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**DOCTORAL SCHOOL
OF MATERIALS SCIENCE AND ENGINEERING**



ESSAY

of the doctoral thesis

Contributions regarding the possibilities of obtaining composites materials with biomedical applications

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Abstract

This PhD thesis includes a theoretical study and a series of experimental research performed in order to develop integrated technological solutions, to obtain composite materials for the manufacture of customized implants to support and improve the bone regeneration process.

The objectives of the thesis were established taking into account the requirements necessary for a product to be considered suitable and ideal for bone reconstruction applications, namely: ensuring bone regeneration through osteoconductive mechanisms, ensuring mechanical properties comparable to those of human bone and good biocompatibility properties.

In this sense, three different types of composite materials were proposed in the PhD thesis, with a common component, namely hydroxyapatite obtained from bovine bone. Hydroxyapatite is a biomaterial with proven clinical performance, and it also has advantages such as low cost and wide availability. Also, the polymeric materials used to obtain the matrices of the composite materials are established and successfully used for various applications in the biomedical field. Another important goal of this PhD thesis was that the obtained materials could be used for the manufacture of customized implants, therefore two of the three types of materials are intended for additive manufacturing. The experimental research carried out in this PhD thesis led to optimistic perspectives regarding the successful use of all three types of materials in the biomedical field.

Keywords: composite materials, hydroxyapatite, customized implants

PART I

CURRENT STATE OF RESEARCH IN THE FIELD

Chapter 1. Introduction

Damage to a part of an organism, or even the loss of an organ, primarily affects their functionality, but it can also generate social and psychological disturbances for an individual. Thus, by combining the knowledge and needs of both doctors and engineers, recently biomaterials have shown an exponential development. In general, a "biomaterial" can be defined as a material of synthetic origin, intended for use in close connection with a living organism, or to replace a part of a living organism.

Biomaterials have been used since ancient times, but constant research in this field has made it possible to develop them both in terms of biocompatibility, functionality and design. Being an interdisciplinary field, based on the response from the interface between a biological system and an advanced material, research is based on knowledge from three different areas: materials science and engineering, biology, and medicine.

Even though in some situations it is enough to change the surface properties by coating with various materials to achieve improved performance, most of the time a single material cannot present a wide range of properties. For this reason, it was necessary to develop complex systems, namely composite materials.

In addition, the development of the field of engineering has also brought improvements to the field of biomaterials through the techniques of additive manufacturing (AM) or 3D printing, and through high-resolution spatial modeling. Thanks to these manufacturing techniques, biomaterials are expected to show even more spectacular development in the coming years.

The materials with applicability in the biomedical field developed up to this point are very numerous and include practically all the classes mentioned above. Regardless of the performance level of biomaterials and medical devices that currently exist, new needs can be identified. Thus, the idea of this doctoral thesis focused on the identification of niches and the development of new composite materials, with applications mainly in the biomedical field, but it is also possible to use them in other fields.

The research carried out in the doctoral thesis involved the use of polymeric materials in three directions, as a matrix for the development of composite materials with various possible applications.

The first aspect addressed consisted in obtaining film-type composite materials, from cellulose acetate reinforced with hydroxyapatite ceramic particles (AC+HAp).

The second stage involved the development of filaments for 3D printing, using as a core two polymer materials common in this field (polylactic acid - PLA and thermoplastic polyurethane - TPU), in the surface of which ceramic particles of hydroxyapatite were introduced at certain temperatures and pressures (PLA/TPU+HAp).

The last stage of the study focused on obtaining composite filament-type materials from PLA and hydroxyapatite (PLA+HAp). They can be used for 3D printing of implantable scaffold products.

Chapter 2. The current state of research regarding composite materials with polymer matrix and addition of ceramic materials, for medical applications

Basically, a composite material is composed of two or more different materials, with a connecting area between them, called the interface. The base material (or continuous phase) is called the matrix, and the material or materials that are added to improve the properties are called reinforcements or reinforcements.

From a macroscopic point of view, these materials differ from each other, but the characteristics and performances obtained from their combination are different from those of the base materials and greatly improved.

Historically, man-made composite materials have been used since ancient times, but there are also elements from nature that can be considered composites, for example wood. And from the point of view of medicine and engineering, one of the most famous and interesting natural composite materials is bone itself. By analyzing nature, people have learned that two completely different compounds can be combined to obtain a new material with improved and significantly better properties than those of the constituent components themselves. Thus, the field of engineering and materials science is in continuous development, focused on research and obtaining new composite biomaterials.

In terms of biomedical applications, composite materials represent one of the best options for treating or replacing certain parts or organs of a living organism. Practically, their use can be unlimited because their physical, chemical and even mechanical properties can make them suitable for any type of application.

Metal alloys, polymers, ceramics, but also composite materials used in biomedical applications, are generally obtained synthetically. Recent research in the field of biomaterials emphasizes the development of biosynthetic (or bioartificial) materials, namely those composite materials that present at least one component of natural origin, thus increasing the degree of biocompatibility and, respectively, healing of the affected area, or acceptance by the human body.

2.1 Polymeric materials usable as a matrix of composite materials

Polymeric materials have properties such as low density, high molecular weight, nontoxicity, good biocompatibility, easy sterilization, respectively good mechanical properties, which makes them suitable for use as biomaterials. Their applicability in the medical field is vast, starting from different types of prostheses and implants, joints, bone cements, artificial organs, membranes and other components of artificial organs, scaffolds for the regeneration of certain tissues, tools for the controlled release of medicines, for making surgical instruments, etc.

Depending on the method of obtaining, polymers can be:

- i. synthetic - obtained as a result of chemical reactions between monomers;
- ii. natural - proteins, polysaccharides, polyhydrocarbons, nucleic acids;
- iii. artificial - obtained as a result of modifications of natural polymers.

2.1.1 Synthetic polymers for biomedical applications

A wide variety of synthetic polymer materials are used in the biomedical field, both as implantable biomedical devices and as coatings for devices made of metallic materials.

The most used polymers in the biomedical field are thermoplastic biopolymers, thermosetting ones being less common. They are of synthetic origin and are inert to the human body, thus being suitable for the applications described previously. Among the most used polymers in the medical field are:

- *nylon* and *polypropylene (PP)* are the polymers used as suture materials.
- *polyethylene terephthalate (PET)* for grafting vessels with large diameters
- *polytetrafluoroethylene (PTFE)* for periodontal ligament grafting
- *PMMA* for cementation in the case of replacing various joints, for rhinoplasty and cranioplasty, or for the manufacture of intraocular lenses
- *PVC* for tubes and bags for blood transport and storage, antibacterial membranes
- *polyacrylamide (PAM)* for medical diagnosis
- *polyvinyl alcohol (PVA)* for drug delivery systems
- *bioresorbable polymers* are materials that degrade without toxic effects on the body, being eliminated naturally and without the need for subsequent surgical interventions
- *polylactic acid (PLA)* has applicability in orthopedic surgery, dentistry and orthodontics, ophthalmology, or in the delivery and controlled release of drugs.
- *polyglycolic acid (PGA)* is used as a surgical suture material
- *hydrogels* are used in artificial hearts or contact lense

2.1.2 Natural polymers for biomedical applications

Natural polymers have as precursors biological systems such as microorganisms, plants and algae or animals. In the biomedical field, the applications of natural polymers are generally for the regeneration of bone tissues, nerves, muscles, cartilage, skin or organs such as the pancreas and liver.

The main groups of natural polymers are:

- i. proteins - collagen, gelatin, fibronectin, fibrin, elastin, fibroins;
- ii. polysaccharides - chitin, chitosan, hyaluronic acid, bacterial cellulose, alginates;
- iii. naturally derived polyesters or polyhydroxyalkanoates (PHA).

Among the most used polymers in the medical field are:

- *collagen* used to improve properties, such as mechanical strength
- *gelatin* for controlled drug release systems, for cell cultures, drug capsules and vitamin preparations
- *fibronectin* for cell adhesion, wound healing and re-epithelialization.
- *fibrin*, with a vital role in homeostasis
- *elastin* used in the engineering of dermal tissues or blood vessels
- *silk fibroin* used for bone and fat tissue, cartilage, ligaments or blood vessels
- *chitosan* used in tissue engineering in the form of hydrogels and porous scaffolds
- *hyaluronic acid* used in applications in tissue engineering, medicine and cosmetics
- *cellulose* for tissue engineering applications
- *chitin* for controlled drug release systems, mucoadhesion, permeation improvement

- *alginate* used in bone tissue engineering

Both synthetic and natural polymers have advantages and disadvantages. Thus, tissue engineering is based on obtaining products with superior properties, resulting from the combination of the two types of polymers.

