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PHD THESIS SUMMARY

MULTIMATERIAL SYSTEMS DEVELOPMENT FOR SPECIFIC ORTHOPEDIC APPLICATIONS

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Abstract

In the context of worldwide bone implant and bone substitute needs to alleviate a large number of bone defects, necessary research steps in the field of regenerative implants are expected. This scientific direction led to the main objective of the PhD thesis: the design, practical implementation, and analysis of a channeled Mg-based implant for the treatment of bone defects. Through an extensive literature review, the main manufacturing routes for Mg-based implants were identified, and a patient-adapted algorithm was proposed for the development of new products. In addition, the importance of artificial intelligence was underlined, and its inclusion in the large production map was considered mandatory. The suitability of two binary Mg-based alloys, Mg-Nd and Mg-Zn, was assessed through material analyses and *in vivo* testing to determine whether these materials could be used safely in implant production. A new manufacturing route was proposed, and dedicated geometries for implant models were numerically investigated based on a virtual laboratory scheme, including computer-aided design, mechanical finite element analysis, and computational fluid dynamics investigation. Last but not least, implant and reference samples produced from cast Mg-based alloys were investigated, fully analyzing their surface properties, mechanical attributes, and *in vitro* cytotoxicity. The main conclusion of the PhD thesis is that the innovative manufacturing route could be successfully applied and further developed by incorporating artificial intelligence, and that the implant samples could serve as a pre-clinical solution.

Keywords: Mg-Based alloys, new manufacturing route, orthopedics, *in vivo* and *in vitro* tests, material test, numerical investigation

Introduction

Today, there is an ongoing need to develop new technologies for biomaterials dedicated to bone regeneration, due to the fact that bone defects are becoming increasingly prevalent in the medical world. Their main causes are fractures that occur as a direct consequence of motor vehicle accidents, tumor resection, or osteoporosis. Some worldwide statistics estimated that by 2040, about 316 million patients will have an osteoporotic fracture [1]. Regarding the other above-mentioned reasons, several 3 million people will be the victims of femoral or other types of bone fractures, which should be fixed [1]. The biomaterial type must be chosen in accordance with the classification of bone defects made by Schemitsch [2]. The small defects have a size smaller than 2 cm and are characterized by a 50% cortical circumference loss, and usually they heal spontaneously based on the human body's resources. Intermediate bone defects exhibit a size between 2 and 6 cm and a cortical loss exceeding 50%, while the large bone defects, which are the hardest to medically address, have a size bigger than 6 cm. Another technical term that must be explained before choosing a certain type of biomaterial, in order to produce a dedicated implant, consists of the critical bone defect that is defined as one with a size larger than 2.5 cm and will never heal based on human body' capabilities, requiring compulsory surgical interventions.

It is well known that the gold standard in orthopedics was considered the use of autologous bone grafts. Unfortunately, this type of intervention is accompanied by important side effects such as donor site morbidity, teratoma, or prolonged hospitalization time. Another solution consists of using allografts or xenografts from other people or animals. However, this technique also implies serious drawbacks such as graft rejection or infection transmission. Taking into consideration the above-mentioned issues, the need to develop new and innovative materials in conjunction with anatomical shape or patient-adapted geometry to be used in bone defect treatment becomes more and more important.

A good definition of an ideal biomaterial that could be used for orthopedic applications was provided by Georgeanu et al. [3]. It is underlined in their work that a single bone substitute must accomplish osteoconductivity, osteoinduction, and osteogenic properties. In addition, ensuring the neurogenesis and angiogenesis provide a real basis for the tissue viability at the bone defect site. Then, the presence of adverse reactions such as allergy, carcinogenicity, teratogenicity, or antigenicity must be carefully analyzed and addressed if any drawbacks are observed. To shortly summarize the ideal attributes of a biomaterial used in bone defect treatment, one should consider a hydrophilic and rougher surface to permit cell adhesion and proliferation, ensuring the implant biocompatibility, adequate mechanical properties with values close to those of the human bone, to prohibit the stress shielding phenomenon, and biodegradability simultaneously with the regenerative potential of the biomaterial in cases where the long exposure to biomaterials is not necessary.

The main objective of the PhD thesis is the design, manufacturing, and testing of a biodegradable implant with special geometry featuring channels, dedicated to being the support for a multimaterial system used for a specific orthopedic application, respectively for the treatment of bone defects. The doctoral study is based on an experimental design presented in Figure 1. Firstly, a prospection of the existing literature in a direct relationship with biological pathways, patient-adapted geometry, and biodegradable implant manufacturing technology was conducted to establish a new and innovative manufacturing route that has never been applied by scholars. Then, the suitability of two Mg-based alloys with high biocompatibility, supported by material analysis and *in vivo* tests, was assessed to determine whether these biomaterials are adequate for the central theme of the doctoral work. A numerical mechanical analysis, together with a computational fluid dynamics investigation for two proposed

geometries, helped decide which to implement in practice. In the last part of the PhD thesis, a new and innovative manufacturing route was proposed and described in detail, along with surface properties, degradability, mechanical properties, and *in vitro* analysis of the implant's performance compared with reference samples made of cast alloys. The innovative and unique character of the PhD thesis is underlined in **four published doctoral papers** with the PhD candidate as first author and **a patent proposal** made in collaboration with the doctoral supervision team and experienced researchers.

