SDS and PHBHV_LDH-SDS biocomposites with 1%, 2%, respectively 3% LDH-SDS

Through DSC analysis it was determined that the melting temperature of the samples is not influenced by the presence or increase of anionic clay content.

The evaluation of the biocompatibility of the obtained materials was performed through the LIVE/DEAD test, observing a better mimicry with cellular environment compared to the reference material used 24 hours after seeding the cells.

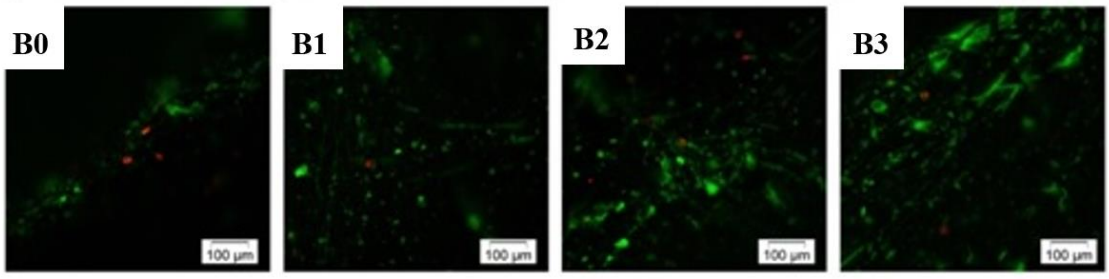


Figure 5.7: Confocal microscopy images after fluorescent labeling of cells to highlight living/dead cells on the control sample of PHBHV (B0) and PHBHV-LDH-SDS biocomposites with a content of 1% (B1), 2% (B2) and 3% (B3) of LDH-SDS.

Due to their good biocompatibility, these biocomposites can be considered as potential candidates for *in vivo* testing for wound healing applications.

CHAPTER 6. Biocomposites based on silk fibroin and magnetite

The medical applications of silk fibroin, protein obtained by regeneration from solution followed by precipitation or chemical modification processes cosists in sutures, materials for wound healing, fibroin-based hydrogels for immobilization or controlled release of active ingredients, reconstruction of cellular tissues etc.

Silk-based supports have been intensively studied for possible applications in the biomedical and biotechnological field. To obtain substrates with improved properties, silk was mixed with synthetic polymers (eg polyvinyl alcohol or polyacrylamide), natural polymers (eg collagen, elastin, gelatin, etc.) or inorganic materials (eg magnetic nanoparticles, hydroxyapatite or clays). In addition, in recent years, magnetic nanoparticles, and especially magnetite, have been used to develop a wide range of biomedical and biotechnological applications such as contrast agents, biosensors, cancer hyperthermia and drug-controlled delivery systems.

In this context, in this chapter are presented the synthesis and characterization of biocomposites based on silk fibroin and magnetite nanoparticles. The process of preparing the biocomposite films included the following steps: obtaining the fibroin solution from natural silk, dispersing the magnetic nanoparticles in the polymer matrix and obtaining the biocomposite films by the solvent evaporation method (**Figure 6.2.**).

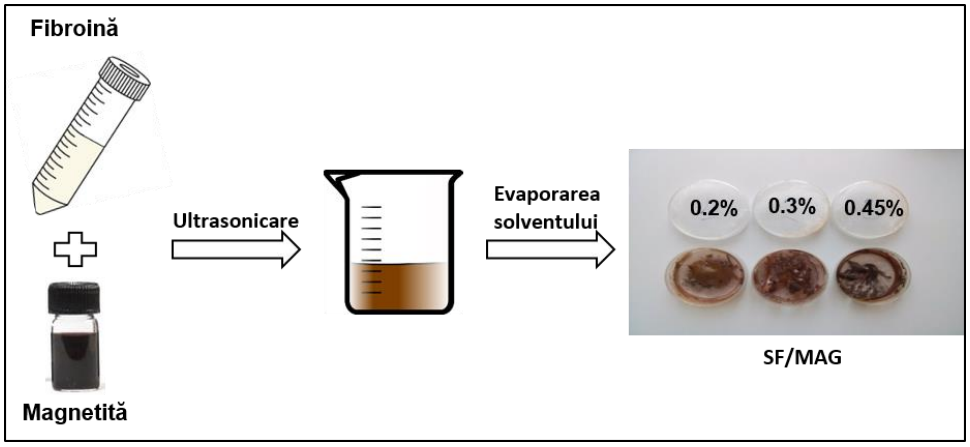


Figure 6.2. Schematic representation of the steps involved in obtaining SF/MAG biocomposite films.

The obtained biocomposites were analyzed in terms of morphological and structural properties. FTIR analysis revealing that amides I and II show a displacement that can be attributed to the obtaining of the β structure of silk fibroin (**Figure 6.3.**).

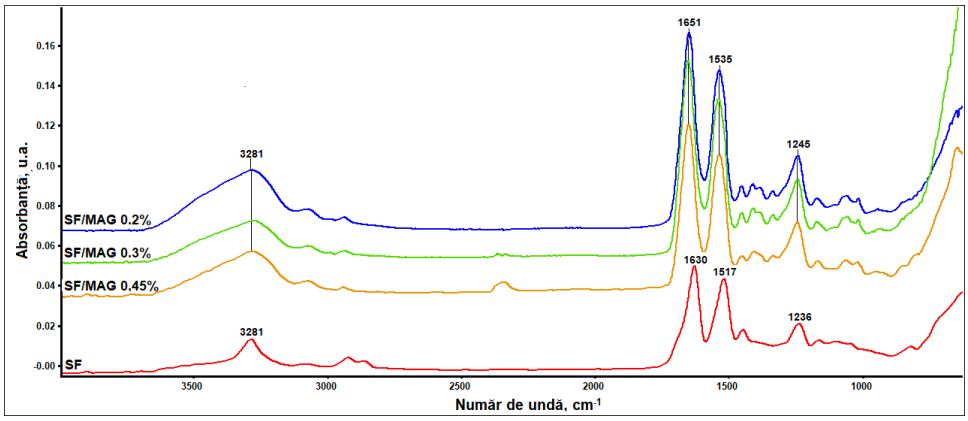


Figure 6.3. FTIR spectra of SF film and SF/MAG biocomposites with 0,2%, 0,3% and 0,45%.

The crystal structure of the biocomposites was highlighted using XRD analysis, the obtained diffractograms of biocomposites shown in **Figure 6.4**. presenting a similar shape to that of fibroin and the presence of magnetite being confirmed by a maximum of 35.7 °.

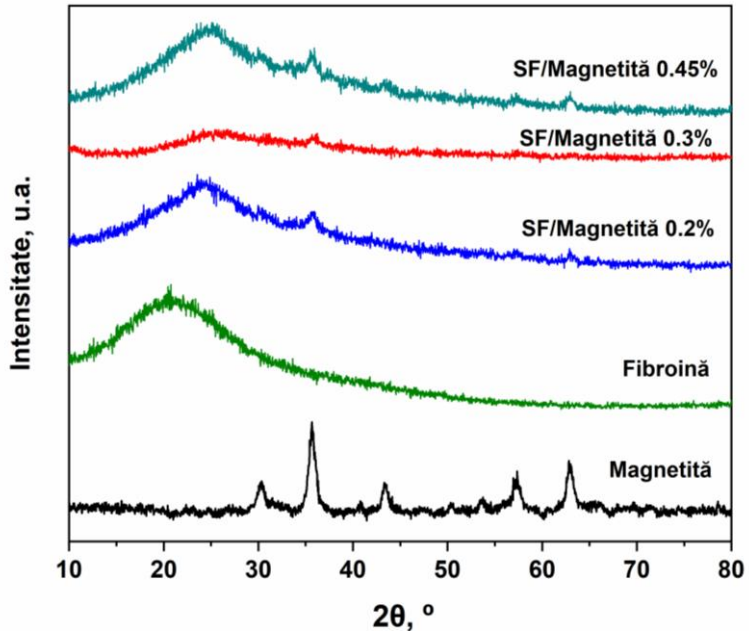


Figure 6.4. X-Ray Difractograms of magnetite nanoparticles, silk fibroin film and SF/MAG biocomposite films with 0.2%, 0.3% and 0.45% magnetite.

SEM analysis highlighted the dispersion of magnetic nanoparticles in the polymer matrix and their tendency to form clusters, with increasing magnetite concentration, biocomposites showing a more uniform dispersion of magnetic nanoparticles, but also a greater tendency to form agglomerations. .

Furthermore, the TEM images of SF/MAG biocomposites, reveals areas where magnetic nanoparticles are well dispersed and areas where clusters have formed. The information provided by the TEM analysis is of particular importance because both the mode of dispersion and the structure of the magnetite can influence the properties of the obtained material (**Figure 6.6.**).

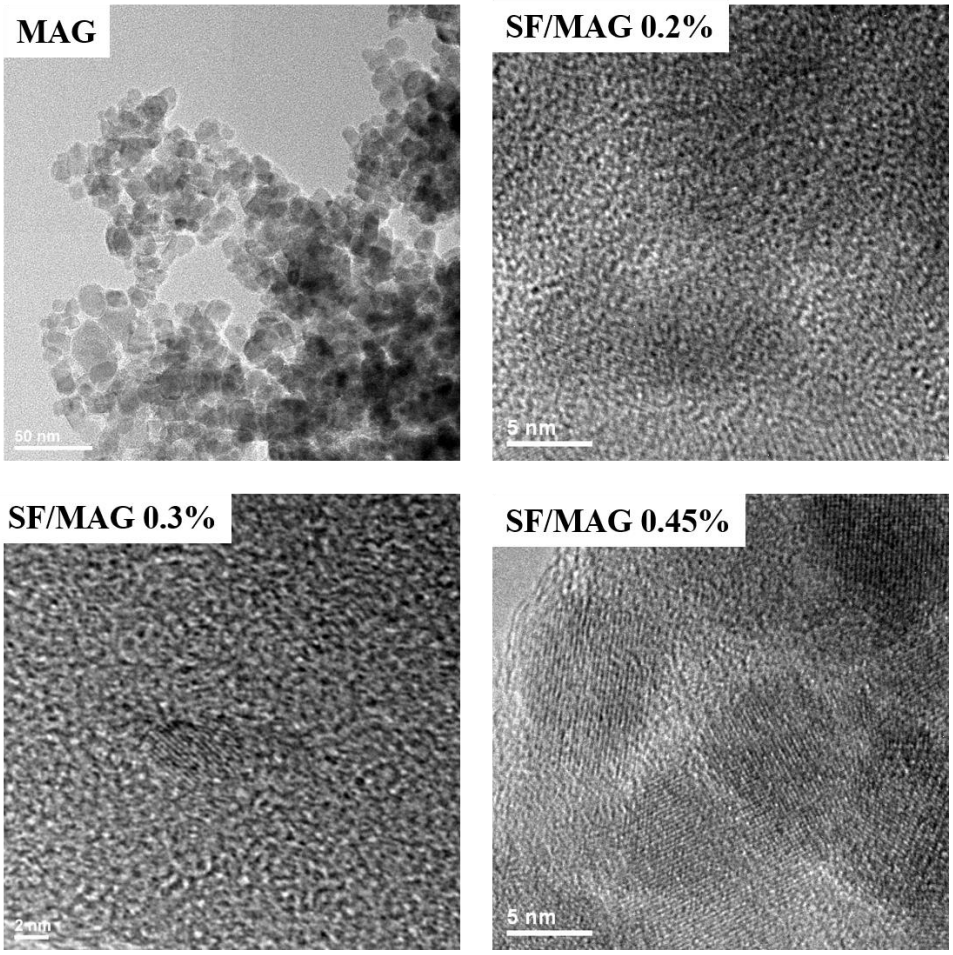


Figure 6.6. TEM and HR-TEM images of magnetite powder and SF/MAG biocomposite films with 0.2%, 0.3% and 0.45% magnetite.

The AFM analysis revealed that the obtained biocomposites have a rough surface compared to the smooth surface of the silk film (**Figure 6.7.**). This property has many

advantages in the field of medical applications such as supporting cell adhesion and proliferation. At the same time, the AFM analysis also showed the relatively uniform distribution of magnetite nanoparticles both on the surface and inside of the polymer matrix through 2D and 3D images (deflection and 3D images).

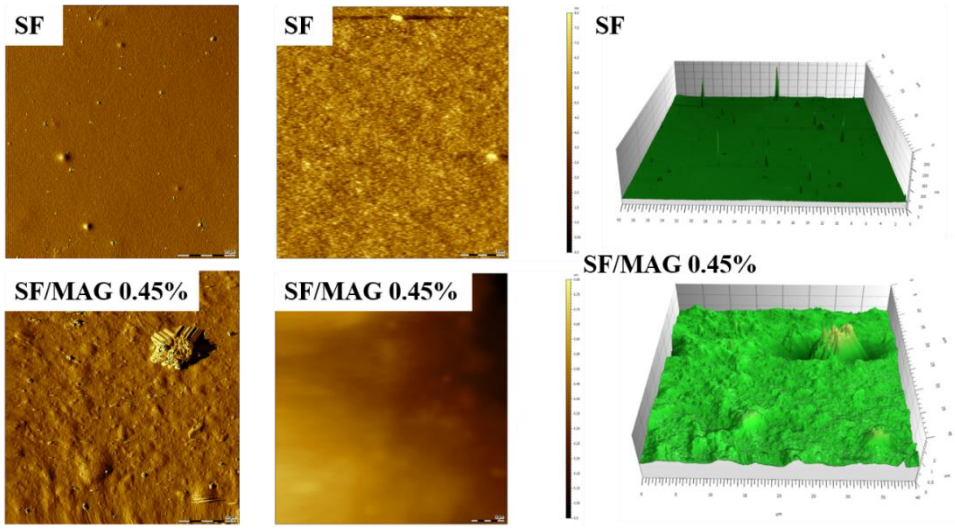


Figure 6.7. AFM images of SF film and SF/MAG biocomposite film with 0.45% magnetite.

Cell viability tests using stem cells derived from human adipocytes performed on the obtained materials showed that all biocomposites have a good biocompatibility *in vitro* (**Figure 6.8.**).

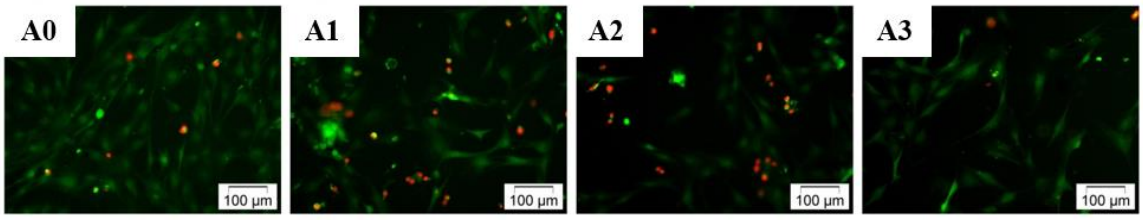


Figure 6.8.: Confocal fluorescence microscopy images of cells cultured on SF (A0), SF/MAG 0.2% (A1); SF/MAG 0.3% (A2) and SF/MAG 0.45% (A3).