2.2 Ceramic materials

Ceramic biomaterials are widely used for the manufacture of biomedical devices, due to the fact that they can be processed into a multitude of shapes, exhibit good mechanical characteristics such as compressive strength, variable porosity, high biocompatibility, good osteoinductive properties and good bioactivity. The medical fields of interest for these types of materials are dentistry, oral and maxillofacial surgery, and orthopedics.

A bioactive material is that material which, when in contact with a physiological environment, allows and facilitates the recovery of damaged tissues, thus leading to the appearance of a strong bonding interface between the bone tissue and the prosthesis. The most important bioactive bioceramic materials are those based on calcium phosphates (components of bone tissue structure) and bioactive glasses.

Depending on the purpose of the medical application for which they are used, bioceramics are classified as follows:

- i. bioinerts - such as alumina and zirconia;
- ii. biodegradable or bioresorbable - such as tricalcium phosphate;
- iii. bioactive - calcium phosphate - hydroxyapatite (the most important).

A bone substitute material must present, in addition to good biocompatibility, other superior properties, such as osteoconductivity, osteoinductivity, bioactivity and bioresorbability.

2.2.1 Hydroxyapatite

Hard tissues, bones and teeth of living organisms are natural composite materials, consisting of a nanocrystalline inorganic component with apatite structure (biological apatite) and an organic component of the calcium phosphate class. A material in the apatite category represents a group of compounds with similar structures but different compositions.

Among the biological apatites, hydroxyapatite represents 65% of the bone structure, being its predominant component, the rest being represented by structures of organic origin, such as collagen and water.

From a biological point of view, bone tissue is a connective tissue intended to support all other body structures, its role being structural. From a structural point of view, bone is a composite material, consisting of an organic matrix of collagen fibers that chemically binds to the inorganic or mineral phase, formed by acicular HAp crystals.

Properties such as high biocompatibility, bioactivity, osteoconductivity and very low toxicity make HAp an excellent substitute and promoter of bone tissue.

2.2.2 Methods of obtaining hydroxyapatite

At the moment there are many studies on obtaining HAp. The methods of obtaining from synthetic precursors are:

- i. dry methods - in solid state and mechano-chemical;
- ii. wet methods - chemical precipitation, hydrolysis, hydrothermal, emulsion, sol-gel and sonochemical;
- iii. high temperature treatments - combustion and pyrolysis.

By means of *dry solid state synthesis methods*, HAp powders with irregular shapes, large grain size (no nanometer sizes are obtained) and a low degree of purity are obtained, due to the fact that the raw material used has a low price, and therefore lower quality.

The *mechano-chemical dry synthesis method* is used in order to obtain HAp powders with a well-defined structure compared to the previously presented method. It is a relatively simple and reproducible method if certain working elements such as the type of reagents, the grinding medium, the rotation speed and the duration of the working steps are carefully followed.

The wet synthesis method by *chemical precipitation* represents one of the easiest techniques to obtain HAp powder by wet method, because at a pH of 4.2 and at ambient temperature it is stable and does not dissolve in aqueous solutions.

Through *hydrolysis*, HAp nanoparticles can be obtained from the two phases of the CaP compound, namely dicalcium phosphate dihydrate (DCPD) and tricalcium phosphate (TCP).

Regarding the *hydrothermal* method, the defining working parameters are high temperature and pressure. Due to these parameters, the HAp powder obtained is of high purity and has an appropriate Ca/P ratio.

The *emulsion method* is used when you want to control the morphology and obtain small particles, without particle agglomerations. This method is quite simple and the reagents used in this case are orthophosphoric acid and calcium nitrate due to their low purchase costs and availability in the market.

The *sol-gel method* is based on molecular level mixing and low temperatures of the reaction products, in order to obtain fine and homogeneous powders of HAp.

The principle on which *sonochemical* methods are based is that chemical reactions are activated by very strong ultrasound waves, thus obtaining a HAp with improved mechanical properties.

The *combustion method* is based on a strongly exothermic and self-sustaining Redox reaction between an oxidant and an organic product in the liquid phase, resulting in a very fine powder, formed by clusters of small particles.

Pyrolysis, or the "spray pyrolysis" method, is a simple and fast process, by which HAp powders formed from stoichiometric, homogeneous and crystalline particles are obtained.

2.2.3 Natural resources for obtaining hydroxyapatite

When naturally occurring resources are used as precursors, what is obtained is known as "natural hydroxyapatite". This type of hydroxyapatite is obtained from biological resources, or organic waste such as: mammal bones, fish bones and scales, aquatic or marine sources, eggshells or clams, vegetable or mineral sources such as limestone.

From a stoichiometric point of view, the Ca/P molar ratio that favors and supports osteogenesis is 1.67. HAp obtained from natural precursors is generally non-stoichiometric, having both Ca and P deficiency.

Animal precursors are reported in the specialized literature as mammalian bones, generally cattle bones, and less often horse or pig bones. To obtain HAp, the cortical part of the femur is generally used, due to its morphological and structural similarity to human bones.

The greatest inconvenience of these resources is the risk of contamination, therefore a pre-treatment of the raw material is necessary and indispensable. Thus, animal bones are subjected to a pre-treatment involving: washing, removal of dirt, removal of fat, bone marrow and soft tissues.

Fish bones and scales are a rich source of precursors for obtaining HAp, due to the high content of calcium, carbonates and phosphates. As in the case of mammalian bones, pre-treatment is required to remove organic matter, and boiling is frequently used.

Aquatic resources, more specifically corals, are used due to their structure (similar to that of a spongy bone). Thus, through heat treatment, organic substances are removed, and the porous microstructure specific to coral is maintained.

Eggshells and clams are of interest due to their high content of calcium carbonate (CaCO₃), about 94%, which can be converted into HAp by various processes.

Regarding *plant sources*, both plants and algae may represent potential precursors for HAp synthase. In this case, the studies focused on certain parts of the plants, such as: leaves, flowers, stem or wood; on plant waste such as fruit and vegetable peels or waste from the fruit processing industry, and also on algae.

Natural mineral sources, such as limestone, are also used to obtain HAp, due to the abundance with which it is found, but also the fact that it is formed from the precipitation of animal skeletons, shells and algae, thus being a rich source of CaCO₃.

2.3 Composite materials with matrix of polymeric origin

A composite material consists of two or more phases, one of which is continuous and is called the matrix or base phase, and the second or other phases are discontinuous and are called the filler(s) or reinforcing phase(s) or reinforcement. These phases present different morphological and compositional properties, but together they give the newly formed material, the composite, superior properties, independent of those of the materials from which it was formed.

Composite materials are classified according to:

- matrix material: with metallic, polymeric, ceramic or organic matrix;
- the reinforcement material: with the addition of particles or fibers

2.3.1 Methods of obtaining film-type composite materials with matrix of polymeric origin

Film-like composite materials are obtained are most often obtained by phase inversion techniques. They involve the controlled transition of the material of polymeric origin from a liquid state to a solid state. This phenomenon occurs when the liquid polymer is immersed in a liquid inert to it.

The methods of obtaining films with matrix of polymeric origin differ from each other depending on the desolvation mechanism, as follows:

- i. thermally induced phase separation (TIPS);
- ii. vapor-induced phase separation (VIPS) - precipitation from the vapor phase;
- iii. phase separation induced by solvent evaporation (SEIPS) - air casting of a polymer in a liquid state;
- iv. non-solvent induced phase separation (NIPS) - immersion precipitation

2.3.2 Methods of obtaining massive composite materials with matrices of polymeric origin

Massive composite materials can be obtained through several techniques such as:

- i. compression molding;
- ii. injection molding;
- iii. resin transfer casting;
- iv. manual stretching;
- v. spray stretching;
- vi. pultrusion;
- vii. vacuum bag casting;
- viii. bag molding under pressure;
- ix. autoclave casting;
- x. filament winding;
- xi. resin film infusion.