The research conducted in the **first doctoral paper**, "**Bone Regeneration Induced by Patient-Adapted Mg alloy-based Scaffolds for Bone Defects: Present and Future Perspectives**", investigated the Mg-based alloys' effect on bone regeneration exhaustively [4]. The bone healing was generally presented, and then host immune response modulation and principal mechanisms that occur during angiogenesis and osteogenesis in the presence of Mg-based implants were analyzed with focus on the recent literature studies [5]. A general strategy dedicated to patient-adapted implants used in bone defect treatment, starting from the medical images and segmentation strategies available in medical software, continuing with different numerical analyses made for estimating the suitability of various geometries, and finalizing with the manufacturing technologies, post-processing solutions, and material and biological investigations, was proposed. In addition, devoted attention was given to modern, already existing manufacturing technologies [6]. This research was performed in order to see what the field has learnt in the Mg-based implant domain and how to develop a new strategy to design a dedicated implant, which should be easy to implement and address the limitations and existing challenges of the Mg-based implants. Last but not least, the impact of artificial intelligence on manufacturing technology was detailed to see how a possible large or medium-scale production of a regenerative implant could be adapted in relation to the commercial markets.

The next objective of the PhD thesis was to investigate the regenerative and osteointegrative potential of two Mg-based alloys. This analysis was developed in **the second doctoral paper** entitled "**Suitability of Mg-Nd and Mg-Zn Alloys to Obtain Biodegradable Structures for Bone Defects**" [7]. Firstly, an experimental program on material property investigations, consisting of microstructural analyses using optical microscopy and scanning electron microscopy with energy-dispersive spectroscopy, and phase identification by X-ray diffraction, was conducted. Then, the mechanical properties of the alloys were determined with compression tests by following the existing standard and computing the compressive strength and Young's modulus. In addition, *in vitro* corrosion was measured using electrochemical methods. Based on the obtained values, a much nobler character for Mg-Zn alloy was noticed compared to Mg-Nd. To establish if the two investigated alloys are characterized by good biocompatibility, an *in vivo* animal study was conducted. Two types of implantation sites were chosen as follows: subcutaneous and bone implantation. Micro-computed tomography and histopathology were performed on samples prepared from animal bones and soft tissue. The superiority of Mg-Zn alloy was obvious, being linked to a much higher mineral density compared to Mg-Nd, which was characterized by a low-density mineral phase. One can conclude that both Mg-alloys exhibited great potential to be used as biodegradable bone substitutes.

Considering the outstanding properties of the previously investigated Mg-based alloys, two implant geometries were developed in the **third doctoral paper**, "**Mechanical and Computational Fluid Dynamic Models for Magnesium-Based Implants**" [8]. Based on computer-aided design (CAD) modeling two implant structures with porosities of 62% and 38% were developed. These geometries were made in a simple way to permit the implementation of the new innovative manufacturing route developed further in the fourth doctoral paper. The mechanical simulation was conducted under the assumption that the stress-

strain relationship is linear and that the applied load remains constant at each simulation stage. The Young's modulus of the models was computed based on the mechanical stress estimation and displacement measurements, by applying a compression test. Both developed structures had values close to the human bone elastic modulus, with a much more adequate behavior of the 62% porosity implant [7]. In order to analyze the biological and hemodynamic performance of the three-dimensional (3D) developed structures, computational fluid dynamics simulation was adopted. All the numerical computations were conducted by considering the following idealizations: the fluid had Newtonian behavior, the fluid velocity respected the natural values, which occur in the case of blood for human bone defects, or fluids that are used in bioreactors, and last but not least, the implant wall roughness was estimated to zero. The permeability was computed as presented in [9]. It is well known that wall shear stress is an important parameter that is usually linked to cell multiplication, viability, and adherence to a certain surface, and it must not exceed 60 mPa, leading to cellular death [10]. It was concluded that both numerical analyses demonstrated the superiority of the 62% porosity geometry. It was decided at the end to practically implement only this geometrical configuration, using both Mg-alloys presented in the second doctoral paper.