The results obtained suggesting that all biomaterials tested have a good in vitro biocompatibility on hASC. Due to the good biocompatibility shown, these materials are potential candidates for *in vivo* testing in the field of wound healing applications.

CHAPTER 7. Biocomposites based on bacterial cellulose and magnetite

Bacterial cellulose is used in wound healing applications due to its remarkable properties such as purity, biocompatibility, high porosity, control of morphology, extracellular collagen-like structure and the ability of BC nanofibers to guide cells. Bacterial cellulose is modified with magnetite in order to improve the wound healing process.

In this context, this chapter presents the obtaining and characterization of new biocomposites based on bacterial cellulose and magnetite nanoparticles with potential applications in the field of wound healing. The biocomposites were obtained by the i*n situ* method, respectively the magnetic nanoparticles were dispersed in the culture medium used for the fermentation of Acetobacter xylinum (**Figure 7.1**.).

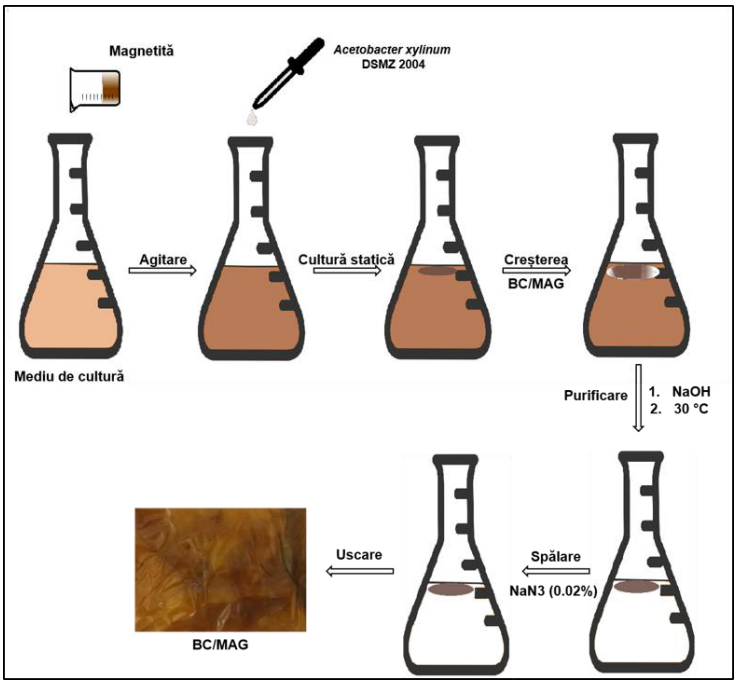


Figure 7.1. Schematic representation of the obtaining of BC/MAG biocomposite membranes by the *in situ* method.

The XRD analysis showed that the modification of bacterial cellulose with magnetite does not influence the crystalline structure of the biopolymer (**Figure 7.2.**), the diffractograms of biocomposites based on bacterial cellulose and magnetite presenting the maxima associated with the two components.

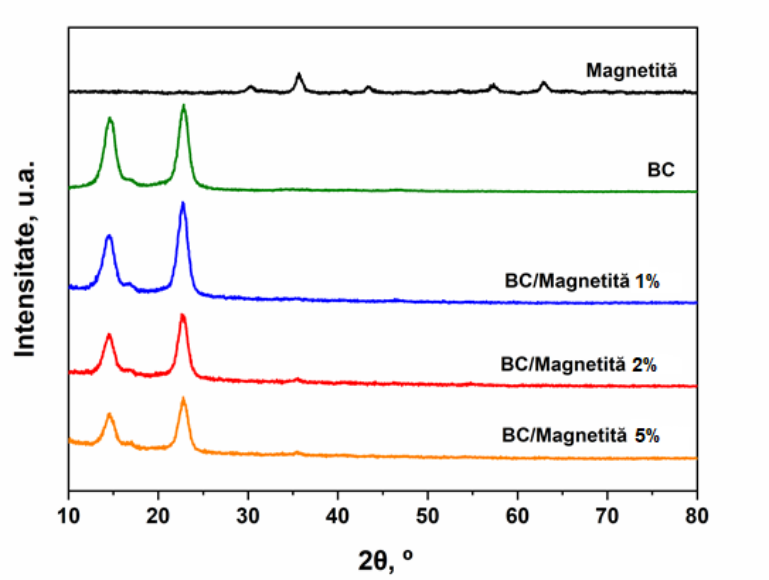


Figure 7.2. X-Ray Difractograms of BC, MAG and BC/MAG biocomposites with different concentrations (1%; 2% and 5%) of magnetite.

The SEM images highlighted the BC-specific fibrillar network as well as the relatively uneven distribution of magnetite particles both on and through the biopolymer fibers.

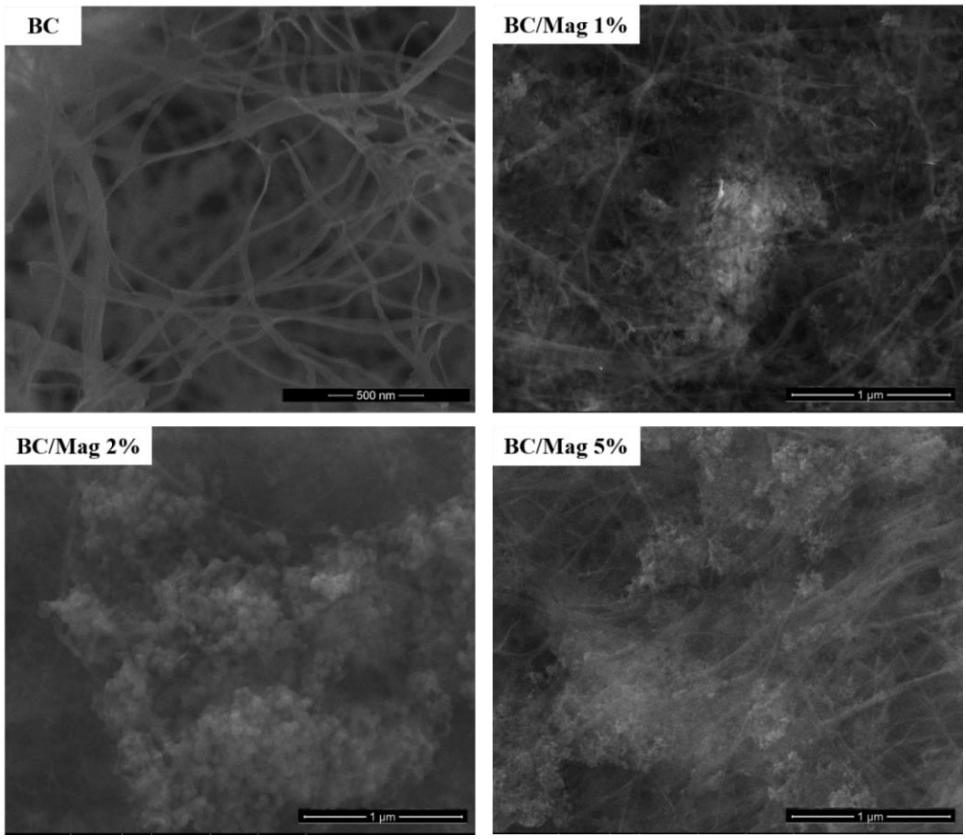


Figure 7.4. SEM images of BC and BC/MAG biocomposites with different concentrations (1%; 2% and 5%) of magnetite.

In order to better highlight the dispersion of magnetite in cellulose membranes, the TEM analysis of biocomposites compared to raw materials was performed, cellulose and magnetite, revealing that the obtained biocomposites have both areas where nanoparticles are well dispersed and areas where clusters were formed.

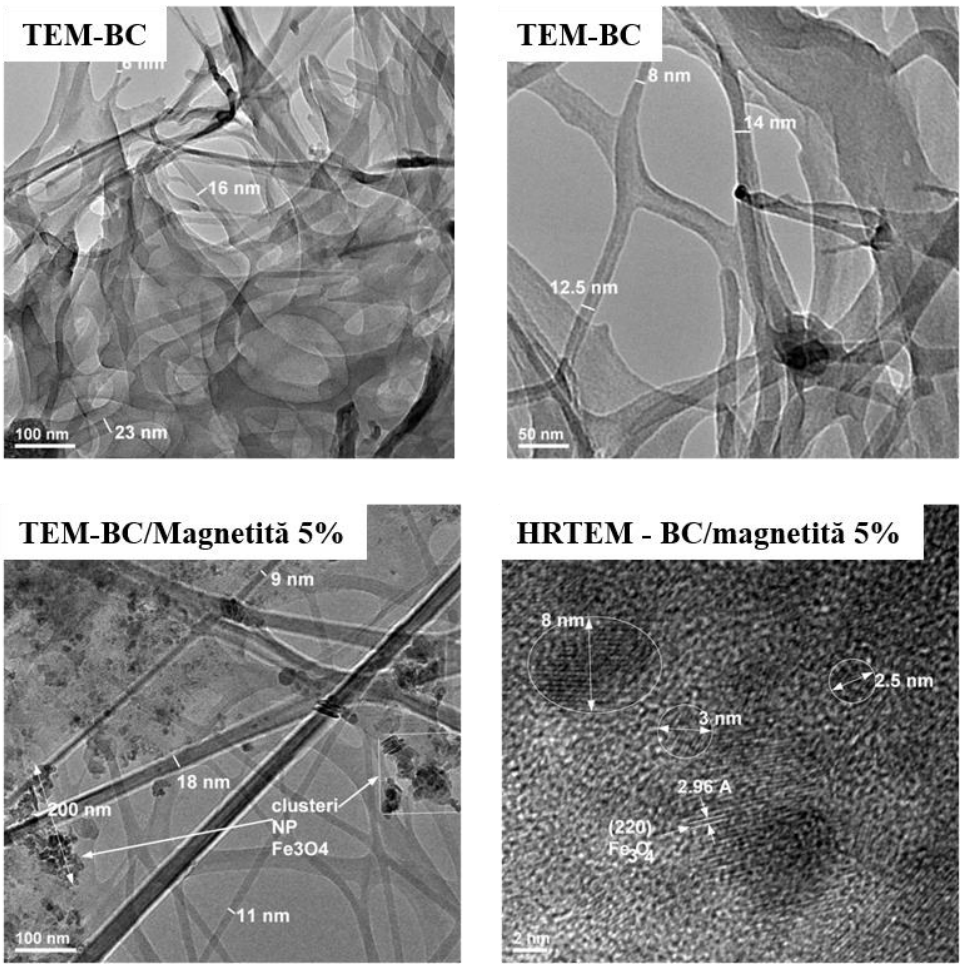


Figure 7.6. TEM images of BC (50 nm and 100 nm) and BC/MAG biocomposite with 5% magnetite; and HR-TEM images of BC/MAG biocomposite with 5% magnetite.

Biocompatibility tests revealed good biocompatibility of this biocomposites on human adipocyte lines. The morphology of hASCs and their ability to interact with the substrate material, BC or BC/MAG bionanocomposites, in terms of cell adhesion and cytoskeleton development were investigated (**Figure 7.7**) and it can be observed that hASCs cells have long and distinct filaments from actin, surrounded by all the magnetic particles in the composites and determines the overall morphology of the cell. The formation of actin microfilaments can be likened to a modeling process that occurs in response to contact between the cell and the biocomposite.

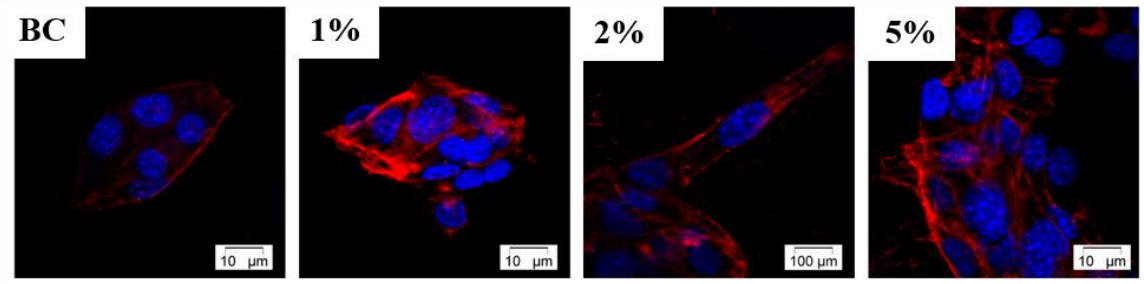


Figure 7.7. Confocal microscopy images of actin filaments (red marking) and nuclei (blue marking) of stem cells derived from human adipocytes in contact with BC and BC/MAG biocomposites with different concentrations of MAG (1%, 2% and 5%).

To study the cell survival rate on the synthesized materials, the viability of hASC 24 hours after inoculation was assessed by LIVE/DEAD assay along with confocal fluorescence microscopy. After 24 hours from seeding, live cells (marked in green) were observed on the surface of all tested materials, but the highest density of live cells was detected on the surface of the BC/MAG biocomposite with 5% magnetite (**Figure 7.8**.).

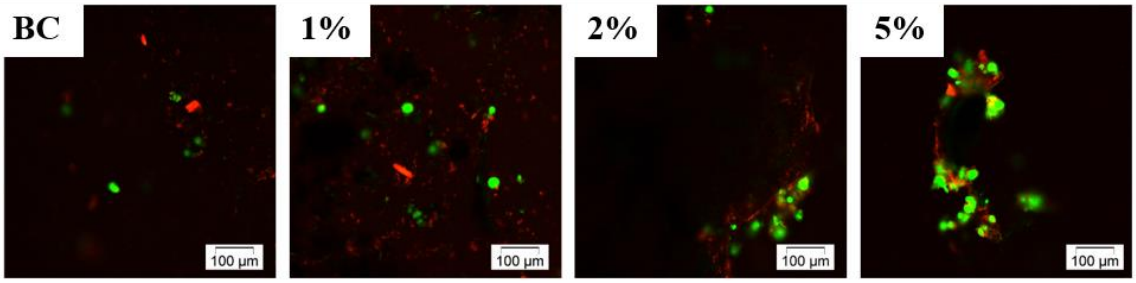


Figure 7.8. Confocal microscopy images after fluorescent labeling of cells to highlight living/dead cells on BC, BC/MAG 1%, BC/MAG 2% and BC/MAG 5%.