2.3.3 Applications of composite materials in the biomedical field

Polymer-based composite materials used recently in the biomedical field are advanced materials, able to ensure, first of all, a very good biocompatibility with the human body. They are used in a multitude of applications for various branches of medicine, such as orthopedics, dentistry or soft tissue engineering.

In the field of orthopedics, polymer composite materials are used in applications such as obtaining fracture fixation screws and plates, bone grafts, bone cements, hip or finger joint replacement or repair, etc. This field is challenging and constantly developing, due to the wide range of applications for which such products are needed.

In the dental field, composite materials have polymer resins as their matrix and are used in restorative applications and as adhesives. These materials must present certain characteristics, such as micromechanical strength, to be suitable for use in small cavities such as those in the oral area. In addition, properties such as the appearance comparable to that of teeth, insolubility in water, insensitivity to dehydration, but also simple processing and relatively low costs, make these materials preferable for the dental field.

In tissue engineering, the most widely used materials are biodegradable synthetic polymers namely PLA, PGA and PLGA (polylactic-co-glycolic acid). They are used as the matrix of the composite material, and hydroxyapatite or bioactive glass in the form of particles or fibers is used as the reinforcing material.

In hard tissue engineering, the most used composite materials are those of the polymer-ceramic type. Originally, bone can be treated as a composite material in which the base is collagen, and the other components are water, hydroxyapatite, organic and inorganic materials.

2.4 Additive manufacturing used to obtain biomedical devices

Additive manufacturing is also known as 3D printing or rapid prototyping and is a technique of converting a computer model into a three-dimensional structure by printing and depositing consecutive layers.

In terms of the biomedical field, polymer printing is advantageous in several directions, due to the diversity of characteristics of polymer materials, the versatility of geometries and the complex architectures that can be obtained, in contrast to traditional manufacturing processes. The term bioprinting is used when the products obtained are intended for use in medical applications.

2.4.1 Types of additive manufacturing technologies with applicability in the biomedical field

There are different additive manufacturing technologies, each of these techniques has both advantages and disadvantages, listed in the following table:

FDM	
Advantage	suitable for many types of materials, high strength, low cost
Disadvantage	anisotropy, nozzle clogging
Mechanisms	material extrusion and deposition
Materials	PC, PLA, ABS, nylon
Precursor type	filament
SLA	
Advantage	high resolution printing
Disadvantage	limitation of materials, high costs, cytotoxicity
Mechanisms	laser scanning and UV induced sintering
Materials	epoxy or acrylate-based resins
Precursor type	liquid photopolymer
SLS	
Advantage	high strength, easy removal of powder support
Disadvantage	high costs, powdery surfaces
Mechanisms	laser scanning and heat induced sintering
Materials	PCL, polyamide powder
Precursor type	powder
PLP	
Advantage	suitable for many types of materials, low cost, easy removal of powder support
Disadvantage	contamination of the binder, clogging of the binder jet
Mechanisms	drop binder printing on demand
Materials	any material in powder form, with a binder

Precursor type	powder
RoboCasting	
Advantage	high resolution printing, obtaining fine materials
Disadvantage	low mechanical strength, slow process
Mechanisms	pressurized orifice and hot or UV induced sintering
Materials	PLA, PCL, hydrogels
Precursor type	liquid or paste

2.4.2 Additive manufacturing of polymer matrix composite materials

By reinforcing with materials in the form of particles, fibers, nanoparticles or ceramics, the mechanical and functional properties are improved and the printing is carried out with high precision.

Composite materials with polymer matrix and reinforcement with particle-type materials present functional and structural properties superior to those of the matrix material and the reinforcement. As for the reinforcing particles, they can be of different types and can be combined with pure or thermoplastic polymers, in the form of liquids or powders. The additive manufacturing techniques used to print such composite materials are FDM, SLA and SLS.

Fiber-reinforced polymer matrix composites are of interest due to their high compressive strength, corrosion resistance, and stiffness. The additive manufacturing techniques used to print these composite materials are FDM and polyJet, other techniques not being used due to the roughness of the surface.

Composite materials with polymer matrix and reinforcement with nanoparticle-type materials are of great interest, because nanotechnologies are part of and influence everyday life, through their applicability in fields such as biomedical, sports, automotive, etc. Thus, nanotechnologies began to have applicability in the field of additive manufacturing by reinforcing polymer matrices with graphite nanoparticles, graphene or carbon nanotubes.

Composite materials with polymer matrix and reinforcement with ceramic materials can be obtained by any additive manufacturing technique that is based on powder-type raw material.

2.4.3 Applications of additive manufacturing technologies in the biomedical field

Since additive manufacturing (AM) techniques are versatile both in terms of the materials used (synthetic and natural) and in the areas of applicability, they are also of great interest in the biomedical field. It is estimated that the applications of AM techniques in the medical field represent approximately 11% of the total, and this percentage is constantly increasing.

Additive manufacturing techniques allow the achievement of all these objectives, thus becoming useful in establishing surgical strategies by improving efficiency and in certain cases by reducing the probability of the need for subsequent interventions. In terms of economics, AM techniques are more affordable compared to traditional ones, also due to aspects such as reduced production time and minimal material losses.

Medical applications successfully achieved through these techniques include obtaining prosthetic joints, temporomandibular joints, customized prosthetic devices for orthopedic surgery, or customizing drug delivery devices.

It can be said that additive manufacturing techniques have experienced an extraordinary development in recent years due to the versatility both in terms of materials and the complexity of the structures obtained. Due to the fact that they are new, modern techniques that are in continuous development, they of course have some disadvantages compared to the classic, established techniques.

A wide range of materials can be used, starting from metals, polymers, ceramics, and up to composite materials with reinforcements such as particles, fibers, nanoparticles or ceramics. The choice of materials depends on the characteristics of the final product, but also on the process. As far as applications for the biomedical field are concerned, materials are chosen according to the anatomical area (hard or soft tissue) and implicitly the mechanical aspects they must fulfill. Thus, composite materials are most often used because they are versatile and can meet the mechanical requirements by rigorously controlling the addition of reinforcing material.

PART II

EXPERIMENTAL RESEARCH

Chapter 3. Research methodology

In this PhD thesis, one of the main objectives was to obtain new or improved materials with applications in the biomedical field, using as a basis the latest results presented in the literature.

Since in the case of performing bone addition operations, regardless of the type of powdered material used, it is necessary to maintain it in the place where it was inserted for long periods of time (on the order of months), film-type materials can be used to fix them. The main idea in this regard consisted in trying to develop a material that has the minimum necessary characteristics in the sense of maintaining the additive material in the place where it was inserted, but also to bring an intake of substances that can contribute to the restoration faster bone. Thus, composite films of cellulose acetate (a bioresorbable material) and hydroxyapatite particles have been developed. For simplicity, the name of these types of materials has been abbreviated, being further used under the pseudonym "CA+HAp".

Another important aspect in the field of surgery, where large bone defects also occur, is related to the materials that can temporarily fill the respective gaps. 3D printing allows obtaining products with complex geometries, as required in some situations, but the available materials do not offer the best conditions for restoring bone tissue. In order to respond to this request, an identified possibility consisted in the use for 3D printing of some filaments made of composite materials resorbable over time, but which after the surgical operation would allow the release of certain amounts of elements necessary for the restoration of the bone (Ca and P). In this sense, two different technologies were chosen.

The first method involved the superficial insertion of hydroxyapatite particles into polylactic acid filaments - PLA (bioresorbable material) and thermoplastic polyurethane - TPU. For simplicity, the name of these types of materials has been abbreviated, being further used under the pseudonym "PLA/TPU+HAp".

The second technology involved the homogenization of some hydroxyapatite particles in a PLA-type bioresorbable polymer matrix. For simplicity, the name of these types of materials has been abbreviated, being further used under the pseudonym "PLA+HAp".

3.1 Materials used to obtain the three types of composite materials

The composite materials investigated in this doctoral thesis were obtained by combining in various forms hydroxyapatite synthesized from natural resources and three different types of polymeric materials: cellulose acetate, polylactic acid and thermoplastic polyurethane. The hydroxyapatite powder was obtained from bovine bone tissue, and the polymeric materials were purchased commercially.

3.2 Technological flow for obtaining hydroxyapatite powder

The ceramic component (HAp powder from bovine bone) was obtained according to a route presented in the specialized literature. Thus, the bovine bones purchased from local slaughterhouses were subjected to heat treatments of boiling to remove organic matter, deproteinization to remove traces of collagen and calcination.