In the **fourth doctoral paper**, "**Biofunctional testing of a degradable implant made by Mg-Nd and Mg-Zn alloys used for bone defects**", the main objective was to develop a new and innovative manufacturing route to obtain two biocompatible implants with channels made of Mg-Nd and Mg-Zn alloys [11]. This last study has two main parts, the first one is dedicated to the exhaustive description of the manufacturing process, while the second one contains material tests and *in vitro* cytotoxicity analysis. Regarding the proposed manufacturing route, the geometry chosen in the third paper was printed in polylactic acid (PLA). Then, a manual weaving procedure was implemented to insert into the polymeric matrix channels biocompatible Ti6Al4V wires. Based on a dedicated solvent in accordance with the literature [12], the PLA matrix was completely dissolved. After that, the remaining Ti6Al4V waved structure was immersed together with small and thin pieces cut from Mg-based alloys, in an induction oven set as presented in the paper [11], in order to obtain a Mg-Ti alloy hybrid. Parallelepipedal samples were prepared. In the last part of the manufacturing procedure, the hybrid metallic samples were immersed in 45% hydrofluoric acid (HF) to remove the Ti alloy wires. In accordance with the literature [12] and as confirmed by X-ray diffraction spectroscopy, a MgF_2 conversion coating formed on the implant surface. In the second part of the paper, material characterization was conducted, based on a comparison between the implant and the reference (prepared from original as-cast Mg-based alloys) samples. The mechanical compression test revealed that the implant samples exhibited lower values of the Young's modulus. It was concluded that the mechanical properties of the implant are in the biological range of the trabecular human bone. On the other hand, immersion analyses in simulated body fluid (SBF) resulted in much lower weight loss for implant samples compared to the reference, due to the positive effect on reducing corrosion caused by the conversion coating. Surface characterization was performed through contact angle measurements, surface free energy (SFE) computations, and roughness tests. Also, in this case, the implant samples exhibited a superior behavior with a super hydrophilic surface, in combination with high SFE values, and increased roughness [11]. *In vitro* cytotoxicity of the developed implant samples was assessed using a custom osteoblast cell line. The main conclusion of this last study, and of the PhD thesis, is that the biocompatible channeled implants developed through an easy, implementable method have high potential for inclusion in pre-clinical studies.

The research presented in these detailed studies shows evidence that the proposed manufacturing route and the biodegradable channeled implants would have a high impact on bone defect treatment. Its high potential was exhaustively proved based on material analysis, *in vivo*, and *in vitro* investigations presented in the four doctoral papers. The elevated

innovative grade of the manufacturing procedure proposed in the PhD thesis is sustained by the patent proposal and hinders the disadvantages of the already existing methods used for Mg-based implants, addressing also the problems linked to the increased pyrophoric effect of Mg alloys. It can be foreseen that the presented manufacturing method could be adapted in the future to a medium-scale production level, and the implant surface and biological properties could be improved by applying different coatings.

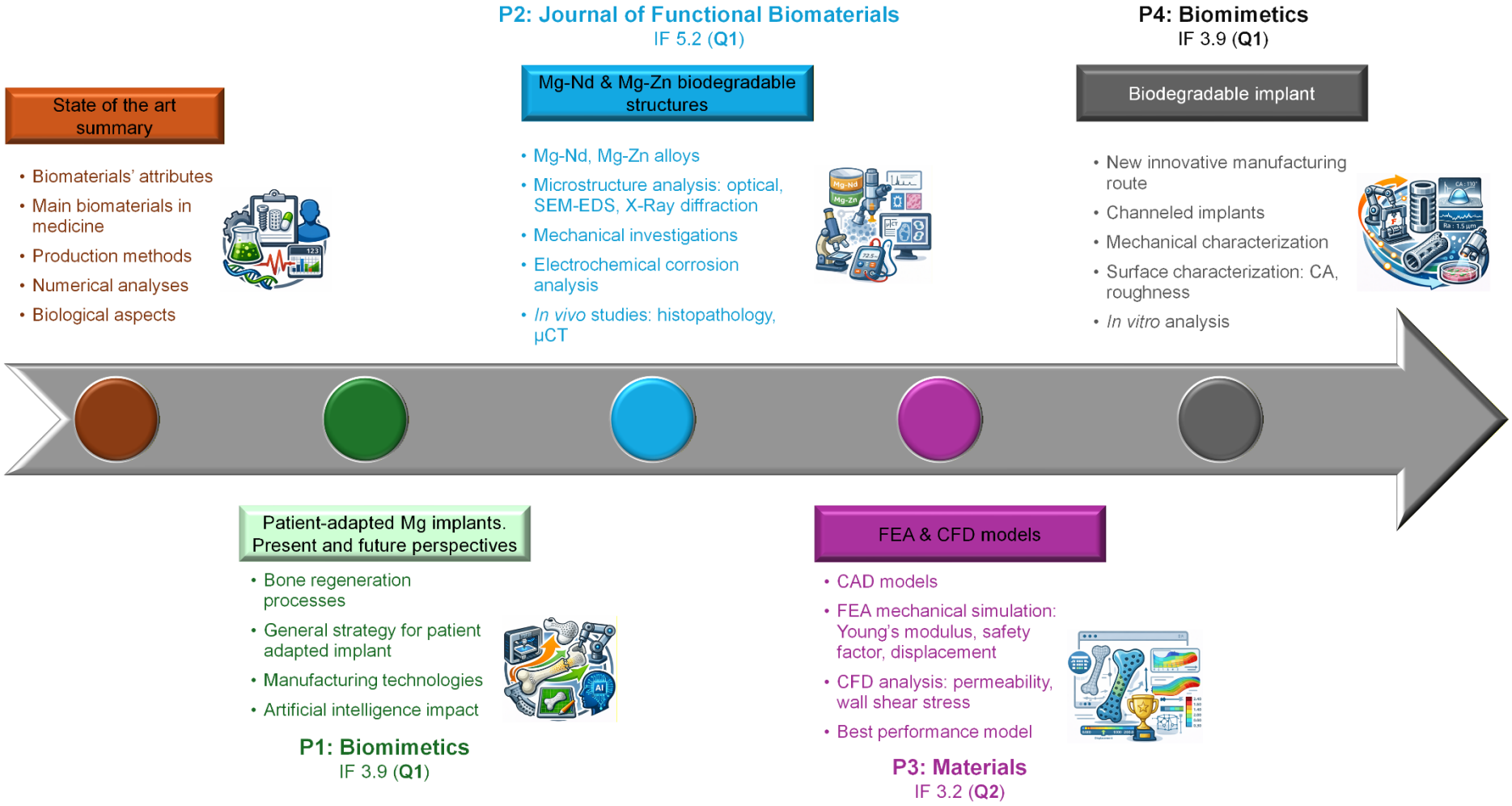


Figure 1. Workflow of the PhD thesis, with published papers for each step.