Biological tests showed excellent biocompatibility and cell viability of BC/MAG biocomposites with 5% magnetite. This sample is considered a good candidate for in vivo studies in the field of wound healing.

CHAPTER 8. Hydrogels based on fibroin, polyacrylamide and graphene oxide with medical applications

The main purpose of this study is to create implant supports that mimic the natural extracellular matrix (ECM) to ensure cell attachment, proliferation and cell differentiation. Therefore, substrates should have adequate biochemistry, appropriate micro/nano scale topography and mechanical properties to ensure binding positions that actively regulate and control the behavior of host cells in their interaction with the material.

Based on these considerations, a new hydrogel based on silk fibroin, polyacrylamide and graphene oxide has been proposed and characterized, in order to obtain a biocomposite with a superior mechanical response to that of fibroin, but with excellent biocompatibility of silk protein. Due to the low content of information on the biological behavior of fibroin/polyacrylamide/graphene oxide materials on osteoblast cells, a study was developed to highlight a potential application of these biocomposites in the field of bone regeneration.

The preparation of biocomposite hydrogels includes the following steps: i)dispersion of graphene oxide (0.1%) in the fibroin solution by ultrasound; ii)preparation of acrylamide (AA) solutions with different concentrations in which the crosslinking agent N, N'-methylenebisacrylamide (N, N'-MBA) was added, iii)mixing the two solutions and adding the redox polymerization system formed from potassium persulfate (KP) and triethanol amine (TEA). The polymerization reaction lasted 24 h and took place at room temperature, then the hydrogels were purified using the method of repeated extractions in distilled water (**Figure 8.1.**).

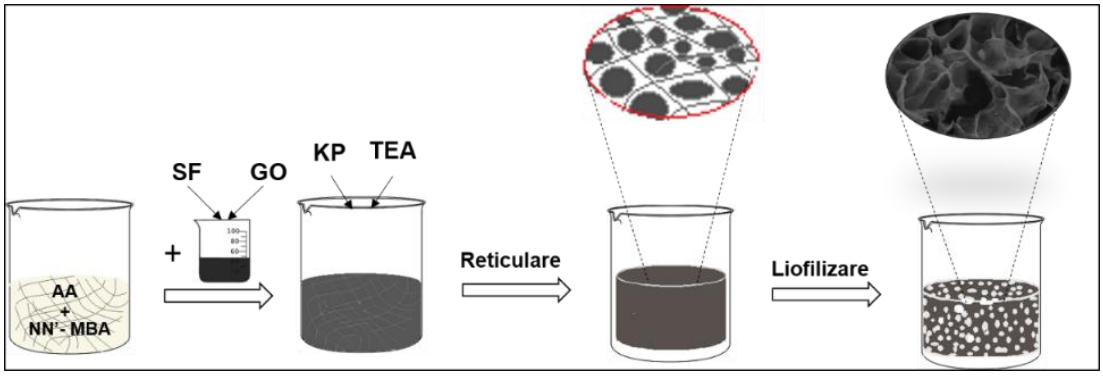


Figure 8.1. Schematic representation of the obtaining of hydrogels based on SF/PAA/GO.

The first step consist in the structural characterization of the hydrogels throught FTIR analysis and showed the formation of the β structure of SF from the biocomposite hydrogels obtained considering both the displacement of the absorption maxima characteristic of amide I and the lack of absorption bands specific to amides II and III. In this case, an important role in the obtaining of the β structure has the components polyacrylamide and graphene oxide (**Figure 8.2.**).

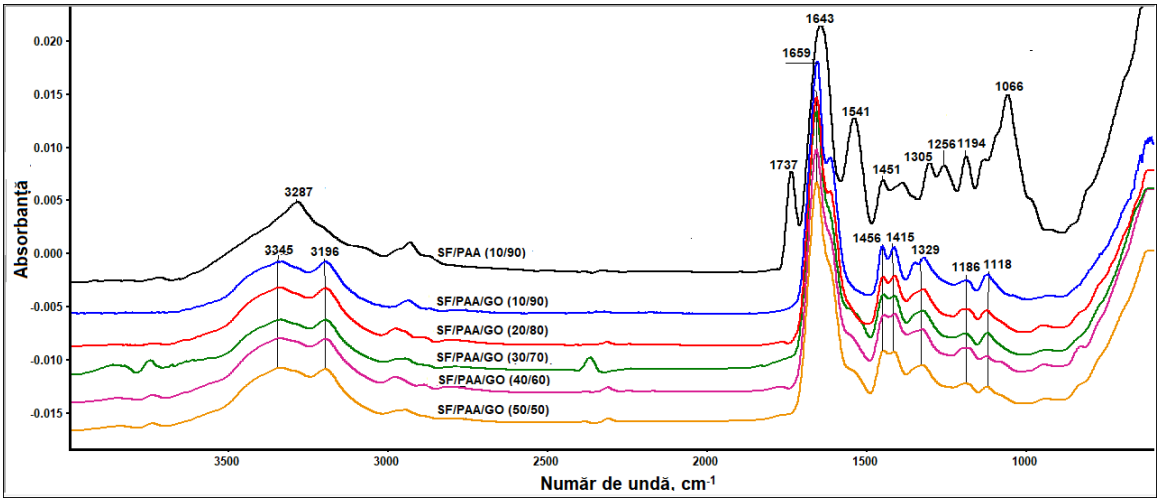


Figure 8.2. FTIR spectra of the SF/PAA hydrogel and of SF/PAA/GO biocomposite hydrogels with different ratios between SF and PAA (10/90, 20/80, 30/70, 40/60, 50/50).

The RAMAN analysis showed a displacement of the GO specific G and D bands in the spectra recorded for biocomposite hydrogels, a displacement that may be due to the intercalation of the polymer chains between the graphene oxide layers.

The study of the swelling capacity of the obtained hydrogels performed through the gravimetric method showed that the incorporation of graphene oxide and the increase of fibroin concentration leads to obtaining higher values of the swelling degree due to the increase in the number of hydrophilic groups. This property is of particular importance because they can have structures similar to tissues that can be easily integrated into host tissues. The graphs of hydrogels based on fibroin, polyacrylamide and graphene oxide with different ratios between fibroin and polyacrylamide (10/90; 30/70; 50/50) containing 0.1% graphene oxide are shown in **Figure 8.4.**

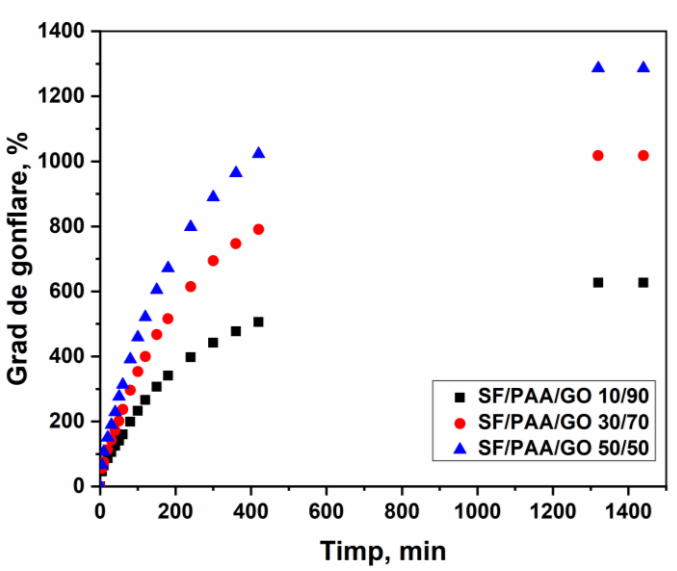


Figure 8.4. The degree of swelling of SF/PAA/GO biocomposite hydrogels with different ratios between SF and PAA (10/90, 30/70, 50/50).

The SEM images realized on completely swollen hydrogels have confirmed the obtaining of three-dimensional porous structures with interconnected pores, along with the increase of the SF concentration in the hydrogels, an increase of the pore size was observed due to the decrease of the crosslinking density.

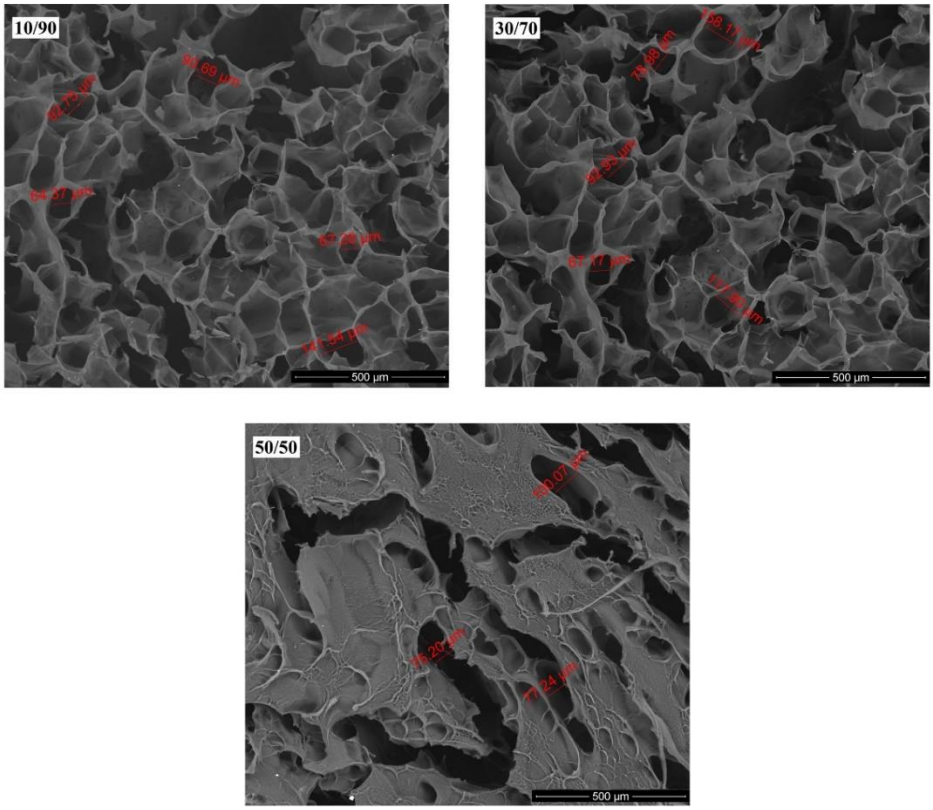


Figure 8.5. SEM images of SF/PAA/GO biocomposite hydrogels with different compositions (10/90, 30/70, 50/50).

The evaluation of the mineralization capacity of the SF/PAA/GO hydrogels was performed by two methods: the alternating cycles method and Kokubo T method. The SEM images of the mineralized hydrogels using the alternating cycles method revealed the obtaining of an acicular type mineral phase close to the hydroxyapatite structure, in this case the composition of the hydrogels did not exert a significant influence on the mineralization capacity of the studied materials **(Figure 8.7.).**

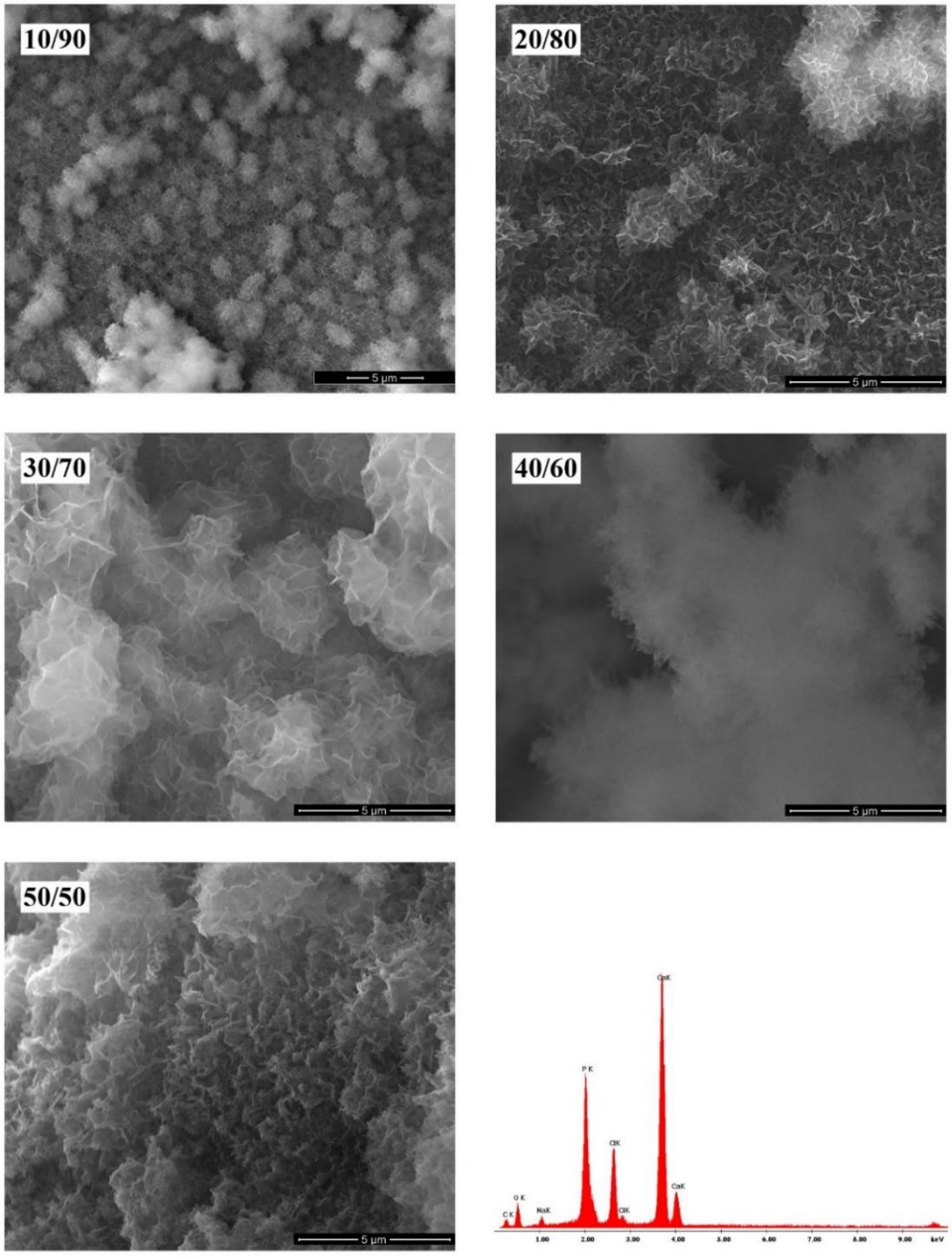


Figure 8.7. SEM images of SF/PAA/GO biocomposite hydrogels with different ratios between SF/PAA (10/90, 20/80, / 30/70, 40/60 and 50/50 and EDX spectrum of hydrogel 50/50) after biomineralization by alternating cycles method.