To obtain the hydroxyapatite powder, the calcined bones were ground, the resulting powder being granulometrically sorted, thus obtaining several granulometric sorts of HAp powder.

3.3 Methods of analysis and characterization of materials

- scanning electron microscopy (SEM)
- energy dispersive X-ray spectroscopy (EDS)
- X-ray photoelectron spectroscopy (XPS)
- Fourier transform infrared spectroscopy (FT-IR)
- microcomputed tomography (μ CT)
- evaluation of the contact angle (CA)
- digital image processing and analysis (pseudo-coloring and roughness profiles)
- evaluation of water permeation and protein retention
- assessment of in vitro biocompatibility
- compressive strength test
- adhesion of particles to the substrate – pull-out type test
- differential scanning calorimetry (DSC)

Chapter 4. Obtaining polymer-ceramic composite materials in the form of a film with matrix of cellulose acetate reinforced with hydroxyapatite

The general aim of this component of the study was to obtain polymer-ceramic structures in the form of a film, from precursors of natural origin, for biomedical applications. These structures consist of a cellulose acetate matrix, extracted from plants, with an addition of different concentrations of hydroxyapatite (HAp) powder.

4.1 Obtaining hydroxyapatite-reinforced cellulose acetate matrix film-form composite materials

To obtain composite materials of this type, HAp powder of two different size grades, namely $<20\ \mu\text{m}$ and $<40\ \mu\text{m}$, was used. The homogenization of the polymer solution with the HAp powder was carried out with an ultrasonic probe for 1 and 4 minutes, so as to ensure the different dispersion of the particles. The film-like composite materials were obtained by phase inversion by immersion in water.

4.2 Results and discussion on characterization of hydroxyapatite reinforced cellulose acetate matrix film composite materials

4.2.1 Morphological and compositional characterization

The AC matrices obtained at a dispersion time of 1 and 4 minutes showed porous structures, with spherical pores and complex shapes, interconnected. By adding different amounts of HAp powders, agglomeration of the particles occurs, which leads to the formation of aggregates.

Regarding the aggregate sizes, they are proportional to the amount of HAp powder added, generally also inducing a completely modified surface. In addition, it was found that there is a direct correlation between the size of the particles and their degree of agglomeration. Regarding the composite materials with added HAp powder <40 μm , there is a strong tendency to clog the pores of the AC matrix. Regarding the influence of dispersion time on the morphology of composite films, clear differences are observed depending on the amount of HAp powder. A longer time (4 minutes) ensured a more homogeneous distribution of the particles in the AC matrix and at the same time prevented the formation of large aggregates.

From a compositional point of view, the EDS analyzes carried out on the reference samples demonstrated the presence of the characteristic AC elements, namely C and O. As for the composite films, it was observed that the signal intensities corresponding to the main elements of HAp (Ca and P) increase proportionally with added powder concentration.

For a better understanding, the compositional uniformity of the surface of the samples was highlighted by using EDS mapping - mapping analysis.

According to previous observations made in EDS spectra, the smaller particle size and prolonged dispersion time result in a uniform dispersion of the particles throughout the matrix volume, with EDS mapping providing the visual representation of the surface composition.

4.2.2 Topographic reconstruction and roughness assessment

3D reconstructions and roughness profiles corresponding to all samples were performed following digital processing of the micrographs. Thus, after the addition of HAp powder and after ultrasonic dispersion at different time intervals, clear changes are observed in the 3D reconstruction images. The tendency of particle agglomeration is confirmed, by the presence of aggregates with variable sizes and shapes depending on the ultrasonication time.

Analysis of surface roughness profiles confirms that dispersion time directly influences surface roughness. The evolution of the roughness parameters, the profiles indicate a decrease in the intensity of the peaks at higher dispersion times. Proper dispersion of HAp particles in the AC matrix prevents particle agglomeration and aggregate formation, thus resulting in a smoother surface..

4.2.3 Evaluation of functional groups

The spectra of the composites were divided into two regions of interest, 500 – 700 cm^{-1} and 950 – 1850 cm^{-1} , each of which was related to the reference spectra of the AC matrix.

In the case of composite materials, the specific vibration bands of the PO_4^{3-} orthophosphate group in the HA structure were found at 573 cm^{-1} and 603 cm^{-1} , 966 cm^{-1} and 1045 cm^{-1} . At 626 cm^{-1} there are bands specific to hydroxyl structural functional units. The characteristic bands of cellulose acetate at 1232 cm^{-1} for C – O bonds, 1373 cm^{-1} for -CH₂, 1598 cm^{-1} (asymmetric stretching mode) and 1654 cm^{-1} were also identified (symmetric stretching mode) for C=C and 1745 cm^{-1} for C=O.

In addition, it was observed that with the increase of ultrasonic dispersion time, the intensities of the bands in the IR spectra intensifies.

Except for some bands appearing at $2600 - 3800\text{ cm}^{-1}$ and characteristic of stretching vibrations of O – H bonds in absorbed water molecules, no other significant changes were recorded in the range $1900 - 4000\text{ cm}^{-1}$.

4.2.4 Analysis by X-ray Photoelectron Spectroscopy (XPS)

At approximately 285 eV and 530 eV, respectively, the presence of C and O atoms is observed for all samples, these being the main elements of AC. The increase in peak intensity is attributed to samples prepared at a dispersion time of 4 min.

Even though HAp was not observed on the surface of the samples, an indirect approach can be made regarding the atomic % C/O ratio, which increased with the addition of the inorganic component. As a general view, the values are higher for samples obtained at a dispersion time of 4 minutes. An explanation can be outlined due to the fact that the C – O bond is flexible and thus can be oriented towards the HAp particles that are embedded in the polymer matrix, inducing an oxygen-saturated surface composition.

It is possible that the acetate functional groups in the polymer chain also form some physical interactions with the HAp particles. During the ultrasonication process, the macromolecular chains gain energy to become more flexible, and thus the increased mobility of the acetate groups can be explained.

4.2.5 Computed microtomography analysis (μCT)

The formation of aggregates through the agglomeration of particles is clearly observed in the entire volume, this evolving depending on the increase in the size of the particles, but also on the dispersion time. With increasing particle size and powder concentration, the clogging tendency of the porous structure becomes more and more prominent. This fact supports the morphological assessment.

3D reconstructions confirm that the association of these effects can be moderated following dispersal over a prolonged period of time. Thus, it can be observed that the larger particles show a preferential dispersion at a period of 1 minute, while at 4 minutes, both the surface and the sections in the volume have a uniform and homogeneous appearance, with a reduced number of aggregates, even at higher powder concentrations.

4.2.6 Surface wettability analysis

The contact angle values were recorded one second after the drop of water deposited on their surface. High contact angle values are directly proportional to increasing HAp powder concentration for both powder types.

This fact is an immediate consequence of the formation of aggregates on the surface of the samples that induce changes both in terms of roughness and local energy of the surface.

From the point of view of the ultrasonic dispersion time, it has a direct influence on the wettability of the sample surfaces, independent of the added concentration. Thus, a longer dispersion time is associated with the uniform dispersion of particles in the AC matrix and the absence of large aggregates, thus resulting in an improved hydrophilic character. All investigated samples showed a hydrophilic character, with contact angle values $<90^\circ$.

Consequently, a controlled surface wettability can be induced by varying the key parameters involved during the synthesis process of composite materials.

4.2.7 Water permeation and protein retention

The aim of the investigations on the flow of water passing through the samples is to demonstrate their stability from a hydrodynamic point of view, consistent with the distribution of HAp particles in the AC matrix. Flow rates decrease proportionally with increasing HAp powder concentration and particle size. In addition, the prolonged dispersion time influences the water flow capacity.

It is observed that the samples obtained at a dispersion time of 4 minutes present higher water flow values than those obtained at 1 minute, a fact due to the absence of large aggregates (so the pores of the matrix are not clogged).

The same arguments apply to protein retention. The proteins retained on the surface of the composite materials and those that passed through its pores were measured every 10 minutes over a period of 120 minutes.

In this sense, two proteins with an important role in the human body were chosen, namely hemoglobin with a role in oxygen transport and serum albumin. An increase in protein retention on the surface of the samples is observed, directly proportional to the increase in HAp powder concentration and to the dimensional increase of the particles. Regarding dispersion time, samples obtained at 4 minutes show a decrease in protein retention, unlike those obtained at 1 minute.