Chapter 1 – State-of-the-art summary related to the PhD thesis objectives

1.1. Generalities regarding modern biomaterials for orthopedic applications

Nowadays, the field of biomaterials is considered one of the most promising in medical sciences. It is well known that implants could be placed directly or indirectly in contact with natural tissue [13]. In addition, this research field requires a multidisciplinary approach involving knowledge from material sciences, medicine, physics, chemistry, biology, mechanical sciences, and electrochemistry. The importance of this scientific direction was also underlined by the World Health Organization (WHO), which estimated that each year a number of 1.5 million medical devices are used for the well-being of the patients, from which about 10000 pieces of equipment are standardized and approved by the Food and Drug Administration (FDA) or are marked under European laws with an European Certificate (CE).

One of the most commonly used definitions of a biomaterial was provided by Xindong Zhang and David Williams and stated that a biomaterial could be considered as "a material designed to take a form that can direct, through interactions with living systems, the course of any therapeutic or diagnostic procedure" [14].

Biomaterials are designed to sustain or replace certain parts of different organs without causing serious adverse reactions, as presented in the Introduction section. Sometimes natural materials are preferred, but medical practice has shown that synthetic biomaterials can also work better and be well tolerated by the human body. They can be found in gel, solid, or liquid form and used in different pathologies. Some of the common applications of biomaterials are for diagnostic (biosensors or gene arrays), medical devices (surgery instruments, blood bags), therapeutic use (external or internal implants), and emerging regenerative medicines (bone, skin and cartilage regeneration, etc.). Some synthetic or natural biomaterials are used to fix bone defects or fractures, for joint replacement, or artificial ligaments and tendons, or simply as dental implants. Others are dedicated to cardiovascular system improvement, such as heart valves or blood vessel prosthesis, while some of them could replace an entire organ, such as an artificial heart, breast prosthesis, or artificial kidney.

In order to achieve the best therapeutic effect, a biomaterial should have the mechanical and chemical attributes of the natural tissue where it will be used to replace the missing parts, must respect a specific geometric architecture to ensure implant integration and cell adherence and viability, and must integrate certain physiological specifications. One of the most important attributes of biomaterials is biocompatibility. This property is defined as the capacity of a biomaterial to accomplish its function as a part of a medical therapy and to generate an appropriate immunologic response without drawbacks such as toxicity, injury, or rejection. The biomaterial is expected to sustain the normal cell activity, and its final goal consists of being incorporated entirely by the human body in the absence of systemic effects. It cannot replace many functions. Another aspect that is worth mentioning is the host response. In the case of inert materials, the foreign body reaction generates a fibrous capsule, which incorporates the implant, while for the bioactive materials, either permanent or biodegradable, they should sustain the tissue development by releasing metallic ions or other types of regenerative substances. The host response in both cases should be moderate and manageable. Usually, attention is devoted to the mechanical properties of a biomaterial or even an implant, such as tensile strength, compression strength, Young's modulus, yield strength, and hardness, due to the fact that materials undergo under the effect of mechanical forces such as primary strain, stress, shear, or a combination of them. Other important attributes, which are analyzed in

clinical practice and are important for the medical use of a biomaterial, are corrosion resistance, wear, and fatigue properties, because the human body is a very corrosive medium, in which implants behave differently from *in vitro* analyses [15]. When a personalized or large production implant is made, its design and manufacturability are of utmost importance. The design must be adapted to the anatomical place where it will be used, while the manufacturability is defined as the possibility of producing a device at a low cost, to serve the initial purpose of fabrication, and to exhibit high reliability.

Biomaterials used in the medical field should mimic the living tissue function and structure, characterized by a given biochemistry and biomechanics. Depending on the chemical bond, three main groups of materials are encountered in practice: metallic, ceramic, and polymeric biomaterials. Devoted attention will be given in this section to materials used in bone defect treatment for implant manufacture. Completions will be provided in the next section of the state of the art, in relation to biodegradable metallic materials used for temporary implants.