SEM images of mineralized hydrogels through Kokubo T method showed a uniform coverage of all biocomposite hydrogels with an apatite like mineral layer, whose morphology is strongly dependent on the composition of the hydrogel, respectively, with increasing fibroin content it can be observed the obtaining of a uniform coating with various forms of hydroxyapatite, increasing the number of microglobules and decreasing their size.

Biocompatibility and cytotoxicity tests were performed on biostructures based on osteoblast cell type MC3T3-E1 and hydrogels with different compositions highlighted that the silk protein plays an important role in the built biosystem leading to improvment of the cells adhesion and proliferation.

The morphology of MC3T3-E1 osteoblasts inside of biocomposite hydrogels based on fibroin, polyacrylamide, graphene oxide and their ability to interact with the substrate material in terms of adhesion and cytoskeleton development were studied 24 h after microscopy inoculation. confocal fluorescence **(Figure 8.8.).**

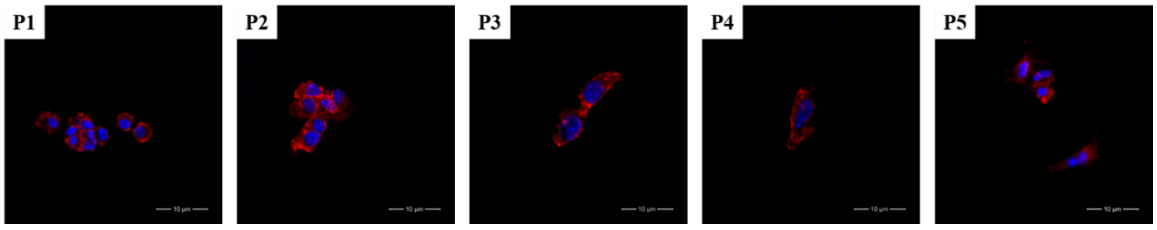


Figure 8.8. Confocal fluorescence microscopy images of actin filaments of MC3T3-E1 osteoblast cells on P1-P5 biostructures.

Thus, MC3T3-E1 cells have distinctive actin filaments surrounding the nuclei in the case of fibroin/polyacrylamide/graphene oxide samples with the following ratios: 30/70; 40/60 and 50/50. This distribution of the components of the cytoskeleton obviously determines the elongation morphology inside the supports with a higher fibroin content. The formation of actin microfilaments can be attributed to a modeling process that occurs in response to direct contact between the cell and the biomaterial. In this context, the results obtained suggest that fibroin in natural silk plays a key role in the adhesion of MC3T3-E1 preosteoblast cells to the biomaterial.

To study cell survival within the tested biomaterials, the viability of MC3T3-E1 cells 7 days after inoculation based on simultaneous labeling of living cells (green) and dead cells (red color) was analyzed using confocal fluorescence microscopy (**Figure 8.9.).**

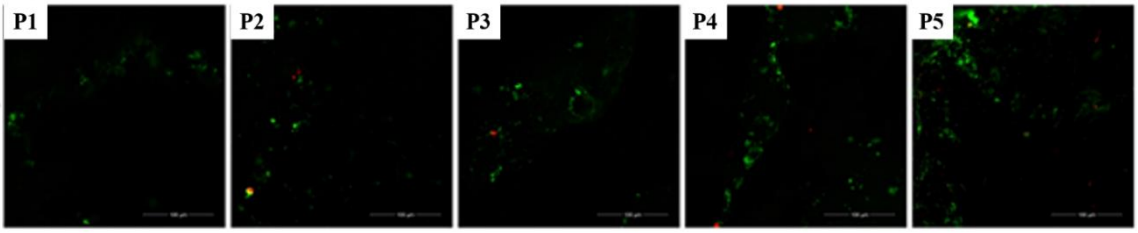


Figure 8.9. Confocal fluorescence microscopy images for the Live/Dead test show both living and dead cells inside P1-P5 biostructures 7 days after seeding. Living cells are marked in green, and dead cells are marked in red.

It can be seen that the ratio between living and dead cells is superunitary for all biocomposites tested. Also, important differences related to cell density inside implant supports are noted, the concentration of living cells increasing with the increasing of the fibroin content. Therefore, the highest number of living cells was obtained in the biostructures fibroin/polyacrylamide/graphene oxide/MC3T3-E1cells with ratios 40/60 and 50/50.

Together with observations related to cell morphology, these data suggest that the protein in natural silk plays a crucial role in the constructed biosystem resulting in both improved adhesion and cell proliferation. In addition, the concentration of fibroin plays an important role in the biocompatibility of implant supports.

CHAPTER 9. DNA biosensors and its interaction with azathioprine

In recent decades, electrochemical biosensors have gained a great importance in the field of analytical devices used to determine the mechanisms of interaction of DNA with various pharmaceutical compounds. Azathioprine (AZA) is an immunosuppressive drug used in the medical field since 1963 [290], initially to prevent kidney transplant rejection and later to treat autoimmune diseases such as ulcerative colitis, rheumatoid arthritis or as a drug against leukemia [314]. This drug is an analogue of purine, its mechanism of action consist to block purine metabolism and DNA synthesis. There are numerous papers in the literature reporting increase of the incidence of skin cancer in patients who received azathioprine and were subsequently exposed to solar radiation; this being one of the reasons that led to the limitation of the use of this drug [315–317]. In order to reduce side effects, it is necessary to use appropriate experimental models at

In this context, the main objective of this study was to investigate the interaction between DNA and azathioprine using a DNA biosensor, and as complementary techniques using UV-Vis spectrophotometry, scanning electron microscopy and mass spectrometry. In situ characterization of the interaction between azathioprine and DNA or purine bases may provide essential information about the effects of this drug on DNA.

In the first place, the redox behavior of azathioprine was studied at a glassy carbon electrode at different pHs using electrochemical techniques such as cyclic voltammetry and differential pulse voltammetry.

The first experiment consisted in recording cyclic voltammograms by scanning in the negative direction (from E_i = 0 V to E_{max} = -1 V) and showed a maximum reduction in E_{pc} = -0.68 V characteristic of the NO_2 group reduction process from azathioprine, and after changing the sweep direction, the appearance of the maxima characteristic of the 2 anodic charge transfer reactions from E_{pa} =+0.45 V and E_{pa} =+1.20 V was observed. All electrochemical measurements were performed using a glassy carbon electrode in azathioprine solutions of 500 μM concentration (**Figure 9.1.**).

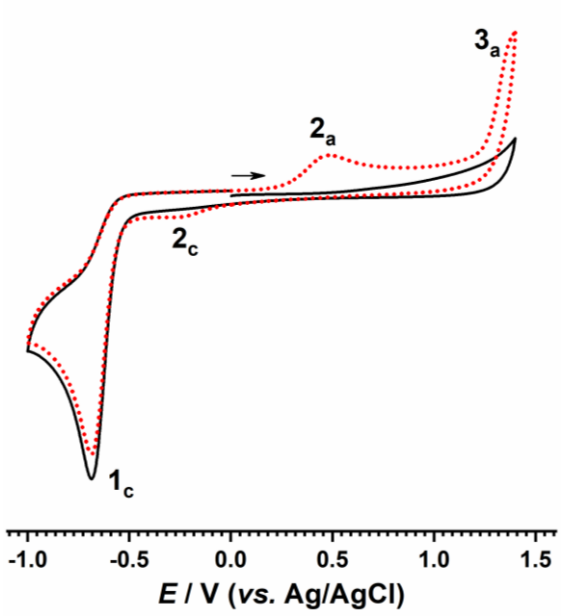


Figure 9.1. Cyclic voltammograms showing, first (—) and second (●●●) scans, recorded in acetate buffer (0.1 M, pH =4.5) containing 500 µM AZA 0.1 M at a scanrate of ν= 50 mVs^{-1}, from E_0=0V to E_{max}=1.4 V.

The next experiment consisted in investigating the effect of the scanrate on the the reduction current of azathioprine, the acquired cyclic voltamograms showed a linear increase of the reduction current with increasing the square of the scanning speed and also a shift of the maximum reduction potential. negative, this type of behavior being characteristic of an irreversibly diffusion-controlled process.

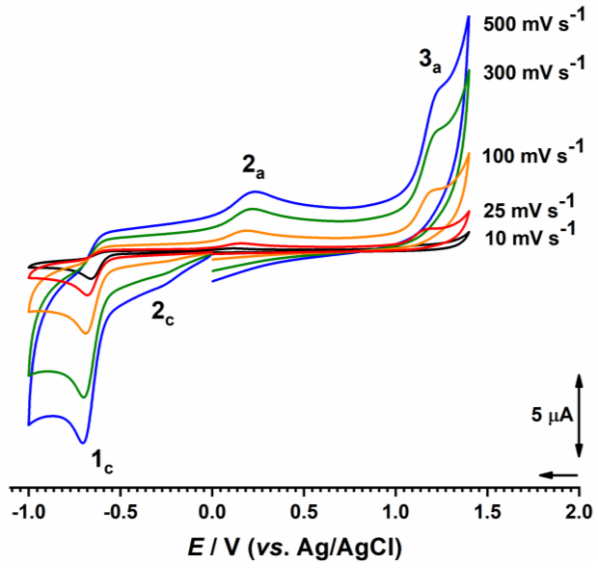


Figura 9.3. Cyclic voltammograms at different scan rates in 500 µM AZA in phosphate buffer (0.1 M, pH = 7.0).

Subsequently, the influence of the pH on the redox behavior of azathioprine by cyclic voltammetry and differential pulse was investigated, the results obtained emphasizing that the reduction potential of azathioprine decreases with increasing pH, this type of behavior suggests that protons are also involved in the reduction process. The electrochemical results presented showed that the reduction of azathioprine takes place in a single step, leading to the formation of reduction products which will be oxidized to positive potentials.

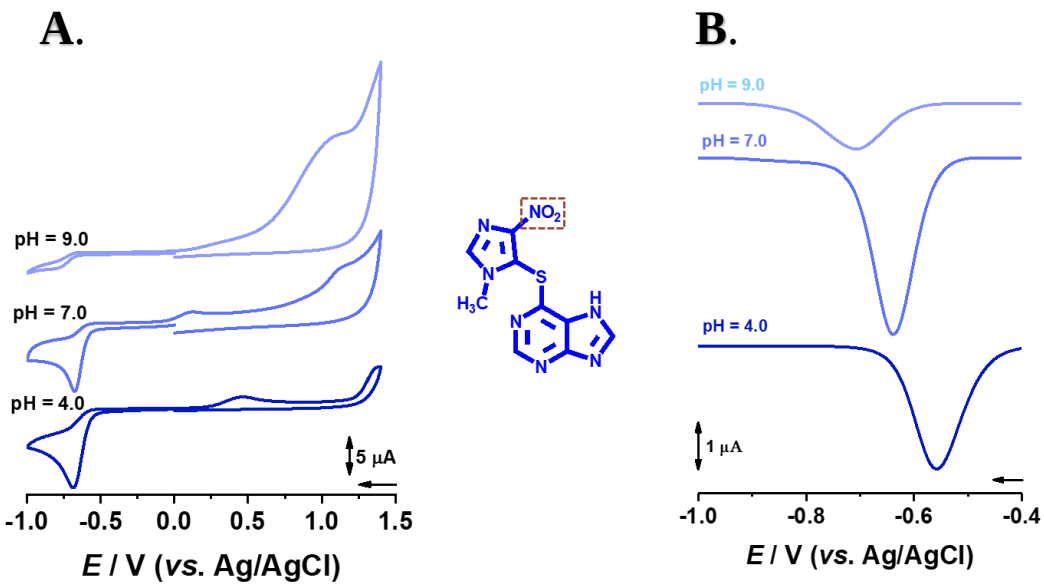
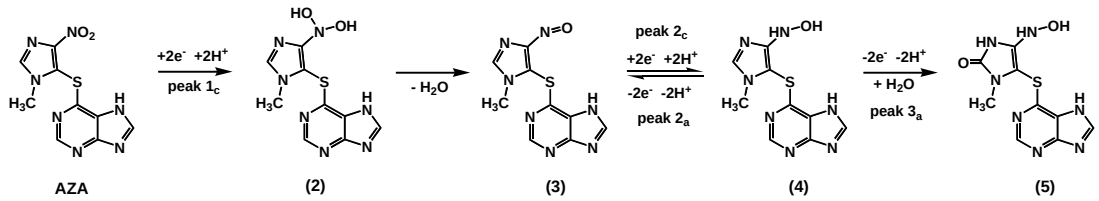


Figure 9.4. A) CV at 100 mV s^{-1} and **B)** DPV recorded in 500 µM AZA in electrolytes with different pH values.

The results presented above showed that the process of reducing azathioprine takes place in a single step. The reduction of azathioprine led to the reduction of products that were subsequently oxidized to positive potential values.

In fact, the voltammetric behavior of azathioprine is dominated by the 1-methyl-4-nitroimidazole compound. In Scheme 1 is proposed to reduce the electrochemical reduction of azathioprine to compound (2) by a mechanism that provides for the conversion of the NO_2 group to a dihydroxylamine compound by a process involving 2 electrons and 2 protons, the maximum value of 1_c, an unstable product, being followed by a dehydration process which led to the obtaining of the compound (3). In turn, compound (3) showed a quasi-reversible redox reaction which was demonstrated by the presence of the two maxima, 2c and 2a, and on the other hand, the process of irreversible oxidation to

positive values of the potential leads to the compound (5), reaction responsible for obtaining the maximum 3a.



Schema 1. Proposed redox mechanism of AZA.

Second, the way in which DNA interacts with azathioprine was investigated using DNA poly [A] or poly [G] electrochemical biosensors,

In the first experiment, differential pulse voltammograms were recorded for the DNA biosensor after incubation in an azathioprine solution 100 µM, varying the incubation time. The electrochemical results showed the appearance of a new oxidation maximum at +0.65 V characteristic to the formation of 8-oxo-dGuo, a compound with a mutagenic effect, while the oxidation maxima of the two purine bases, guanine and adenine, do not present significant changes (**Figure 9.7.**).