4.2.8 *In vitro* biocompatibility testing

The research focused on determining the response of MC3T3-E1 pre-osteoblasts, and was studied from the point of view of cell adhesion and morphology as well as viability.

First, the ability of the composite materials to support cell proliferation and morphology was investigated by fluorescent labeling of cytoskeletal actin with phalloidin. Thus, 24 hours after seeding, osteoblasts cultured on the composite substrates showed various distributions and morphological features. Predominantly, MC3T3-E1 cells show a typical polygonal morphology with numerous lamellipodia and well-represented actinic stress fibers.

In the case of composite materials, on their surface the MC3T3-E1 cells present a predominantly spindle-shaped morphology. This pattern of cell spreading and morphology can be explained by differences in the surface characteristics of the analyzed substrates. The same assumptions are valid for a period of 7 days after sowing.

The biological performance of the obtained composite substrates was also evaluated by investigating their potential to support the viability of MC3T3-E1 pre-osteoblasts, by performing the CCK-8 test after 24 hours of culture. The number of viable metabolic assets on the composite materials obtained was almost similar to the reference materials, without statistical significance, for both experimental groups studied. Regarding cell viability after 7 days of culture, an increasing potential to sustain MC3T3-E1 cells can be observed..

4.2.9 Preliminary conclusions

Modulation of the acquisition parameters had a direct influence on both surface and volume characteristics. Of these, the prolonged dispersion time of the ceramic particles proved to be the key element in achieving a uniform and homogeneous surface and volume appearance. In terms of hydrophilicity, water permeability and protein retention, the combined results highlighted the viability of the obtained materials for bone regeneration applications. In addition, for an adequate dispersion in volume and optimal cell adhesion, it is recommended to use particles of the smallest possible size to obtain the composite materials.

Moreover, in vitro investigations with MC3T3-E1 pre-osteoblasts demonstrated that all developed composite substrates elicited a good osteoblast response in terms of adhesion, morphology and cell viability, thus denoting promising prospects for guided bone regeneration applications.

Chapter 5. Obtaining core-shell polymer-ceramic composite materials with polylactic acid/thermoplastic polyurethane matrices reinforced with hydroxyapatite particles

The aim of the second part of the experimental study was to obtain core-shell polymer-ceramic structures, from two established polymers in the field of biomaterials, namely polylactic acid (PLA) and thermoplastic polyurethane (TPU). As for the ceramic component, hydroxyapatite, it was obtained from bovine bones by the same route described in the previous chapter. The obtained filaments are intended to be used for 3D printing.

5.1 Obtaining composite materials in core-shell form

To obtain the core-shell composite materials, namely the polymer filament coated with bioceramics, two types of polymer materials, PLA filament and TPU filament, were used. The filaments were placed on a bed of HAp powder, particle size $>125\ \mu\text{m}$, over which a new layer of powder was added (required for complete filament coverage).

The filaments completely covered in a volume of HAp were pressed under the action of different pressing forces, and heat treated in an electric furnace for different time periods (30, 60 and 120 minutes) and different temperatures (195 °C, 205 °C, 215 °C). The final step

involved ultrasonically vibrating the samples to remove particles that did not adhere to the polymer surface.

5.2 Results and discussions regarding the characterization of core-shell composite materials with polylactic acid/thermoplastic polyurethane matrix reinforced with hydroxyapatite particles

5.2.1 Caracterizare din punct de vedere morfologic și compozițional

From a morphological point of view, the differences between the samples can be clearly observed. Regarding the influence of the working parameters on the morphology, it is observed that the temperature is the main influencing factor. At the temperature of 195°C, different amounts of small particles adhere to the surface of both polymer materials. In the case of PLA filaments, a higher amount of particles generates a rougher surface appearance, compared to TPU filaments where a lower amount of adhered particles results in a smoother looking surface with an approximately uniform distribution.

With increasing temperature, a change in coating morphology is observed, so that at 205°C, both small and large particles adhere to the surface of both types of polymers. In the case of the PLA filament, the small particles adhered to the polymer surface, while in the case of the TPU filament, the predominance is attributed to the particles in the large size range of the HAp powder used.

The maximum temperature used in this study (215°C) has a defining influence on the appearance of the material coatings, so that, in its case, on the surface of both types of polymers, only large particles finally adhered.

Regarding the compositional analysis, the EDS spectra of both types of structures revealed specific peaks of the main elements of HAp, especially Ca and P. Regarding the presence of the carbon peak in the EDS spectra, it denotes the existence of the polymer substrate.

5.2.2 Filament shape variation analysis

From the point of view of the influence of all the factors involved in the synthesis process of core-shell materials, namely pressing force, time and temperature, different types of investigations have been carried out. First, a cross-sectional morphological analysis of both types of materials was performed.

The shape factor represents the deviation from the initial cylindrical shape of the filaments, to indeterminate and irregular shapes. Due to the incrementally applied pressing force, the cross-sectional shape of the samples becomes more complex and distinct from the ovoid shape present in the reference materials (obtained without pressing force). The maximum and minimum measured perpendicular to the longitudinal axis of each micrograph were used to account for the degree of deformation. Accordingly, the x and y axes were chosen according to the preferential direction of deformation and not as horizontal and perpendicular to the page.

The lower values obtained (from the maximum value of 1.0 corresponding to the reference material to zero) are directly proportional to the increase in irregularity of the samples.

Polymer filaments melt in the supply hose of commercial 3D printers after traveling a defined path. Therefore, the shape of the cross-section should be considered an important parameter.

Therefore, it is important that the original round shape of the filament is preserved and as faithful as possible, without being affected by the bulk insertion of the HAp powder. Considering and maintaining this aspect ensures that these types of materials are suitable for additive manufacturing applications.

It can be concluded that:

- PLA-based materials show a sharp variation in the form factor compared to TPU ones;
- increasing exposure time is directly proportional to decreasing form factor.

5.2.3 Analysis of ceramic particle incorporation by measuring mass variation

Regarding the mass variation, it is directly proportional to the degree of incorporation of HAp particles into the surface of the polymeric material, thus resulting in the bioceramic coating. In the case of PLA filaments, as previously observed in Figure 5.5A at the temperature of 195°C, the reference samples showed the highest amount of incorporated powder regardless of the exposure time. At this temperature, the reference material showed the largest mass variation, i.e. 100%. In conclusion, the final mass of the core-shell material is double that of the original PLA polymer.

Regarding the degree of incorporation of HAp particles at 195°C, in the case of TPU filament, it is slightly different compared to that of PLA, thus resulting in a rather low mass variation. The temperature of 205°C determines, in the case of PLA filaments, a good incorporation of the ceramic particles, directly proportional both to the increase in exposure time and to the increase in pressing force. From the point of view of the final mass of the obtained core-shell materials, it can vary up to 120% of the initial mass of the polymer, thus suggesting an increased degree of HA powder incorporation. Furthermore, at this temperature, the incorporation of particles into the surface of the TPU filament follows the same downward trend with an unpredictable pattern, independent of changing the acquisition parameters. Moreover, their mass varies very hard, so the final mass values were at most 50% higher than those of the initial polymer.

As the temperature increases to 215°C, the final mass of core-shell materials is drastically changed. In the case of composite materials based on PLA, a significant increase in mass is observed. With the increase in exposure time and pressing force, their mass increases proportionally, up to values of 200%.

In addition, for TPU filaments, significant changes in mass variation are observed. As the temperature is high, the particles easily adhere to the polymer surface and therefore the mass of the final composite materials increases up to 160% at an increased exposure time, namely 120'.

5.2.4 Compressive strength test

The most promising evidence, namely:

- PLA-based materials obtained at a temperature of 205°C, an exposure time of 60' and a maximum pressing force of $0.5 \cdot 10^{-4}$ MPa,

- TPU-based materials obtained at a temperature of 215°C, 60' exposure time and a pressing force of $2.5 \cdot 10^{-4}$ MPa

were mechanically tested for compressive strength together with a sample of cortical bone tissue to support the preliminary investigations carried out in this study.

In the bends of HAp-inserted PLA and TPU filaments, a malleable behavior with significant plastic deformation is observed. For compressive loading on cortical bone, rapid hardening occurs after yielding, followed by softening. Bone is considered an ideal brittle solid that yields at an elastic limit that coincides with the compressive strength.