The metals exhibit a crystalline structure and good mechanical properties. However, not all metallic materials are suitable for use in bioengineering due to their high elastic modulus, which can lead to stress shielding that generates a delayed healing of the bone defect. In addition, for metals used in permanent implant manufacture, the metallic ion emission is a supplementary concern, which is strongly linked to metallosis apparition or tissue necrosis. One of the most used in practice biomaterials is Titanium (Ti) and its alloys. They are characterized by increased biocompatibility, high corrosion resistance, and durability. On the other hand, their Young's modulus is high compared to that of the human bone, so porous structures are in most cases preferred. Some studies [16], [17], proved the Ti6Al4V osteointegrative potential by conducting *in vivo* studies on merino sheep, or *in vitro* investigations performed on different cell lines (MC3T3-E1, CC-2538). During this time, surface coatings or modifications were applied to Ti-based implants, increasing their biocompatibility and reducing the grade of foreign body reactions or cytotoxicity. Another biomaterial used in clinical practice is stainless steel (SS). This metal exhibits an increased corrosion resistance in association with high biocompatibility. Being an inert material, its osteoconduction and osteoinduction are limited, and exactly as in the case of Ti-based alloys, coatings, or a combination with other metals are usually required. One of the newest and most expensive metals used in orthopedics is Tantalum (Ta). It is characterized by important durability, high strength, and good biocompatibility. In addition, it was proved in different investigations [18], [19] that tantalum undergoes a self-passivation process by forming a bone-like apatite layer. Sometimes, bone morphogenetic proteins or growth factors could be added to increase the new bone formation when Ta implants are used.

Another class of biomaterials is that of ceramics, which have a similar chemical composition to that of human bone and, in most cases, are characterized by a bioactive behavior. Their main drawback consists of reduced mechanical properties, being very brittle, a fact that hinders their use in load-bearing zones of the human body. A large part of the literature studies was dedicated to hydroxyapatite (HAp) $Ca_{10}(PO_4)_6(OH)_2$, which is a main inorganic component of the human bone. Usually, this biomaterial is used as a nano coating to increase the osteoblast differentiation, but in other cases, despite its reduced mechanical properties personalized scaffolds were manufactured with high biocompatibility, demonstrated by various *in vitro* studies performed on MC3T3-E1 and RAW264-7 cell lines. Another ceramic with outstanding biological properties is the beta-tricalcium phosphate (β-TCP) $Ca_3(PO_4)_2$. Despite its important regenerative effects, some studies [20], [21] reported an important side effect consisting of bone defect delay healing due to a rapid dissolution of the implant or applied coating. In [22] β-TCP scaffolds were developed. When combined with exosomes or had a surface modification, or by adding antibacterial chemical elements such as Ag or copper (Cu),

a very good differentiation of the stem cells towards osteoblasts, combined with a property against pathogens was achieved. Last but not least, the class of bioglasses (BG) is worth mentioning. These materials are characterized by reduced mechanical properties and increased bioactive behavior and are dedicated to manufacturing implants for non-bearing load zones. By prospecting the literature [23] it can be concluded that BGs are characterized by a tunable chemical composition and dissolution rates, being adequate for bone ingrowth and remodeling. In addition, if they are intended to be used in load-bearing zones, reinforcement with metallic ions and a careful control of the pore sizes should be maintained.

The last class of biomaterials met in the present is that of polymeric ones. They have the following advantages: application-adapted mechanical properties, reduced cytotoxicity, increased life duration, and controllable chemical compositions. Unfortunately, in some cases, drawbacks such as foreign body reactions or low degradation rates are reported in clinical practice. Polymers could be classified as synthetic or natural ones. The synthetic class showed good mechanical properties, high prelucrability, adaptable morphology and degradation, and increased biocompatibility [24]. Regarding their natural counterparts, they are considered highly compatible with human tissue, by preserving the water and exceeding outstanding osteoconduction and osteoinduction properties [25].

Some studies also mentioned the use of composite materials and sustained their efficiency [26], [27]. They stated that biocompatibility with the medical image technique, or being lightweight, should always be considered in conjunction with a certain bioactivity, which promotes new bone formation and bone defect treatment. Figure 2 summarizes some of the most commonly used implants, together with the main classes of biomaterials involved in orthopedics.

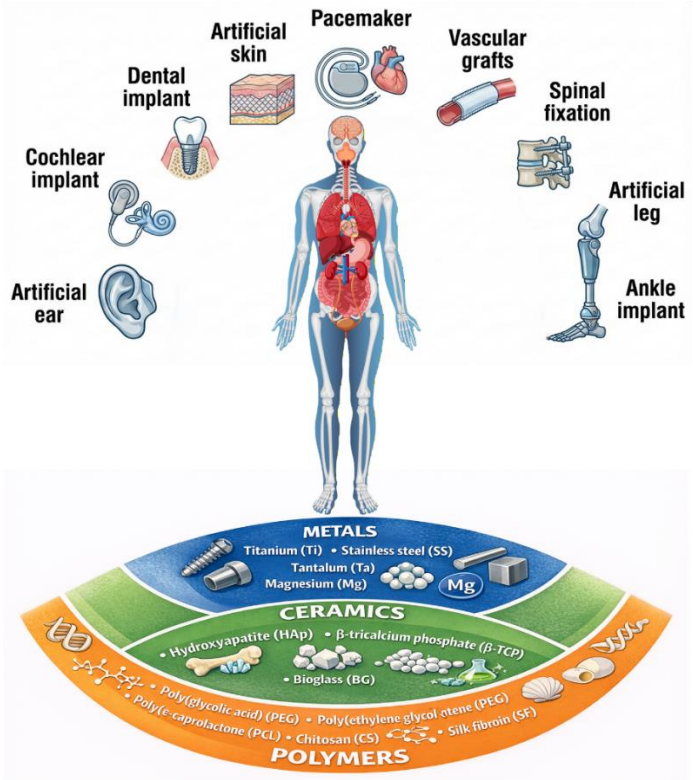


Figure 2. Regular implants used in practice, and the main biomaterials involved in orthopedics.