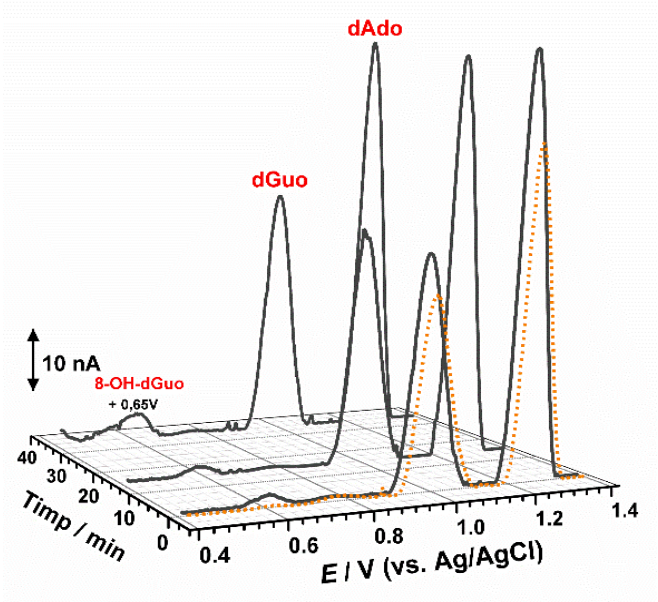


Figura 9.7. DP Voltammogramms recorded in acetate buffer (0.1 M, pH 4.5) with the DNA biosensor after incubation at -0.60 V in 100, 250 and 500 µM AZA for 10 min. Note: the orange dotted curve represents the DP voltammogram with the DNA biosensor after incubation at -0.60 V in buffer.

The second experiment consists in incubating the DNA biosensor in azathioprine solutions of different concentrations, for 10 minutes, under these conditions a gradual increase was observed in accordance with the increase of azathioprine concentration this behavior being induced by the gradual increase of DNA degradation generated by the increase of the drug concentration in the incubation solution also observing the appearance of the maximum from +0.65 V characteristic of the formation of 8-oxo-dGuo (**Figure 9.8.**).

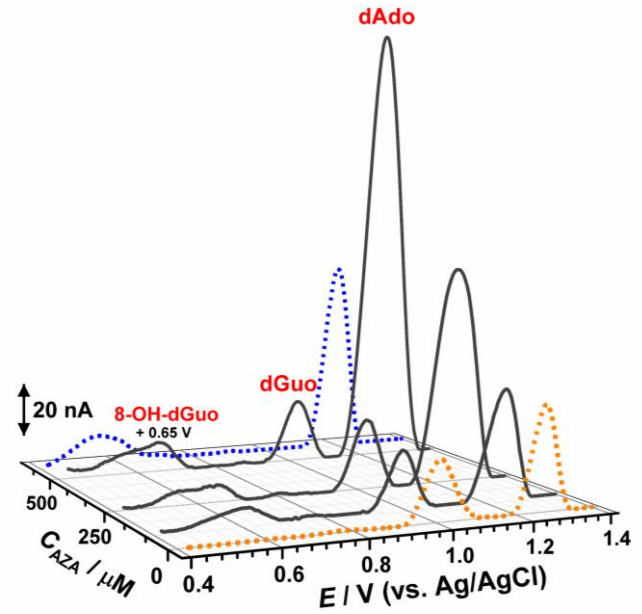


Figure 9.8. DP voltammograms recorded in acetate buffer (0.1 M, pH 4.5) with de DNA biosensor after incubation at -0.60 V in 100 µM AZA for 1, 10 and 35 min. Note: the blue dotted curve represents the DP voltammogram in AZA after applying -0.60 V and the orange dotted curve represents the DP voltammogram with the DNA biosensor after incubation at -0.60 V in buffer.

In order to have a better perspective related to the origin of the maximum from +0.65 V, voltammetric studies were performed using poly [A] (**Figure 9.9.)** and poly [G] (**Figure 9.10.**) biosensors.

Differential pulse voltamograms obtained after the interaction of the poly [A] biosensor with azathioprine showed a significant increase of the anodic potential characteristic to adenine residues, a maximum that can be assigned to both the degradation process of azathioprine and purine residues.

The differential pulse voltamograms obtained after the interaction of the poly [G] biosensor with azathioprine showed both an increase in the anodic potential specific to

the oxidation process of guanine residues and the specific maximum 8-oxo-dGuo (+0.65V).

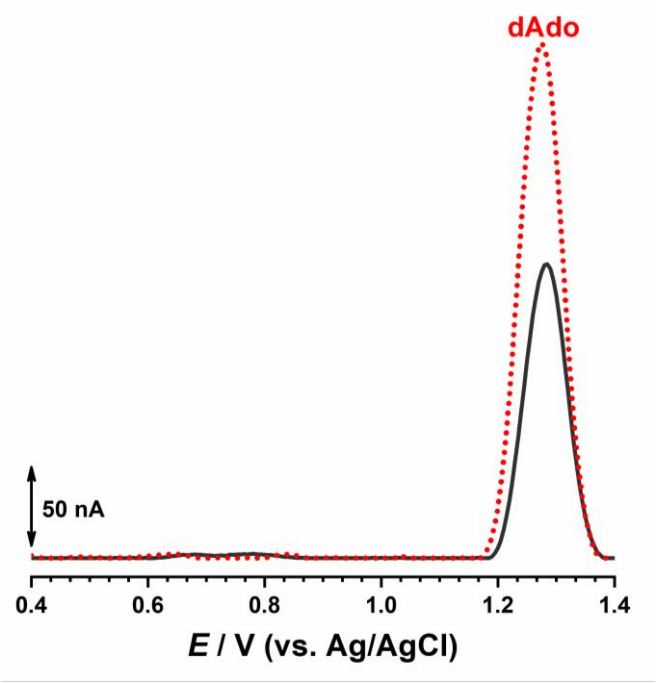


Figure 9.9. DP voltammograms in acetate buffer (0.1 M, pH 4.5) with poly(A) biosensors before and after incubation (red curves) in 500 µM AZA over 10 min.

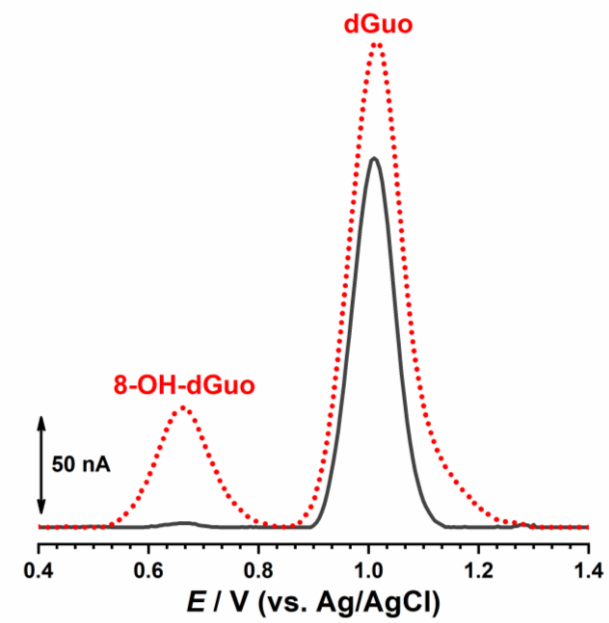


Figure 9.10. DP voltammograms in acetate buffer (0.1 M, pH 4.5) with poly(G) biosensors before and after incubation (red curves) in 500 µM AZA over 10 min.

Furthmore, the mass spectra of DNA sample after the digestion process highlighted the presence of signals specific to free nucleotides. The mass spectra of DNA samples studied after the interaction with azathioprine, both DNA-specific signals and new signals corresponding to fragments of azathioprine were obtained, the presence of 8-oxo-dGuo being also underlined (**Figure 9.11.).** In addition, the mass spectra recorded under these conditions highlighting the presence of new signals that can be assigned to the formation of adducts between purine residues and azathioprine fragments (96 m/z).

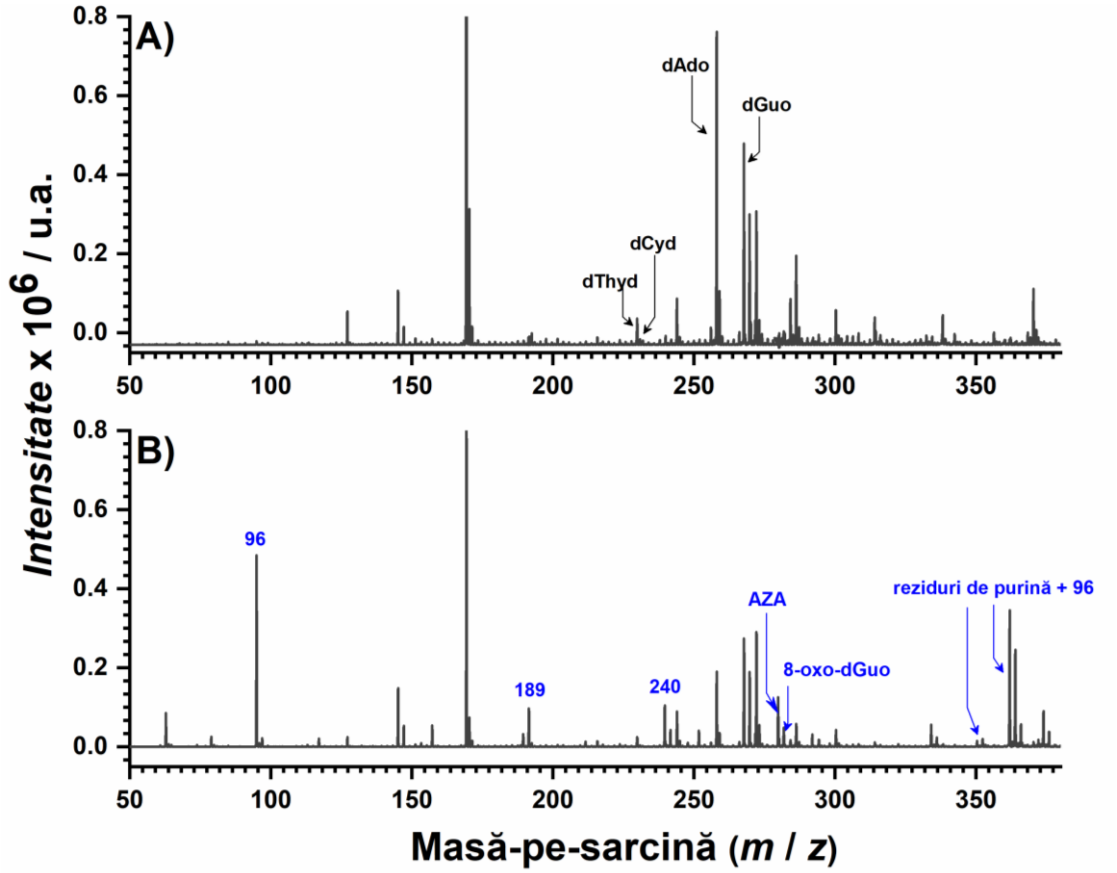


Figure 9.11. Mass spectra of DNA after acidic digestion **A)** bfore and **B)** after incubation at -0.60 V in 500 μM AZA for 30 min.

Subsequently, in order to investigate the interaction between DNA and azathioprine after degradation of the immunosuppressive drug in the presence of light, UV-Vis measurements were performed on degraded samples at the solar simulator using two pHs, the results obtained highlighting that the degradation process was amplified by the presence of protons, in the case of DNA-AZA samples observing the appearance of

the hyperchromic effect characteristic of the DNA denaturing effect effect due to the denaturing action of the immunosuppressive drug on the nucleic acid (**Figure 9.12.)**

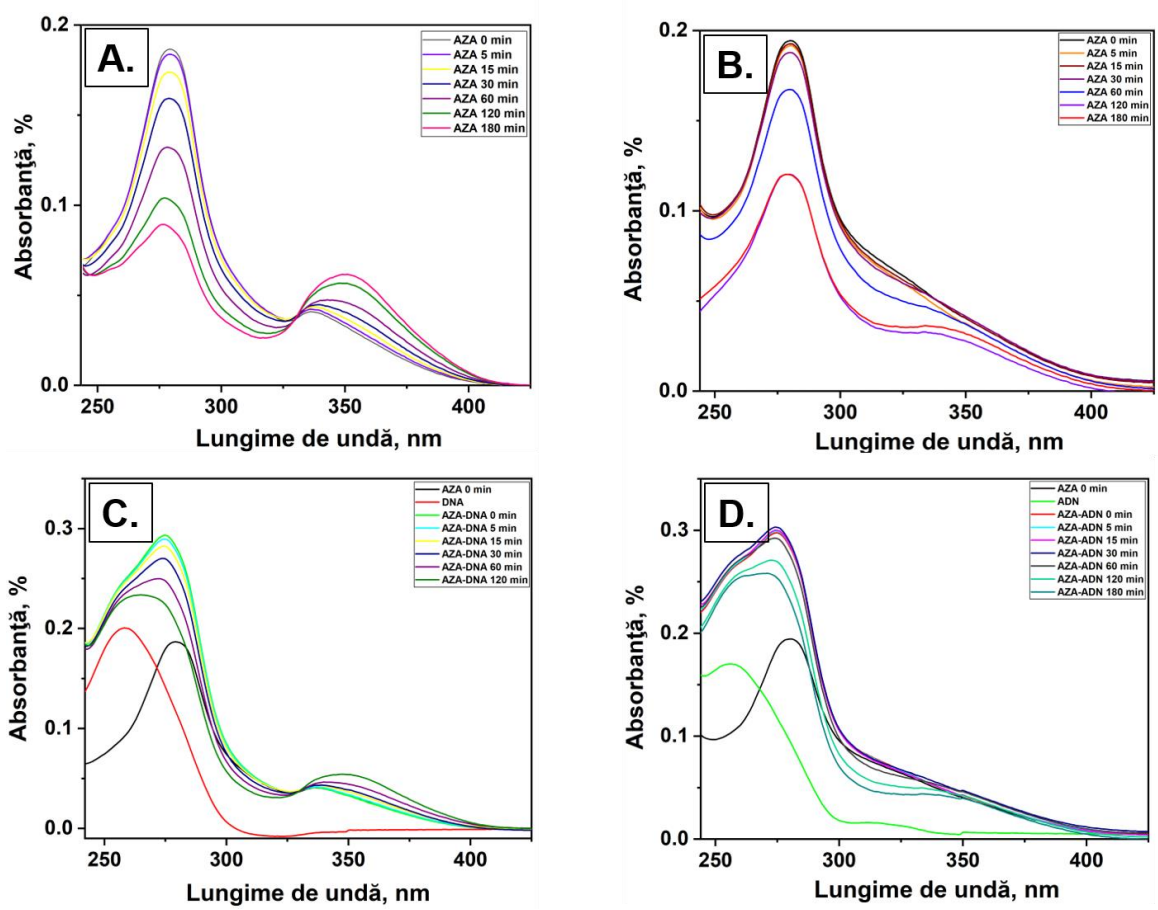
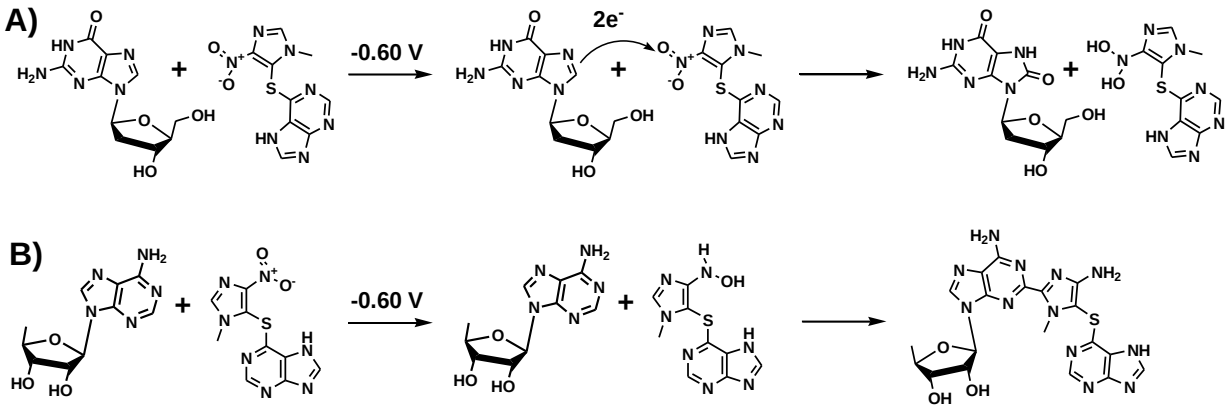


Figura 9.12. Spectrele de absorbție ale: AZA preparate în soluții tampon A) pH 4.5 și B) pH 7 și ale ADN-ului incubat cu AZA după degradarea prin expunerea la lumină între 0 și 180 min folosind probe preparate în soluții tampon C) pH 4,5 și D) pH 7.