Comparing the compressive strength values of the selected core-shell composite materials with those of cortical bone, values about 4 times lower than those of cortical bone in the longitudinal direction are observed. From a comparative point of view, the results obtained show lower values than those of established metallic materials used in medicine but higher than those of ceramics.

5.2.5 Analysis of particle adhesion by pull-out testing

The pull-out test was also performed on the most promising samples (PLA-based materials obtained at 205°C, 60' holding time and a maximum pressing force of $0.5 \cdot 10^{-4}$ MPa and materials based of TPU obtained at 215°C, 60' holding time and a maximum pressing force of $2.5 \cdot 10^{-4}$ MPa).

A uniform strain distribution with a concentrated loading zone at the adhesive-filament interface is observed for the PLA samples. In contrast, the deformation of the TPU samples occurred in the early part of the filament and was later transferred to the adhesive-filament interface. This non-uniform behavior can be explained based on the different elastic moduli of PLA, TPU and the chosen adhesive, since PLA exhibits an elastic modulus about three times higher than that of the adhesive, while TPU values are close to those of the adhesive.

A similar behavior is observed for core-shell composite materials. In this case, the presence of HAp particles changes the state of stresses and strains, because they are mainly concentrated in the contact region of the particle-adhesive layer and have been gradually transferred to the particle-coating-substrate interface. In this case, any reorientation / slippage of the particles can be encountered, together with the decrease in stiffness of the assembly and increase in displacement. Also, breakage occurs at the particle-substrate coating junction and is predominantly cohesive in nature, as the particles occur both on the adhesive and on the substrate surface.

Following the analysis of the values of the maximum displacement forces, a decrease of 61.82% of the displacement force is observed in the case of PLA-HAp core-shell samples and 67.14% in the case of TPU-HAp core-shell samples, compared to displacement forces of the initial polymer filaments (without incorporated HAp particles).

5.2.6 Preliminary conclusions

All the changes induced on the working parameters had a direct influence on all the properties of the final core-shell composite materials, especially on their coating.

In this sense, for PLA-based materials, the obtained results recommend the use of a temperature of 205°C, an exposure time of 60' and a maximum pressing force of $0.5 \cdot 10^{-4}$ MPa, compared to materials on base TPU for which an elevated temperature of 215°C, 60' exposure time and higher pressing forces (up to $2.5 \cdot 10^{-4}$ MPa) were determined to be adequate for the final purpose due to the fact that the filament it retains its cylindrical shape and the coverage is uniform and well defined. In addition, a comparative study with cortical bone, for compressive strength, was performed for these samples to demonstrate their applicability in dentistry and orthopedics. These technological parameters represent an excellent possibility towards an optimal route to obtain core-shell composite materials for FDM additive manufacturing.

Chapter 6. Obtaining filament-shaped composite materials with polylactic acid matrix reinforced with hydroxyapatite particles

The aim of the third part of the study consisted in obtaining filamentary structures from polymer-ceramic composite materials, from a polymer established in hard tissue engineering, namely polylactic acid (PLA) and a ceramic component, namely hydroxyapatite, obtained from bones of bovine origin, through the same synthesis route described in previous chapters.

6.1 Obtaining composite materials in filament form

To obtain the composite materials in the form of PLA-HAp filament, PLA granules of approximately 2 mm in size, and HAp powder of bovine origin were used. As for the hydroxyapatite of bovine origin, it was obtained by the same synthetic route as in the case of the other composite materials obtained in the previous chapters.

In terms of size, the HAp used in this study belonged to three different grades, namely: $<40 \mu\text{m}$, $<100 \mu\text{m}$ and $>125 \mu\text{m}$. Compositionally, six different types of filaments were obtained, namely: 100% PLA - control sample, 90% PLA + 10% HAp, 80% PLA + 20% HAp, 70% PLA + 30% HAp, 60% PLA + 40% HAp, 50% PLA + 50% HAp.

The polymer granules and the ceramic powder component were homogenized in a three-axis rotary mixer. The next type of homogenization was by mechanical mixing in the melt, on the plate of a magnetic stirrer at a temperature of 250° C. Following this stage, foils and flat strips were obtained that were mechanically discharged, thus obtaining pellets of approx. 2 mm.

The pellets were inserted into an extruder, and thus the composite filaments of PLA and HAp were obtained, with a diameter of approximately 1.75 mm, intended for FDM additive manufacturing.

6.2 Results and discussion on the characterization of hydroxyapatite particle-reinforced polylactic acid matrix filament composite materials

6.2.1 Morphological and compositional characterization

The third part of this study is based on establishing the necessary parameters for obtaining filaments from composite materials intended for the field of biomedical engineering. These, in turn, aim to create biomedical devices obtained through modern techniques, namely FDM-type additive manufacturing.

A. Compositional and morphological characterization at the macroscopic level

From a macroscopic point of view, for the composite materials obtained with HAp powder of the <40 μm grade, the homogeneity of the filaments can be observed. The control sample, 100% polymer material, has a cylindrical shape, ideal and absolutely necessary for the final purpose, with a homogeneous structure both on the surface and inside. The addition of HAp powder changes the internal structure of the filaments, observing the particles uniformly and homogeneously distributed throughout the polymer mass. The increase in HAp powder concentration is visible both in the internal structure of the filaments and in their external appearance.

From a compositional point of view, the EDS spectrum of the control material, i.e. 100% PLA, revealed peaks specific to the main elements (C and O). As for the filament composite materials, the EDS spectra show specific peaks of both the main elements of HAp, especially Ca and P, and peaks specific to the main elements of PLA. The radical change of the spectra is observed, primarily with the addition of HAp, but also with the increase in its concentration, and the decrease in the content of PLA.

As for the materials obtained with HAp of the <100 μm grade, the particles are much more visible in the structure of the filament-type composite materials. Thus, from the point of view of homogeneity, a uniform dispersion of particles can be observed both in the internal structure of the materials and on their exterior. Although the filaments retain their cylindrical shape, they undergo some surface changes with increasing particle size.

From a compositional point of view, the EDS spectra show the same directly proportional increase in the intensity of the peaks of specific HAp elements with the increase of its concentration. At the same time, the increase in the intensity of the peaks of the HAp specific elements is directly proportional to the decrease in the intensity of the PLA specific elements, which indicates the homogeneity of the obtained bicomposite material.

In the case of materials obtained with HAp powder with dimensions >125 μm , the macroscopic appearance of the filament-type materials is completely modified. Regarding the internal structure of the filaments, large-sized particles are observed approximately uniformly distributed throughout the structure of the composite materials

Regarding the shape of the filaments, they retain their cylindricality, but their external appearance is rough. The outer roughness is present even with small additions of powder (20%), a fact also due to the large size of the HAp particles.

From a compositional point of view, the EDS spectra show, as in the case of the other materials, the same proportionality between the decrease in the intensity of the peaks of the elements specific to the polymer (C and O) and the increase in the intensity of those of the elements specific to hydroxyapatite (Ca, P and Mg).

B. Compositional and morphological characterization at the microscopic level

Morphological characterization at the microscopic level is essential for the definition and validation of these types of materials for use in medical engineering through the additive manufacturing technique. In this sense, the composite materials obtained were characterized at a microscopic level, from a morphological point of view, as follows:

- i. cast samples or foils obtained after the homogenization of the polymer and hydroxyapatite to determine the degree of uniformity, and to validate the technique used;
- ii. section of filament obtained from foils by extrusion;
- iii. the surface of the obtained filament.

In the case of cast samples, with added powder from the $<40\ \mu\text{m}$ grade, compared to the control sample, 100% PLA, the samples with added HAp show a homogeneous structure regardless of the amount of added powder. This homogeneity is also maintained in the case of filaments. In the filament section, a change in structure is observed with the increase in the amount of HAp powder added, but this fact does not affect the homogeneity of the filament. Characterization of the filament surface indicates and supports the same assumptions of a uniform composite structure.

Samples with added powder from the $<100\ \mu\text{m}$ grade show a high level of homogeneity, even from the stage of cast samples, and this is also maintained in the case of filaments, both in section and on the surface. Comparatively, the particle size directly influences the surface morphology, so in the case of materials with high addition of HAp powder, the structure is completely changed.