1.2. Biodegradable metals for specific orthopedic applications

1.2.1. Prerequisite properties necessary for the biodegradable metals

An important research direction is that of biodegradable metals, such as Mg-based alloys, which are the primary topic investigated in the literature, followed by Fe-based and Zn-based alloys [28], [13]. An updated definition of a degradable metal was provided by Liu et al. [29], and it stated that "metals expected to corrode gradually *in vivo*, with an appropriate host response elicited by released corrosion products, which can pass through or be metabolized or assimilated by cells and/or tissue, and then dissolve completely upon fulfilling the mission to assist with tissue healing with no implant residues". This new concept developed the old one provided by Y.F. Zheng in [30]. One can immediately notice that this recent approach is similar to that of "absorbable metals" provided by ASTM-F3160 [29].

In [29], some important criteria for considering a metal as a biodegradable one were provided. For instance, the biodegradability criteria were linked to the alloy corrosion process in physiological media, being entirely described by parameters such as electrode potential, galvanic and reactivity series, Pilling-Bedworth ratio, and Pourbaix diagram. As biodegradable metals are considered the ones that have a standard electrode potential below the zero value of hydrogen. Many metals met this criterion, but magnesium (Mg), iron (Fe), zinc (Zn), calcium (Ca), and strontium (Sr) were considered the most adequate to be used in medical sciences, due to their increased biocompatibility. The galvanic series of biodegradable metals was established based on their corrosion potential. Regarding the reactivity series, it was found that there are metals with a much-reduced reactivity than Mg that react with different acids, such as Ti alloys, which are usually very slowly degradable in the human body. The Pourbaix diagram evidenced the values for the pH and potential, for which the material degrades faster, and also the slow corrosion zones with associated metals [29].

Biocompatibility criteria must be considered when establishing which biodegradable metals should be used in clinical practice. Firstly, the cellular biocompatibility based on 50% inhibitory concentration (IC_{50}) is analyzed, then tissue biocompatibility through lethal dose 50% (LD_{50}) is measured, being linked to the acute cytotoxicity with *in vivo* organisms. Regarding the Mg-based alloys, there are just a few animal models in which long-term chronic toxicity was noticed [31].

To summarize the key biodegradable materials' attributes, a careful analysis of their chemical composition must be undertaken. Of utmost importance is the corrosion type, galvanic or pitting, and the applied local stresses, which facilitate the alloy degradation profile. After that, the biological properties related to the tissue regeneration process, the establishment of organ function, anti-tumor effects, and anti-inflammatory responses should be investigated. In the biodegradable metals domain, good results were obtained for Mg-Zn or Zn-Mg alloys [32], [33] with outstanding mechanical properties. In the case of medical devices, the following physical properties must be considered as supplementary requirements: compatibility with medical images, good wettability, increased surface roughness, well-defined radiopacity, and electrical conductivity for electrical stimulation [29].

1.2.2. Biodegradable metals used in preclinical and clinical studies

Recently, as previously presented, three classes of biodegradable materials were intensively analyzed, including Fe-based, Zn-based, and Mg-based alloys. It is worth mentioning that these materials are used to manufacture temporary implants, which are gradually replaced by human tissue, providing proper mechanical support during the early stages of natural tissue healing. Biodegradable metals offer the advantage of not requiring a second surgery, as with a permanent implant, thereby avoiding the risk of infection.

Iron is considered an interesting alternative in the biodegradable metal domain with respect to the ultimate candidate Mg-based alloys, because it is characterized by the highest ultimate tensile strength of about 550 MPa and good ductility. It is not usually used in orthopedics but is more often involved in cardiovascular stents or thin struts manufacturing. Some *in vivo* and *in vitro* investigations [34], [35] proved that cardiac stents made of pure Fe did not generate important toxic effects when they were implanted into the aorta of a pig animal model or exert cytotoxic effects on different cell lines. However, it was also observed that Fe is characterized by a very reduced corrosion speed, being in some cases similar in side effects to permanent metallic implants. An ideal degradation period was established by Serruys et al. [36], who stated that a time between 12 and 24 months would be considered safe. Anyway, in some cases, iron oxides that are not easily accommodated by the human body can remain inside the tissue for a long time. In order to increase the corrosion speed of iron, alloying with different chemical elements, such as manganese (Mn), is required. Manganese has the property to lower the Fe standard electrode and leads to the formation of a solid solution. In addition, the Fe-Mn alloys exhibit antiferromagnetic properties, making the material more suitable for use in the magnetic resonance imaging domain. Other solutions met in practice consist of achieving a galvanic coupling with noble metals, by generating microgalvanic coupling points similar to chemical batteries that fasten the corrosion of iron.

Just to summarize the main critical challenges associated with iron used as a biodegradable metal, one should notice once again the degradation mismatch linked to a very slow corrosion rate of Fe (0.1 - 0.5 mm/year), MRI incompatibility due to the ferromagnetic character of Fe, if not alloyed with Mn, and last but not least the iron oxide long clearance from the human body.