Also, a morphological characterization of the DNA biosensors before and after the interaction with azathioprine was performed, the FESEM images obtained in the case of DNA biosensors after the interaction with azathioprine highlighting the formation of clusters assigned to the action of the immunosuppressive drug on DNA.

The electrochemical results correlated with the morphological results obtained by FESEM and with the spectrometric results obtained by mass spectrometry, confirms that with AZA reduction, the formation of 8-oxo-dGuo takes place among the DNA layers, a mutagenic compound which can generate oxidative lesions of DNA, based on

these data a mechanism of interaction between DNA and azathioprine has been proposed, **Scheme 9.2.**



Scheme 9.2. Proposed mechanism of interaction between AZA and DNA with DNA bases **(A)** formation of 8-oxo-deoxyguanosine and **(B)** adducts with adenine bases

In this study, the electrochemical behavior of azathioprine was evaluated through cyclic voltammetry measurements and a complex redox mechanism was proposed for it. At the same time, in order to determine the mechanism of interaction between azathioprine and DNA, a DNA biosensor was used was incubated under different conditions in azathioprine solutions, the results showing that this drug leads to DNA damage, oxidation of guanine residues and obtaining 8-oxo-dGuo, a product with mutagenic potential. The electrochemical results obtained being confirmed with the help of mass spectrometry.

CHAPTER 10. General conclusions and perspectives

The research topics studied in the doctoral thesis led to the development of biocomposites based on natural polymers that have the suitable characteristics for the medical applications field. In this context, all synthesized biocomposites were characterized by different techniques in terms of structural, morphological, thermal, watering, swelling, mineralization, biocompatibility and cytotoxicity properties. Biological tests performed on different cell lines, depending on the intended application, showed a good biocompatibility of the obtained materials with the biological environment.

In chapter 4, organic/organic biocomposites based on bacterial cellulose and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with different concentrations were obtained, the novelty factor being both the use of this type of polyester and the use of dried membranes of bacterial cellulose. The prepared materials were characterized in terms of morphology, structure, thermal stability, biological behavior and watering properties. The modification of bacterial cellulose with poly(3-hydroxybutyrate-co-3-hydroxyvalerate) had two main objectives, namely to improve the barrier properties and to improve the biocompatibility and biodegradability of the synthesized materials. By performing contact angle measurements on biocomposites based on bacterial cellulose and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) it was shown that the surface tends to a hydrophobic character compared to the surface of bacterial cellulose characterized by hydrophilicity, this confirming at the same time the improvement of the barrier properties. From the point of view of biological tests, it was shown that the obtained biocomposite membranes presents biocompatibility and, respectively, good cell viability on human adipocyte cell lines, thus giving the possibility to consider this type of materials potential candidates for applications in the field of wound healing.

In the following study, new biocomposites based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and organophilized double-layered hydroxides with different concentrations were developed by ultrasonic dispersion of anionic clay in the polymer solution. Biocomposite films were obtained by the solvent evaporation technique and were characterized in terms of structural, morphological, thermal stability and in vitro biological behavior. In this case, the novelty factor comes from the use of this type of

material in the field of wound healing, taking into account the fact that, until this study, materials based on polyhydroxyalkanoates and double layered hydroxides targeted only industrial applications such as water treatment. Biological tests showed a good biocompatibility of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) biocomposite materials/organophilized double-layered hydroxides by LIVE/DEAD tests, observing a better mimicry with cellular medium compared to the reference material used, taking into account the better viability of the cells 24 hours after seeding. Due to the good biocompatibility shown, the biocomposites based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and organophilized double-layered hydroxides are potential candidates for *in vivo* testing in the field of wound healing applications.

Regarding the next chapter, new biocomposites based on natural silk and magnetic nanoparticles with different compositions were prepared by dispersing the magnetite nanoparticles in the silk fibroin solution. The development of this type of biocomposite materials is an original idea considering that, this type of materials have not been previously reported. The biocomposites were characterized from a structural and morphological point of view in order to highlight the influence of the magnetic nanoparticles to the polymeric matrix and the dispersion of magnetic nanoparticles in the polymer matrix. Biological tests showed a good biocompatibility of fibroin/magnetite biocomposite films, through the LIVE/DEAD test showing that the biocomposites have a better mimicity with the cell environment compared to the reference material used, considering the viability of the cells was better 24 hours after seeding. Due to the good biocompatibility shown, fibroin and magnetite biocomposites are possible candidates for in vivo tests for wound healing applications.

Next, new biocomposites based on bacterial cellulose and magnetic nanoparticles with different compositions obtained by the *in situ* method, respectively, by adding the magnetite nanoparticles in the cellulose culture medium, were synthesized and characterized. Structural and morphological analyzes highlighted the deposition/penetration of magnetite nanoparticles on/between cellulose fibers and the fact that at higher concentrations of magnetite, magnetic nanoparticles presents a uniform coverage of cellulose fibers. Biological tests showed a good biocompatibility and a good cellular viability of bacterial cellulose/magnetite composites on human adipocyte cell lines, the best results being obtained for the materials containing 5%

magnetite, a material that can be considered a good candidate for development of *in vivo* studies in the field of wound healing.

In the following study, the obtaining of composite hydrogels based on silk fibroin, polyacrylamide and graphene oxide was presented. Morpho-structural analyzes showed both the formation of the β structure of fibroin and the influence of fibroin and graphene oxide on the properties of hydrogels obtained. The increase in fibroin content and the introduction of graphene oxide led to increased swelling behavior and improved mineralization capacity. The best results were obtained on biocomposites based on silk fibroin, polyacrylamide and graphene oxide with the following compositions: 40/60/0.1 and 50/50 /0.1. Biological tests have shown that the morphology of osteoblasts varies in the hydrogels fibroin/polyacrylamide/graphene oxide with different compositions, the data obtained suggesting that silk fibroin improves the organization of the cytoskeleton and cell distribution. The viability and proliferation results together with those on cytotoxic potential suggested that osteoblasts have a higher proliferation rate in contact with hydrogels with a higher content of silk fibroin. Therefore, the biocomposites fibroin/polyacrylamide/graphene oxide with compositions 40/60/0.1 and 50/50/0.1 have been shown to be biocompatible and have the most balanced ratio between proliferative and cytotoxic potential. Excellent biocompatibility, good mechanical properties and biomineralization potential recommend biocomposites based on fibroin, polyacrylamide and graphene oxide for potential biomedical applications in the area of bone regeneration. However, the long-term cytotoxic potential and non-biodegradability of graphene oxide and the specific effects of graphene on cells, tissues or organs and in vivo metabolism require future studies.

In the last chapter of original contributions of this doctoral thesis dedicated to DNA-based biosensors and their interaction with azathioprine, the redox behavior of azathioprine was studied at glassy carbon electrode using different pHs and electrochemical techniques such as cyclic voltammetry and differential pulse voltammetry leading to the proposal of a redox mechanism of this immunosuppressive drug. It has also been shown that the reduction of azathioprine takes place in a single step, leading to the formation of reduction products which in turn will be oxidized to positive potentials. Various DNA, poly [A] or poly [G] electrochemical biosensors were used in this study to invastigate interaction mechanism between DNA and azathioprine. The results obtained by electrochemical methods were correlated with the morphological

results obtained by FESEM and with the spectrometric results obtained by mass spectrometry, thus confirming that with the reduction of azathioprine, the formation of 8-oxo-dGuo takes place among the DNA layers, a compound with mutagenic potential, which generates oxidative damage to DNA.

In conclusion, during the present doctoral thesis biocomposites and biosensors based on natural polymers with adequate characteristics for medical applications were developed and characterized.

In the future work, the aim will be to improve the characteristics of biocomposites based on polyhydroxyalkanoate by electrophilating the polymer to obtain three-dimensional porous structures with a large specific surface area suitable for use in the medical field. Also, the biocompatibility, biodegradability, mechanical and antimicrobial properties of natural polyester can be improved by modifying with different types of inorganic structures such as zinc oxide or magnetic nanoparticles. For bacterial cellulose and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) biocomposites, the aim will be to improve the dispersion of natural copolyester among and on cellulose nanofibers and the purification process of biocomposites will be streamlined. To improve the dispersion of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) the porosity of the cellulose membrane will be modified by lyophilization and a vacuum chamber will be used during impregnation. In the case of silk and nanoparticle biocomposite films, the aim will be to improve the dimensional stability by optimizing experiential parameters such as concentration of the polymer solution, drying temperature, ultrasonic time, polymer film thickness or reinforcing agent concentration. In the case of fibroin/polyacrylamide/graphene oxide hydrogels, investigated the mechanical properties should be considered, as they have particular importance in the field of hard tissue engineering applications. Given that all biocomposites obtained in this doctoral thesis target medical applications, all proposed optimizations may involve restoring biocompatibility and cytotoxicity and performing in vivo tests for materials with appropriate properties. The use of natural polymers will also be pursued for the development of biosensor-type devices modified with natural polymers to facilitate the immobilization of enzymes or other types of bioreceptors and to improve the electrochemical response and to develop actuators based on natural polymers for applications in the field of artificial muscles.

Selective bibliography

[1] A.K. Błedzki, A. Jaszkiewicz, M. Urbaniak, D. Stankowska-Walczak, Biocomposites in the past and in the future, Fibres Text. East. Eur. 96 (2012) 15–22.

[2] P. Jayakumar, L. Di Silvio, Cellular response to biocomposites, Biomed. Compos. (2009) 354–384. https://doi.org/10.1533/9781845697372.3.354.

[3] K. Haraguchi, Encyclopedia of Polymeric Nanomaterials, Encycl. Polym. Nanomater. (2021) 1–8. https://doi.org/10.1007/978-3-642-36199-9.

[4] M. Pumera, Electrochemistry of graphene, graphene oxide and other graphenoids: Review, Electrochem. Commun. 36 (2013) 14–18. https://doi.org/10.1016/j.elecom.2013.08.028.

[5] D. Wei, Y. Liu, Controllable synthesis of graphene and its applications, Adv. Mater. 22 (2010) 3225–3241. https://doi.org/10.1002/adma.200904144.

[6] L. Horváth, A. Magrez, M. Burghard, K. Kern, L. Forró, B. Schwaller, Evaluation of the toxicity of graphene derivatives on cells of the lung luminal surface, Carbon N. Y. 64 (2013) 45–60. https://doi.org/10.1016/j.carbon.2013.07.005.

[7] P.C. Henriques, A.T. Pereira, A.L. Pires, A.M. Pereira, F.D. Magalhães, I.C. Gonçalves, Graphene Surfaces Interaction with Proteins, Bacteria, Mammalian Cells, and Blood Constituents: The Impact of Graphene Platelet Oxidation and Thickness, ACS Appl. Mater. Interfaces. 12 (2020) 21020–21035. https://doi.org/10.1021/acsami.9b21841.

[8] T.H. Lee, C.T. Yen, S.H. Hsu, Preparation of Polyurethane-Graphene Nanocomposite and Evaluation of Neurovascular Regeneration, ACS Biomater. Sci. Eng. 6 (2020) 597–609. https://doi.org/10.1021/acsbiomaterials.9b01473.

[9] M. Silva, N.M. Alves, M.C. Paiva, Graphene-polymer nanocomposites for biomedical applications, Polym. Adv. Technol. 29 (2018) 687–700. https://doi.org/10.1002/pat.4164.

[10] C. Cheng, S. Li, A. Thomas, N.A. Kotov, R. Haag, Functional Graphene Nanomaterials Based Architectures: Biointeractions, Fabrications, and Emerging Biological Applications, Chem. Rev. 117 (2017) 1826–1914. https://doi.org/10.1021/acs.chemrev.6b00520.

[11] D.A. Dikin, S. Stankovich, E.J. Zimney, R.D. Piner, G.H.B. Dommett, G. Evmenenko, S.T. Nguyen, R.S. Ruoff, Preparation and characterization of graphene oxide paper, Nature. 448 (2007) 457–460. https://doi.org/10.1038/nature06016.

[12] D.R. Dreyer, S. Park, C.W. Bielawski, R.S. Ruoff, The chemistry of graphene oxide, Chem. Soc. Rev. 39 (2010) 228–240. https://doi.org/10.1039/b917103g.

[13] M.Z.I. Nizami, S. Takashiba, Y. Nishina, Graphene oxide: A new direction in dentistry, Appl. Mater. Today. 19 (2020) 100576. https://doi.org/10.1016/j.apmt.2020.100576.