The samples with added powder from the $>125\ \mu\text{m}$ grade show a high level of homogeneity, even from the stage of the cast samples, this is also maintained in the case of the filaments, both in section and on the surface. From a dimensional point of view, the HAp powder has different sizes, this fact being especially evident in the case of the filaments analyzed in the section.

From a compositional point of view, comparative EDS analysis performed on all extruded filaments in cross-section revealed the elements characteristic of the composition of calcium phosphate and polymeric materials, namely C, O, Ca and P. The small amounts of Mg detected in the case of samples with addition of 10-50% ceramic particles is due to the HAp precursor, namely bovine bone.

Furthermore, independent of the particle size ranges, the amount of carbon decreased drastically with increasing HAp ratio and, implicitly, a higher degree of coverage was provided. As for the HAp characteristic elements, calcium, phosphorus and magnesium, their variable concentrations increased in an upward trend line with increasingly higher addition of HA. These minor differences may be attributed to variation in surface roughness, particle sizes, or general method inaccuracy.

6.2.2. Differential scanning calorimetry analysis (DSC)

According to the DSC curves, all sample analyzes show two endothermic peaks at approx. $140\text{--}142\ \text{°C}$ and $151\ \text{°C}$ due to the progressive melting of PLA, independent of the HAp ratio. However, the incorporation of HA particles led to the appearance of a specific exothermic peak for each sample in the range $\sim 86\text{--}93\ \text{°C}$ (at minimum and maximum HAp concentrations, split-type appeared) corresponding to crystallinity—cold crystallization of the polymer (crystallization temperature, T_c). According to the evolution of the T_c parameter, it can be appreciated that its downward trend with increasing HAp ratio is related to the heterogeneous crystallization of samples with more HAp particles, which can act as nucleating agents for PLA crystals.

Furthermore, all composite samples showed another endothermic peak indicative of the glass transition temperature (T_g). It was found that T_g increases with the gradual increase in HAp ratio from ~53 °C to ~57 °C. This phenomenon was previously reported and explained based on the influence of the amount of HAp particles per unit volume in the composite, and it was concluded that T_g is dependent on the high surface area of the HAp particles.

Therefore, it can be concluded that due to the tendency of HAp particles to form nucleation centers in the polymer matrix, the rigid phases are modeled and further govern the final characteristics of the products, as was also demonstrated by morpho-compositional analysis.

6.2.3 Surface analysis by measuring contact angle and surface energy

Surface hydrophilicity was evaluated by contact angle (CA) measurements with three wetting agents, namely different test liquids (water – H₂O, diiodomethane (DIM), and ethylene glycol (EG)) on pellets of cast mixtures.

In addition, the SFE was calculated by the method developed by Owens, Wendt, Rabel, and Kaelble (OWRK) using the previously measured contact angle values. The surface free energy indicates the sum of the dispersive and polar interactions recorded at the solid-liquid interface. To obtain good experimental results, water and EG were used as polar liquids, while DIM was used as nonpolar/dispersive liquid.

The CA values induced a constant downward slope with increasing incorporated HAp concentration from 10% to 50%, independent of the test liquid or HAp particle sizes.

The CA for water, DIM, and EG ranged from ~66 to 20, ~67 to 22, and ~70 to 21 for HAp particles in the <40 m, <100 m, and >125 m ranges, respectively. Thus, all results revealed a significantly improved hydrophilic character (CA < 90°) of the composites compared to pure PLA. Even though the incorporation of smaller particles (<40 μm) led to smoother surfaces, their surface microporosity and regular distribution, together with low CA values compared to those of PLA, attest to their beneficial role in improving wettability characteristics of the evidence.

The deduced evolution of the SFE values revealed an increasing trend with increasing HAp ratio, for all dimensional ranges of HAp. A high value of SFE is a corresponding indicator of a pronounced wetting, both of which are necessary in the field of additive manufacturing, to print continuous lines without the occurrence of the phenomenon of fusion. Moreover, the higher the SFE value, the higher the chances of improved protein adsorption and overall cellular response for the implantable products that could be obtained.

6.2.4 Micro-CT analysis of the distribution of ceramic powders in composite materials

3D reconstructions of all filaments obtained from composite materials provided a complete overview of the internal distribution of ceramic particles in the PLA matrix. Based on the CT technique, having the ability to correlate the typed material with the corresponding atomic number, it was possible to present the areas of interest differently due to the two materials involved.

The particle-by-particle dispersion phenomenon of HAp highlighted in the cross-sectional, surface view of the filaments was preserved and extended to the entire volume,

independent of the ceramic particle size. Incorporation of increasingly higher concentrations of HAp radically reduced the free space between the particles (polymeric matrix) while maintaining the degree of surface-to-volume homogeneity. Thus, once the size of the HAp particles increased concomitantly with the added concentration, the distribution was more uniform, and led to the formation of complex, organized and gradual internal structures that evolved in the section and along the length of the extruded filaments.

In addition, no agglomerations in the form of aggregates were identified on the surface of the samples, or in their volume. Moreover, the individual outline of each particle can be observed in the 3D triangular cut area of all samples and distinctly highlighted in yellow, especially for samples with addition of HAp particles with sizes $>125\ \mu\text{m}$. Also, the rounding effect of the edges of the particles during extrusion is confirmed throughout the volume of the filaments, which is also in good agreement with the morphological evaluation.

Therefore, 3D reconstructions established that composite filaments of constant diameter can be obtained by modulating both the size and aspect ratio of HAp particles. Through a homogeneous surface-volume distribution of the ceramic particles in the polymer matrix, a ceramic network can be gradually induced inside the filaments. As such, the print nozzle, print size and speed could be modulated depending on the type of filament - in this case determined by the size and proportion of incorporated HAp particles.

6.2.5 Evaluation of mechanical characteristics

The degree of deformation by height and diameter (ie, degree of barreling) was calculated by measuring the samples before and after the compressive force was applied. The variation of compressive strength and modulus of elasticity, along with shortening and degree of barreling, showed linearly increasing trends, similar to increasing incorporated HAp concentration, independent of ceramic particle size.

Compared to samples obtained from PLA alone, both compressive strength and elastic modulus show high values of $\sim 1-1.18$ ($<40\ \mu\text{m}$), $\sim 1.1-1.19$ ($<100\ \mu\text{m}$), $\sim 1-1.10$ ($>125\ \mu\text{m}$), $\sim 1-1.81$ ($<40\ \mu\text{m}$), $\sim 1.1-1.96$ ($<100\ \mu\text{m}$), and $\sim 1.1-1.43$ ($>125\ \mu\text{m}$), proving that HAp particles derived from natural sources indeed acted as a reinforcing material for the PLA matrix. Another argument in this direction concerns their ability to sustain correspondingly higher degrees of deformation than PLA before cracking, recorded with an amplification factor of $\sim 1.34-1.38$ ($<40\ \mu\text{m}$), $\sim 1.35-1.46$ ($<100\ \mu\text{m}$), $\sim 1.31-1.40$ ($>125\ \mu\text{m}$) for shortening, and $\sim 1.60-1.88$ ($<40\ \mu\text{m}$), $\sim 1.47-2.06$ ($<100\ \mu\text{m}$) and $\sim 1.46-2$ ($>125\ \mu\text{m}$) for barrels.

The modulus of elasticity is also representative of the degree of stiffness, and therefore, the gradual addition of HAp particles led to more rigid composite materials. A certain degree of interaction between the ceramic particles and the polymer matrix is expected, as they connect in two different ways: physically due to the high porosity of the particles, which increases the surface tension, and chemically based on the interactions between the polar groups of PLA and HAp. Both elements, together with the (low) degree of crystallinity, increase the adhesion between polymer and ceramic materials, thus increasing the stiffness of composite products, even at low HAp concentrations, regardless of particle size. Moreover, the lower values recorded for samples with HAp particles $>125\ \mu\text{m}$ can be attributed to the decrease in the degree of dispersion and the tendency of the particles to form aggregates.

Considering the obtained results, it can be assumed that the addition of HAp has a great influence on the increase of the elastic modulus of the composite materials, while the larger particle sizes tend to decrease it.