Another important class of biodegradable metals used in orthopedics is that of Zn-alloys, which have the lowest degradation rate compared to Mg-based and Fe-based alloys. Mutar et al. [37] found that Zn is usually characterized by a tunable degradation rate, with a full alloy dissolution in a time between 6 to 12 months. This period is adequate for human bone tissue regeneration [37]. From the Zn-based alloy degradation processes, it was learnt that their degradation rate exhibits values very close to the latest clinical requirements [38]. In addition, exactly as in the case of Fe-based alloys, no hydrogen emissions are present during the degradation of Zn [39].

The remaining challenges are still related to a creep phenomenon that occurs in the case of Zn, at the human body temperature. Some toxicity levels for Zn^{2+} ions are very important, as [40], [41] showed that concentrations above 100 μM were toxic to endothelial cells. The mechanical properties of alloys such as Zn-Li or Zn-Mg must be carefully controlled, as these materials are very brittle and implants could fracture.

One of the most commonly used in clinical practices for biodegradable implant manufacture is the Mg-based alloys. Antoniac et al. [28], [13], [42], [43] underlined very clearly the attributes of Mg alloys as follows: high biocompatibility, good mechanical properties with a Young's modulus of about 45 GPa, a value considered very close to that of the human bone (15÷25 GPa), and last but not least a mass density between 1.74 and 1.84 g/cm^3, again very close to the human bone density (1.8÷2.1 g/cm^3). In addition, Mg^{2+} ions activate about 300 enzymes in the human body and important signaling pathways that promote osteoblast differentiation and migration concomitantly with osteoclast activity inhibition. These biological processes were already extensively described by V. Manescu (Paltanea) et al. [4] in the first doctoral paper.

Nowadays, there are Mg-based implants that received approval to be used on humans. For example, Mg-Y-RE-Zr (MAGNEZIX®) alloy from Syntellix AG (Germany) received CE marking in 2023 [13]. In Asia, the Mg-Ca-Zn (RESOMET™) alloy was used in South Korea for hand fracture treatment, obtaining the Korea Food and Drug Administration (KFDA)

approval in 2015 [13] and pure Mg was involved in human clinical trials in China to treat the osteonecrosis of the femoral head with excellent outcomes.

The main alloying elements that are introduced in the chemical composition of Mg-based alloys are: Zn, Ca, Mn, rare earths (RE), and Yttrium (Y). Regarding the new trend observed worldwide, the corrosion process is no longer controlled by alloying magnesium with rare earths such as Neodymium and Yttrium due to toxicity reasons. A shift towards Mg-Zn and Mg-Ca with reduced corrosion rate, as presented by V. Manescu (Paltanea) et al. [7].

The main critical challenges are associated with the H_2 emission that occurs during the magnesium corrosion process, delaying the defect healing, generating pain, or even tissue necrosis. Another aspect that must be considered consists of the pitting corrosion, which in some cases is characterized by an accelerated dissolution of the alloy in certain points that could initiate a crack formation, followed by implant fracture. The alkaline microenvironment, which occurs during the corrosion of Mg-based alloys, must be addressed because high pH spikes are linked to cell damage at the implant insertion site [44].

In the next sections of the Introduction chapter, attention will be devoted exclusively to Mg-based alloys, because these biodegradable materials are considered today as one of the best candidates for temporary implant manufacturing when regenerative properties and bioactive character of the material is regarded as key features.

Figure 3 presents the primary biodegradable metals used for implant manufacture, along with the main criteria for inclusion in the biodegradable class and the most important properties.