[14] S.H. Ku, C.B. Park, Myoblast differentiation on graphene oxide, Biomaterials. 34 (2013) 2017–2023. https://doi.org/10.1016/j.biomaterials.2012.11.052.

[15] M.P. Figueiredo, V.R.R. Cunha, F. Leroux, C. Taviot-Gueho, M.N. Nakamae, Y.R. Kang, R.B. Souza, A.M.C.R.P.F. Martins, I.H.J. Koh, V.R.L. Constantino, Iron-Based Layered Double Hydroxide Implants: Potential Drug Delivery Carriers with Tissue Biointegration Promotion and Blood Microcirculation Preservation, ACS Omega. 3 (2018) 18263–18274. https://doi.org/10.1021/acsomega.8b02532.

[16] C. Li, M. Wei, D.G. Evans, X. Duan, Recent advances for layered double hydroxides (LDHs) materials as catalysts applied in green aqueous media, Catal. Today. 247 (2015) 163–169. https://doi.org/10.1016/j.cattod.2014.05.032.

[17] Y. Liu, Y. Wu, R. Zhang, J. Lam, J.C. Ng, Z.P. Xu, L. Li, H.T. Ta, Investigating the Use of Layered Double Hydroxide Nanoparticles as Carriers of Metal Oxides for Theranostics of ROS-Related Diseases, ACS Appl. Bio Mater. 2 (2019) 5930–5940. https://doi.org/10.1021/acsabm.9b00852.

[18] X. Lu, L. Meng, H. Li, N. Du, R. Zhang, W. Hou, Facile fabrication of ibuprofen-LDH nanohybrids via a delamination/ reassembling process, Mater. Res. Bull. 48 (2013) 1512–1517. https://doi.org/10.1016/j.materresbull.2012.12.057.

[19] V. Rives, M. del Arco, C. Martín, Intercalation of drugs in layered double hydroxides and their controlled release: A review, Appl. Clay Sci. 88–89 (2014) 239–269. https://doi.org/10.1016/j.clay.2013.12.002.

[20] F. Barahuie, M.Z. Hussein, P. Arulselvan, S. Fakurazi, Z. Zainal, Drug delivery system for an anticancer agent, chlorogenate-Zn/Al-layered double hydroxide nanohybrid synthesised using direct co-precipitation and ion exchange methods, J. Solid State Chem. 217 (2014) 31–41. https://doi.org/10.1016/j.jssc.2014.04.015.

[21] M.S. San Román, M.J. Holgado, B. Salinas, V. Rives, Drug release from layered double hydroxides and from their polylactic acid (PLA) nanocomposites, Appl. Clay Sci. 71 (2013) 1–7. https://doi.org/10.1016/j.clay.2012.10.014.

[22] M. Baikousi, A. Stamatis, M. Louloudi, M.A. Karakassides, Thiamine pyrophosphate intercalation in layered double hydroxides (LDHs): An active bio-hybrid catalyst for pyruvate decarboxylation, Appl. Clay Sci. 75–76 (2013) 126–133. https://doi.org/10.1016/j.clay.2013.02.006.

[23] M. Chakraborti, J.K. Jackson, D. Plackett, D.M. Brunette, H.M. Burt, Drug intercalation in layered double hydroxide clay: Application in the development of a nanocomposite film for guided tissue regeneration, Int. J. Pharm. 416 (2011) 305–313. https://doi.org/10.1016/j.ijpharm.2011.06.016.

[24] A. Jaiswal, M.C. Chattopadhyaya, Synthesis and characterization of novel Co/Bi-layered double hydroxides and their adsorption performance for lead in aqueous solution, Arab. J. Chem. 10 (2017) S2457–S2463. https://doi.org/10.1016/j.arabjc.2013.09.010.

[25] T.H. Kim, J.M. Oh, Dual nutraceutical nanohybrids of folic acid and calcium containing layered double hydroxides, J. Solid State Chem. 233 (2016) 125–132. https://doi.org/10.1016/j.jssc.2015.10.019.

[26] W.Y. Wang, H. Liu, S.P. Li, X.D. Li, Synthesis of methotrexatum intercalated zinc-aluminum-layered double hydroxides and the corresponding cell studies, Appl. Clay Sci. 121–122 (2016) 103–110. https://doi.org/10.1016/j.clay.2015.11.025.

[27] Y. Kuthati, R.K. Kankala, C.H. Lee, Layered double hydroxide nanoparticles for biomedical applications: Current status and recent prospects, Appl. Clay Sci. 112–113 (2015) 100–116. https://doi.org/10.1016/j.clay.2015.04.018.

[28] T. Zhan, X. Wang, Y. Zhang, Y. Song, X. Liu, J. Xu, W. Hou, Direct electrochemistry and electrocatalysis of hemoglobin immobilized in layered double hydroxides modified with amino functionalized ionic liquid through coprecipitation technique, Sensors Actuators, B Chem. 220 (2015) 1232–1240. https://doi.org/10.1016/j.snb.2015.07.043.

[29] C.F. Dai, D.Y. Tian, S.P. Li, X.D. Li, Methotrexate intercalated layered double hydroxides with the mediation of surfactants: Mechanism exploration and bioassay study, Mater. Sci. Eng. C. 57 (2015) 272–278. https://doi.org/10.1016/j.msec.2015.07.040.

[30] J. Perera, M. Weerasekera, N. Kottegoda, Slow release anti-fungal skin formulations based on citric acid intercalated layered double hydroxides nanohybrids, Chem. Cent. J. 9 (2015) 0–7. https://doi.org/10.1186/s13065-015-0106-3.

[31] I. Gualandi, E. Scavetta, Y. Vlamidis, A. Casagrande, D. Tonelli, Co/Al layered double hydroxide coated electrode for in flow amperometric detection of sugars, Electrochim. Acta. 173 (2015) 67–75. https://doi.org/10.1016/j.electacta.2015.04.172.

[32] N. Carolina, C. Hill, Magnetite : Structure , Properties and Applications, (2015).

[33] H. El Ghandoor, H.M. Zidan, M.M.H. Khalil, M.I.M. Ismail, Synthesis and some physical properties of magnetite (Fe 3O 4) nanoparticles, Int. J. Electrochem. Sci. 7 (2012) 5734–5745.

[34] K.Y. Castrejón-Parga, H. Camacho-Montes, C.A. Rodríguez-González, C. Velasco-Santos, A.L. Martínez-Hernández, D. Bueno-Jaquez, J.L. Rivera-Armenta, C.R. Ambrosio, C.C. Conzalez, M.E. Mendoza-Duarte, P.E. García-Casillas, Chitosan-starch film reinforced with magnetite-decorated carbon nanotubes, J. Alloys Compd. 615 (2015) S505–S510. https://doi.org/10.1016/j.jallcom.2013.12.269.

[35] M. Mahdavi, M. Bin Ahmad, M.J. Haron, F. Namvar, B. Nadi, M.Z. Ab Rahman, J. Amin, Synthesis, surface modification and characterisation of biocompatible magnetic iron oxide nanoparticles for biomedical applications, Molecules. 18 (2013) 7533–7548. https://doi.org/10.3390/molecules18077533.

[36] E. Tanasa, C. Zaharia, A. Hudita, I.C. Radu, M. Costache, B. Galateanu, Impact of the magnetic field on 3T3-E1 preosteoblasts inside SMART silk fibroin-based scaffolds decorated with magnetic nanoparticles, Mater. Sci. Eng. C. 110 (2020) 1–13. https://doi.org/10.1016/j.msec.2020.110714.

[37] J.L. Kirschvink, A. Kobayashi-Kirschvink, B.J. Woodford, Magnetite biomineralization in the human brain, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 7683–7687. https://doi.org/10.1073/pnas.89.16.7683.

[38] A. Pohl, F. Berger, R.M.A. Sullan, C. Valverde-Tercedor, K. Freindl, N. Spiridis, C.T. Lefèvre, N. Menguy, S. Klumpp, K.G. Blank, D. Faivre, Decoding Biomineralization: Interaction of a Mad10-Derived Peptide with Magnetite Thin

Films, Nano Lett. 19 (2019) 8207–8215. https://doi.org/10.1021/acs.nanolett.9b03560.

[39] B. Kalska-Szostko, M. Rogowska, A. Dubis, K. Szymański, Enzymes immobilization on Fe 3 O 4 -gold nanoparticles, Appl. Surf. Sci. 258 (2012) 2783–2787. https://doi.org/10.1016/j.apsusc.2011.10.132.

[40] S. Sathiyavimal, S. Vasantharaj, F. LewisOscar, R. Selvaraj, K. Brindhadevi, A. Pugazhendhi, Natural organic and inorganic–hydroxyapatite biopolymer composite for biomedical applications, Prog. Org. Coatings. 147 (2020) 105858. https://doi.org/10.1016/j.porgcoat.2020.105858.

[45] E.E. da Silva, H.H.M. Della Colleta, A.S. Ferlauto, R.L. Moreira, R.R. Resende, S. Oliveira, G.T. Kitten, R.G. Lacerda, L.O. Ladeira, Nanostructured 3-D collagen/nanotube biocomposites for future bone regeneration scaffolds, Nano Res. 2 (2009) 462–473. https://doi.org/10.1007/s12274-009-9042-7.

[50] J.P. Reddy, S. Ahankari, M. Misra, A. Mohanty, A new class of injection moulded structural biocomposites from PHBV bioplastic and carbon fibre, Macromol. Mater. Eng. 298 (2013) 789–795. https://doi.org/10.1002/mame.201200087.

[52] W.S. Pietrzak, B.L. Eppley, The effect of high temperature intraoperative molding on bioabsorbable PLLA-PGA craniofacial fixation, J. Craniofac. Surg. 17 (2006) 920–925. https://doi.org/10.1097/01.scs.0000229554.27823.fa.

[58] A.M. Al-Ghraibah, M. Al-Qudah, F.M. AL-Oqla, Medical Implementations of Biopolymers, Elsevier Inc., 2020. https://doi.org/10.1016/b978-0-12-819661-8.00010-x.

[67] I. Sulaeva, U. Henniges, T. Rosenau, A. Potthast, Bacterial cellulose as a material for wound treatment: Properties and modifications: A review, Biotechnol. Adv. 33 (2015) 1547–1571. https://doi.org/10.1016/j.biotechadv.2015.07.009.

[70] M.G. Albu, Z. Vuluga, D.M. Panaitescu, D.M. Vuluga, A. Căşărică, M. Ghiurea, Morphology and thermal stability of bacterial cellulose/collagen composites, Cent. Eur. J. Chem. 12 (2014) 968–975. https://doi.org/10.2478/s11532-014-0545-z.

[98] B. Galateanu, M.-C. Bunea, P. Stanescu, E. Vasile, A. Casarica, H. Iovu, A. Hermenean, C. Zaharia, M. Costache, In Vitro Studies of Bacterial Cellulose and Magnetic Nanoparticles Smart Nanocomposites for Efficient Chronic Wounds Healing, Stem Cells Int. 2015 (2015). https://doi.org/10.1155/2015/195096.

[102] L. Lamboni, C. Xu, J. Clasohm, J. Yang, M. Saumer, K.H. Schäfer, G. Yang, Silk sericin-enhanced microstructured bacterial cellulose as tissue engineering scaffold towards prospective gut repair, Mater. Sci. Eng. C. 102 (2019) 502–510. https://doi.org/10.1016/j.msec.2019.04.043.

[156] D.M. Panaitescu, I. Lupescu, A.N. Frone, I. Chiulan, C.A. Nicolae, V. Tofan, A. Stefaniu, R. Somoghi, R. Trusca, Medium Chain-Length Polyhydroxyalkanoate Copolymer Modified by Bacterial Cellulose for Medical Devices, Biomacromolecules. 18 (2017) 3222–3232. https://doi.org/10.1021/acs.biomac.7b00855.

[158] T. Tsujimoto, N. Hosoda, H. Uyama, Fabrication of porous poly(3-hydroxybutyrate-co-3-hydroxyvalerate monoliths via thermally induced phase

separation, Polymers (Basel). 8 (2016). https://doi.org/10.3390/polym8030066.

[186] Y. Wang, J. Guo, L. Zhou, C. Ye, F.G. Omenetto, D.L. Kaplan, S. Ling, Design, Fabrication, and Function of Silk-Based Nanomaterials, Adv. Funct. Mater. 28 (2018) 1–24. https://doi.org/10.1002/adfm.201805305.

[187] R.K. Pal, S.C. Kundu, V.K. Yadavalli, Fabrication of Flexible, Fully Organic, Degradable Energy Storage Devices Using Silk Proteins, ACS Appl. Mater. Interfaces. 10 (2018) 9620–9628. https://doi.org/10.1021/acsami.7b19309.

[217] W. Sheng, J. Liu, S. Liu, Q. Lu, D.L. Kaplan, H. Zhu, One-step synthesis of biocompatible magnetite/silk fibroin core-shell nanoparticles, J. Mater. Chem. B. 2 (2014) 7394–7402. https://doi.org/10.1039/c4tb01125b.

[218] J. Kundu, L.A. Poole-Warren, P. Martens, S.C. Kundu, Silk fibroin/poly(vinyl alcohol) photocrosslinked hydrogels for delivery of macromolecular drugs, Acta Biomater. 8 (2012) 1720–1729. https://doi.org/10.1016/j.actbio.2012.01.004.

[219] C. Zaharia, M.R. Tudora, I.C. Stancu, B. Galateanu, A. Lungu, C. Cincu, Characterization and deposition behavior of silk hydrogels soaked in simulated body fluid, Mater. Sci. Eng. C. 32 (2012) 945–952. https://doi.org/10.1016/j.msec.2012.02.018.

[220] J.C. Bragg, H. Kweon, Y.Y. Jo, K.G. Lee, C.C. Lin, In situ formation of silk-gelatin hybrid hydrogels for affinity-based growth factor sequestration and release, RSC Adv. 6 (2016) 114353–114360. https://doi.org/10.1039/c6ra22908e.

[221] J. Wang, SURVEY AND SUMMARY From DNA biosensors to gene chips, 2000.

[222] A. Yang, F. Yan, Flexible Electrochemical Biosensors for Health Monitoring, ACS Appl. Electron. Mater. 3 (2021) 53–67. https://doi.org/10.1021/acsaelm.0c00534.

[223] M. Arnold, G. Rechnitz, Biosensors based on plant and animal tissue, Biosens. Fundam. Appl. 8 (1989) 45.

[224] K. Suzuki, Biosensing, Anal. Sci. 23 (2007) 3. https://doi.org/10.2116/analsci.23.3.