In the case of incorporating HAp derived from natural resources, at different concentrations and with variable particle sizes into the PLA matrix, all three premises were met and confirmed throughout the preliminary investigation program. All the results obtained for the mechanical characteristics are suitable for biomedical applications, considering that they far exceed the lower strength limit for cortical bone, but not the upper one

6.2.6 Preliminary conclusions

The possibility of obtaining by extrusion filaments from composite materials with PLA polymer matrix and HAp reinforcement derived from natural resources was confirmed. The influence of simultaneous modulation of HAp particle size, proportion and shape on the morphology, surface wettability, free energy, internal distribution and mechanical behavior of blends cast into cylindrical shapes and extruded into filaments were investigated for the first time. All the changes made regarding the ceramic component directly influenced the final characteristics of the filaments extruded from composite materials. In this regard, a homogeneous dispersion was observed for all experimental samples, independent of the size or proportion of HAp particles, by morphological investigations — for the surface and in the cross-section of the filaments and by micro-CT — for the internal distribution and arrangement of the particles in the polymer matrix. In any case, to keep an invariable diameter and a smooth and uniform surface of the filaments, the proportion of HAp should be limited to a maximum of 40% for particle sizes <40 μm , 30% for particles <100 μm and 20% for particles > 125 μm .

Regarding the adhesion at the HAp/PLA interface, the particular polyhedral shape and microporous surface texture of the natural ceramic particles played a significant role in establishing a strong bond between the two components. Furthermore, the absence of any porosity on the contour of the interface of the ceramic particles with the polymer was also clearly observed. In combination with the improved mechanical characteristics, it can be seen that HAp acted advantageously as a mechanical reinforcement element for the polymeric material, leading to values of compressive strength and modulus of elasticity/stiffness within bone tissue compatible limits.

Chapter 7. Conclusions and personal contributions

7.1 Conclusions following the experimental results

In this doctoral thesis, the development of composite materials with polymeric matrices and reinforcement with hydroxyapatite particles of natural origin was pursued, for applications aimed at the biomedical field, specifically bone regeneration.

Following the thorough analysis of specialized literature carried out for the theoretical study of the doctoral thesis, it was demonstrated that the practical applicability in the biomedical field of bone reconstruction products developed as a result of research in the field of materials science and engineering is difficult. Until now, no studies are clearly reported to certify a material completely suitable for bone regeneration, which exhibits good surface and

biodegradation properties and fulfills all the necessary mechanical properties, comparable to those of human bone. Being a relatively new field, clinical studies are very limited, thus resulting in a high degree of risk regarding the success of the products in terms of biocompatibility and mechanical behavior.

Regarding the techniques for manufacturing products from composite materials with polymer matrix and ceramic reinforcement, additive manufacturing has been of recent interest. The increased interest in these techniques is due to the fact that one hundred percent customized products can be obtained, with various geometries and sizes, in a relatively short time compared to classical methods. However, being modern technologies, the costs involved are high, and thus their profitability is not yet fully justified.

Thus, the experimental research presented in this doctoral thesis aimed at obtaining composite materials with polymer matrix reinforced with ceramic powders for applications in the biomedical field, with a view to guided bone regeneration, by varying both the parameters of the obtaining routes, as well as the quantity and size added hydroxyapatite particles. The common element of these materials was the ceramic part (hydroxyapatite) obtained by a well-established synthesis route and reported in the specialized literature. As for the polymer component, it was different, in order to obtain improved composite materials. So:

- i. the first part aimed at obtaining composite materials with cellulose acetate matrix and hydroxyapatite reinforcement in film form;
- ii. the second part aimed at obtaining composite materials with PLA and TPU matrices and hydroxyapatite reinforcement in core-shell filament form;
- iii. the third part involved obtaining composite materials with PLA matrix and filamentous hydroxyapatite reinforcement.

The research continued with the complex characterization from several points of view, of all these types of composite materials.

The main conclusions resulting from the experimental research carried out as part of the doctoral thesis are presented below.

- i. With regard to composite materials in film form:
 - by modulating the ultrasonic dispersion time but also the particle sizes and the concentration of added hydroxyapatite powder, the surface and volumetric characteristics of the final products are directly influenced;
 - preliminary biocompatibility investigations, namely hydrophilicity, water permeability and protein retention, have highlighted the viability of these types of products in the intended applications;
 - following in vitro investigations with MC3T3-E1 pre-osteoblast cells, it was demonstrated that all types of composite materials obtained in this part of the study had a response in osteoblast studies, which confirms the potential of applicability in the biomedical field;
 - by varying the dispersion time of the hydroxyapatite particles in the cellulose acetate matrix, it was found that a longer time ensures a uniform, adequate dispersion throughout the polymer volume;
 - following the variation of the sizes of the hydroxyapatite particles, it was found that the smaller sized particles are more suitable for these types of applications;

- changing the concentration of added hydroxyapatite led to the finding that a lower concentration ensures a uniform dispersion, while a higher concentration leads to the formation of aggregates, independent of the dispersion time.
- ii. With regard to core-shell filament composites:
- by varying the technological parameters (treatment time and temperature in the oven, pressing force) but also by varying the material of the polymer matrix (PLA and TPU), all the properties of the final products were directly influenced, especially in terms of the ceramic coating (shell));
 - following the investigations carried out on PLA matrix materials, it was found that the optimal results are obtained at the following working parameters - temperature of 205°C, exposure time of 60 minutes and maximum pressing force of $0.5 \cdot 10^{-4}$ MPa;
 - investigations carried out on TPU matrix materials have shown that optimal results are obtained at the following working parameters: elevated temperature of 215°C, 60 minutes exposure time and higher pressing forces (up to $2.5 \cdot 10^{-4}$ MPa);
 - through the comparative study of compression resistance with cortical bone, the possible applicability of these types of composite materials in the field of dentistry and orthopedics was demonstrated;
 - the modulation of these technological parameters represents a possibility of an optimal synthesis route of core-shell filament-shaped materials intended for the biomedical field, but also for additive manufacturing.
- iii. With regard to filament composites:
- following the simultaneous modulation of the characteristics of the ceramic component (particle size and added powder concentration) a direct influence on the final characteristics of the composite material was observed in terms of morphology, surface wettability, internal architecture and mechanical aspects;
 - a homogeneous dispersion of the ceramic reinforcement in the polymer matrix was found for all the materials obtained in this part of the experimental study;
 - the absence of contour porosities was observed at the interface of the ceramic particles with the polymer;
 - following the characterizations from a mechanical point of view, it was demonstrated that the hydroxyapatite powder acts as a reinforcing material, by significantly improving the mechanical properties;
 - the hydrophilic nature of all the composite materials developed in this part of the study demonstrates their potential to respond favorably to cell adhesion.

The production methods presented in this study regarding composite materials with polymer matrix and addition of ceramic reinforcement material (hydroxyapatite) led to the creation of products with applicability in the biomedical field.

7.2 Original contributions

This PhD thesis includes original contributions in the field of materials engineering. The main goal was to obtain new materials with superior properties aimed at applicability in both dental and orthopedic fields. As for the original contributions resulting from experimental research, they are represented by:

- obtaining for the first time composite materials in film form that can stimulate bone regeneration through the cellulose acetate matrix (bioresorbable polymer) and the addition of hydroxyapatite (rich source of Ca and P - elements that stimulate osteogenesis);
- the development of an original technological route to obtain composite filaments in core-shell form, through the superficial insertion of hydroxyapatite particles into the surface of bioresorbable polymer filaments of polylactic acid and thermoplastic polyurethane;
- the development of a technological route for obtaining in two stages, which involves the homogenization of the particles (used as reinforcement material) in a polymer matrix of polylactic acid. The main goal of this part was to obtain biocompatible composite materials that can be used to extrude filaments for the additive manufacturing of scaffolds by the FDM method.

The research presented in this doctoral thesis has practical applicability in order to manufacture products from composite materials intended for the regeneration of bone tissue. The presented strategies can be implemented in order to achieve several objectives aimed at manufacturing customized products in series, especially through FDM-type additive manufacturing techniques.

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Regarding the importance of the original contributions from this doctoral thesis, it is supported in 8 papers presented at national and international conferences and recognized by the publication of 3 scientific articles in the field of materials engineering, in specialized journals rated Clarivate Analytics with a factor of cumulative impact equal to 15, according to WoS (Web Of Science).

The success of these types of composite materials intended for bone regeneration also depends on the continuation of experimental research, which may include: additional testing of the biological and mechanical performances of all three types of composite materials, preclinical testing on animal models, validation of the materials through reproducibility studies in order to obtaining certified reference materials to be used as a standard in the quality control of products intended for the biomedical field.