[225] Q. Liu, C. Wu, H. Cai, N. Hu, J. Zhou, P. Wang, Cell-based biosensors and their application in biomedicine, Chem. Rev. 114 (2014) 6423–6461. https://doi.org/10.1021/cr2003129.

[226] C.N. Kotanen, F.G. Moussy, S. Carrara, A. Guiseppi-Elie, Implantable enzyme amperometric biosensors, Biosens. Bioelectron. 35 (2012) 14–26. https://doi.org/10.1016/j.bios.2012.03.016.

[227] Nurlely, M. Ahmad, L. Yook Heng, L. Ling Tan, Potentiometric enzyme biosensor for rapid determination of formaldehyde based on succinimide-functionalized polyacrylate ion-selective membrane, Measurement. 175 (2021) 109112. https://doi.org/10.1016/j.measurement.2021.109112.

[228] A. Liu, K. Wang, S. Weng, Y. Lei, L. Lin, W. Chen, X. Lin, Y. Chen, Development of electrochemical DNA biosensors, TrAC - Trends Anal. Chem. 37 (2012) 101–111. https://doi.org/10.1016/j.trac.2012.03.008.

[229] S. Cagnin, M. Caraballo, C. Guiducci, P. Martini, M. Ross, M. Santaana, D. Danley, T. West, G. Lanfranchi, Overview of electrochemical DNA biosensors: New

approaches to detect the expression of life, Sensors (Switzerland). 9 (2009) 3122–3148. https://doi.org/10.3390/s90403122.

[230] K. V, DNA Biosensors-A Review, J. Bioeng. Biomed. Sci. 07 (2017). https://doi.org/10.4172/2155-9538.1000222.

[231] Q. Wu, Y. Zhang, Q. Yang, N. Yuan, W. Zhang, Review of electrochemical DNA biosensors for detecting food borne pathogens, Sensors (Switzerland). 19 (2019). https://doi.org/10.3390/s19224916.

[232] M.M. Aleksić, V. Kapetanović, An overview of the optical and electrochemical methods for detection of DNA - Drug interactions, Acta Chim. Slov. 61 (2014) 555–573.

[233] M. Pohanka, Overview of piezoelectric biosensors, immunosensors and DNA sensors and their applications, Materials (Basel). 11 (2018). https://doi.org/10.3390/ma11030448.

[234] J. Wang, Glucose biosensors: 40 Years of advances and challenges, Electroanalysis. 13 (2001) 983–988. https://doi.org/10.1002/1521-4109(200108)13:12<983::aid-elan983>3.0.co;2-%23.

[235] J. Švorc, J. Miertuš, M. Stred'ansky´, M. Stred'ansky´, Composite transducers for amperometric biosensors. The glucose sensor, Anal. Chem. 69 (1997) 2086–2090. https://doi.org/10.1021/ac9609485.

[244] M. Xu, V.K. Yadavalli, Flexible Biosensors for the Impedimetric Detection of Protein Targets Using Silk-Conductive Polymer Biocomposites, ACS Sensors. 4 (2019) 1040–1047. https://doi.org/10.1021/acssensors.9b00230.

[248] V.C. Diculescu, A.M. Chiorcea-Paquim, A.M. Oliveira-Brett, Applications of a DNA-electrochemical biosensor, TrAC - Trends Anal. Chem. 79 (2016) 23–36. https://doi.org/10.1016/j.trac.2016.01.019.

[250] E. Paleček, M. Bartošík, Electrochemistry of nucleic acids, Chem. Rev. 112 (2012) 3427–3481. https://doi.org/10.1021/cr200303p.

[252] F. Crick, J. Watson, © 1953 Nature Publishing Group, (1953).

[253] T.T. Wu, Secondary structures of DNA., Proc. Natl. Acad. Sci. U. S. A. 63 (1969) 400–405. https://doi.org/10.1073/pnas.63.2.400.

[258] S. Tombelli, G. Marrazza, M. Mascini, Recent advances on DNA biosensors, Int. J. Environ. Anal. Chem. 80 (2001) 87–99. https://doi.org/10.1080/03067310108044375.

[259] A.M. Oliveira-Brett, V.C. Diculescu, Electrochemical study of quercetin-DNA interactions: Part I. Analysis in incubated solutions, Bioelectrochemistry. 64 (2004) 133–141. https://doi.org/10.1016/j.bioelechem.2004.05.003.

[262] V.C. Diculescu, T.A. Enache, P.J. Oliveira, A.M. Oliveira-Brett, Electrochemical oxidation of sanguinarine and of its oxidation products at a glassy carbon electrode - relevance to intracellular effects, Electroanalysis. 22 (2010) 113–120. https://doi.org/10.1002/elan.200900367.

[266] A.M. Oliveira-Brett, V.C. Diculescu, Electrochemical study of quercetin-DNA

interactions: Part II. In situ sensing with DNA biosensors, Bioelectrochemistry. 64 (2004) 143–150. https://doi.org/10.1016/j.bioelechem.2004.05.002.

[268] C. Zaharia, E. Vasile, B. Galateanu, M.-C. Bunea, A. Casarica, P.O. Stanescu, Bacterial cellulose-polyhydroxyalkanoates composites synthesis, physico-chemical characterization and biological evaluation for tissue engineering, Mater. Plast. 51

[274] R. Zhang, H. Huang, W. Yang, X. Xiao, Y. Hu, Preparation and characterization of bio-nanocomposites based on poly(3-hydroxybutyrate-co-4-hydroxybutyrate) and CoAl layered double hydroxide using melt intercalation, Compos. Part A Appl. Sci. Manuf. 43 (2012) 547–552. https://doi.org/10.1016/j.compositesa.2012.01.005.

[275] M.-C. Bunea, B. Galateanu, E. Vasile, C. Zaharia, P.-O. Stanescu, C. Andronescu, I.-C. Radu, R. Fuchs, H. Iovu, Novel biocomposites based on polyhydroxyalkanoates-layered double hydroxides for tissue engineering applications, UPB Sci. Bull. Ser. B Chem. Mater. Sci. 78 (2016).

[279] M.C. Bunea, E. Vasile, B. Galateanu, A. Hudita, M. Serban, C. Zaharia, Silk fibroin films decorated with magnetic nanoparticles for wound healling applications, Mater. Plast. 54 (2017).

[284] L. Wang, D. Song, X. Zhang, Z. Ding, X. Kong, Q. Lu, D.L. Kaplan, Silk-Graphene Hybrid Hydrogels with Multiple Cues to Induce Nerve Cell Behavior, ACS Biomater. Sci. Eng. 5 (2019) 613–622. https://doi.org/10.1021/acsbiomaterials.8b01481.

[286] B. Galateanu, A. Hudita, C. Zaharia, M.-C. Bunea, E. Vasile, M.-R. Buga, M. Costache, Silk-Based Hydrogels for Biomedical Applications, (2019) 1791–1817. https://doi.org/10.1007/978-3-319-77830-3_59.

[295] B. Galateanu, S. Dinescu, A. Cimpean, A. Dinischiotu, M. Costache, Modulation of adipogenic conditions for prospective use of hADSCs in adipose tissue engineering, Int. J. Mol. Sci. 13 (2012) 15881–15900. https://doi.org/10.3390/ijms131215881.

[301] C. Zaharia, E. Vasile, B. Galateanu, Bacterial Cellulose-polyhydroxyalkanoates Composites, (2014) 2016–2019.

[302] M.E. Schrader, Young-Dupre Revisited, Langmuir. 11 (1995) 3585–3589. https://doi.org/10.1021/la00009a049.

[303] H.Y. Erbil, The debate on the dependence of apparent contact angles on drop contact area or three-phase contact line: A review, Surf. Sci. Rep. 69 (2014) 325–365. https://doi.org/10.1016/j.surfrep.2014.09.001.

[304] C. Andronescu, S.A. Garea, E. Vasile, H. Iovu, Synthesis and characterization of polybenzoxazine/layered double hydroxides nanocomposites, Compos. Sci. Technol. 95 (2014) 29–37. https://doi.org/10.1016/j.compscitech.2014.01.012.

[305] N. Mahmed, O. Heczko, O. Söderberg, S.P. Hannula, Room temperature synthesis of magnetite (Fe3-δO 4) nanoparticles by a simple reverse co-precipitation method, IOP Conf. Ser. Mater. Sci. Eng. 18 (2011) 3–7. https://doi.org/10.1088/1757-899X/18/3/032020.

[306] Y. Zhu, Q. Wu, Synthesis of magnetite nanoparticles by precipitation with forced

mixing, J. Nanoparticle Res. 1 (1999) 393–396. https://doi.org/10.1023/A:1010091625981.

[311] R. Deepachitra, M. Chamundeeswari, B. Santhosh Kumar, G. Krithiga, P. Prabu, M. Pandima Devi, T.P. Sastry, Osteo mineralization of fibrin-decorated graphene oxide, Carbon N. Y. 56 (2013) 64–76. https://doi.org/10.1016/j.carbon.2012.12.070.

[312] Y. Zhu, S. Murali, W. Cai, X. Li, J.W. Suk, J.R. Potts, R.S. Ruoff, Graphene and graphene oxide: Synthesis, properties, and applications, Adv. Mater. 22 (2010) 3906–3924. https://doi.org/10.1002/adma.201001068.

[313] R.K. Layek, A.K. Nandi, A review on synthesis and properties of polymer functionalized graphene, Polymer (Guildf). 54 (2013) 5087–5103. https://doi.org/10.1016/j.polymer.2013.06.027.

[315] C.M. Perrett, S.L. Walker, P. O'Donovan, J. Warwick, C.A. Harwood, P. Karran, J.M. McGregor, Azathioprine treatment photosensitizes human skin to ultraviolet A radiation, Br. J. Dermatol. 159 (2008) 198–204. https://doi.org/10.1111/j.1365-2133.2008.08610.x.

[316] C. Ulrich, E. Stockfleth, Azathioprine, UV light, and skin cancer in organ transplant patients do we have an answer?, Med. Monatsschr. Pharm. 23 (2000) 261–266. https://doi.org/10.1093/ndt/gfl762.

[317] I. Smaranda, A. Nila, C.M. Manta, D. Samohvalov, D. Gherca, M. Baibarac, The influence of UV light on the azathioprine photodegradation: New evidences by photoluminescence, Results Phys. 14 (2019). https://doi.org/10.1016/j.rinp.2019.102443.

[318] E. Asadian, A. Iraji Zad, S. Shahrokhian, Voltammetric studies of Azathioprine on the surface of graphite electrode modified with graphene nanosheets decorated with Ag nanoparticles, Mater. Sci. Eng. C. 58 (2016) 1098–1104. https://doi.org/10.1016/j.msec.2015.09.022

Disemination of results

Articles published in ISI Thomson Reuters journals

M.-C. Bunea, V.-C. Diculescu, M. Enculescu, H. Iovu, T.-A. Enache, "Redox mechanism of azathioprine and its interaction with DNA". International Journal of Molecular Science, 2021, 22(13), 6805. DOI:10.3390/ijms22136805. **I.F.=5,923**

B. Gălățeanu, **M.- C. Bunea**, P.- O. Stănescu, E. Vasile, A. Căşărică, H. Iovu, A. Hermenean, C. Zaharia, M. Costache, "In vitro studies of bacterial cellulose and magnetic nanoparticles smart nanocomposites for efficient chronic wounds healing", Stem cells International, 2015, DOI:10.1155/2015/195096. **I.F.= 3,687.**

M.-C. Bunea, B. Gălățeanu, E. Vasile, A. Hudita, M.Șerban, C. Zaharia, Silk fibroin films decorated with magnetic nanoparticles for wound healling applications, Revista de Materiale Plastice, 2017, 54(1), 83-87. **I.F.= 1,248.**

C. Zaharia, E. Vasile, B. Gălățeanu, **M.-C. Bunea,** A. Căşărică, P.- O. Stănescu, "Bacterial Cellulose-polyhydroxyalkanoates Composites Synthesis, physico-chemical characterization and biological evaluation for tissue engineering", Revista de Materiale Plastice, 2014, 51(1), 1-5. **I.F.= 0,824.**

Articles sent to publication

M.-C. Bunea, V.-C. Diculescu, M. Enculescu, H. Iovu, T.-A. Enache, " On the interaction of DNA with azathioprine metabolites: spectrophotometric and electrochemical studies". International Journal of Molecular Science

Articles published in UPB Scientific Bulletin

M.-C. Bunea, B. Gălățeanu, E. Vasile, C. Zaharia, P.-O. Stănescu, C. Andronescu, I.-C. Radu, R. Fuchs, H. Iovu, Novel biocomposites based on polyhydroxyalkanoates-layered double hydroxides for tissue engineering applications, U.P.B. Sci. Bull., Series B, 78 (2), 2016.

Book Chapters published

B. Gălățeanu, A. Hudita, C. Zaharia, **M.-C. Bunea**, E. Vasile, M.-R. Buga, M. Costache,"Silk-Based Hydrogels for Biomedical Applications", Polymers and Polymeric Composites: A Reference Series, Springer Science and Business Media LLC, 2019, 1791-1817.

Participation to national and international conferences

M.-C. Bunea, T.-A. Enache, M. Enculescu, V.C. Diculescu, *Mecanismul redox al azatioprinei şi interacţia acesteia cu ADN-ul*, Workshop 58PCCDI-SANOMAT, București, România, 19 mai 2020, Prezentare orală.

M.-C. Bunea, T.-A. Enache, *Redox behavior of azathioprine and its interaction with DNA*, XXV International Symposium on Bioelectrochemistry and Bioenergetics of the Bioelectrochemical Society, Limerick, Irland, 26-30 Mai 2019, Prezentare orală.

M.-C. Bunea, C. Zaharia, E. Vasile, B. Gălățeanu, P.-O. Stănescu, H. Iovu, *Development of magnetic silk fibroin scaffolds for tissue engineering applications,* 19th Romanian International Conference on Chemistry and Chemical Engineering – RICCCE 19, Sibiu, Romania, 02-05.09.2015, Prezentare orală.

M.-C. Bunea, C. Zaharia, B. Gălățeanu, E. Vasile, A.-M. Pandele, H. Iovu, *Silk Fibroin-Graphene Oxide Hydrogels for Bone Tissue Engineerig*, Young Scientists Joining Forces For Excellence In Biomaterials Research, București, România, 28-29.05.2015, Poster.

M.-C. Bunea, C. Zaharia, P.-O. Stănescu, B. Gălățeanu, E. Vasile, A.-M. Pandele, H. Iovu; *Development Of Graphene/Silk Fibroin Hydrogels With Potential Uses In Bone Tissue Engineering*, Fourth International Symposium Frontiers in Polymer Science, Riva del Garda, Italia, 20-22.05.2015, Poster.