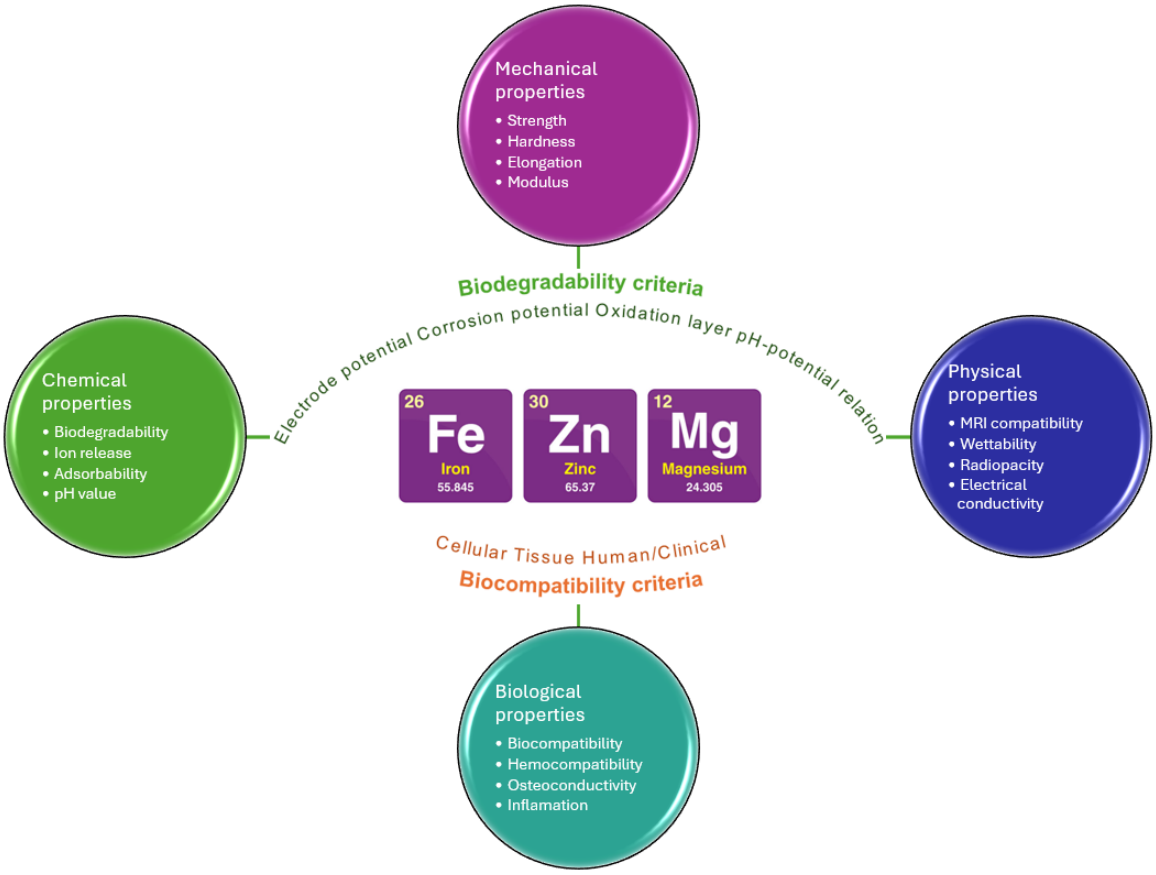


Figure 3. Main biodegradable metal attributes met for clinical implementation.

1.3. Manufacturing methods for obtaining biodegradable structures from magnesium-based alloys for bone defect treatment

1.3.1. Classic manufacturing methods

In this section of the PhD thesis, one of the most commonly used methods for producing Mg-based implants will be briefly described, as exhaustively presented by V. Manescu (Paltanea) et al. [4].

The first technology found in the literature is based on the titanium space holder method. The main steps of this technology consist of developing a 3D Ti wire structure using different approaches, then inserting this metallic geometry into liquid Mg-based. After that, the metallic hybrid is immersed in hydrofluoric acid, which initiates the dissolution of the Ti wire. As a direct consequence, an implant made of Mg-based alloys is achieved, exhibiting a protective conversion coating of MgF_2 and a channel structure where Ti wires previously existed. The channeled implants proved beneficial for rapid osteointegration in various types of bone defects, as presented in [6]. Some studies [45], [46] described a modified version of this technology by replacing the Ti wires with other materials.

Another method used is hydrogen injection technology. It is based on the molten Mg that is poured under advanced vacuum conditions into a dedicated recipient. Then, high-pressure hydrogen is injected into the recipient, while the Mg melt is heated [47]. By reaching the hydrogen saturation state and under the effect of a water-cooled mold, the metallic melt is upward solidified. As a result of this method, straight pores occurred.

A classical technology, used a long time ago, is powder metallurgy. It is described as mixing Mg-based powders with space holder materials. Then two heating stages are applied to obtain firstly the space holder burning and elimination, and then to achieve a sinterization process of Mg-based green products. In this way, porous implants could be developed [48].

Laser perforation is a simple method that is composed of a programmable laser machine that acts directly on Mg pieces, by generating pores into the bulk material [49].

Other technologies that could lead to perfect pore size and shape control are laser-based additive manufacturing technologies [50]. It could be noticed that these methods represent adequate technologies when complicated geometrical shapes are needed, although it is an expensive technology.

The last classical method found in the literature is the indirect additive manufacturing method [51]. It is based on generating a 3D CAD model, then printing a positive template and infiltrating it with NaCl paste. Then, high-temperature treatments are applied to remove the template and increase the density of NaCl. The last step of this method consists of casting liquid Mg into the achieved pattern and finally of dissolving the NaCl, resulting in a porous implant as previously designed.

As a conclusion, there are various methods described in the literature, which lead to patient-adapted geometries. However, ones of the methods are expensive, manifesting the necessity of complex equipment with vacuum or inert working conditions and leading to precise geometries and porosity values for the implants, while others are much simpler and do not require high investments, but they are leading to imprecise geometries, which are characterized by the application of supplementary processing steps.

1.3.2. Innovative workflow proposed

To summarize, the medical images of the affected area are acquired using magnetic resonance imaging (MRI) or computed tomography (CT) techniques. Two-dimensional images representing the intensity map of grey between white and black colors are obtained. They are in the digital imaging and communications in medicine (DICOM) format, from which, based

on different segmentation procedures, 3D images of the defect, organs, or even tumors can be constructed. After that, the models could be easily transferred to finite element analysis (FEA) software or converted to a standard triangular language or stereolithography (STL) file. Using a slicer program, the STL model becomes a G-code, which is usually used by dedicated 3D printer devices.

Regarding the implant design, the unit cell is selected and it is then multiplied by various spatial orientations as required by the medical application. In almost all cases, a porosity greater than 50% is necessary in bone defect treatment.

Mechanical FEA and computational fluid dynamics (CFD) investigations should be conducted to determine whether the designed implant has adequate mechanical properties and is biologically compatible with blood, other body fluids, or living tissue.

Figure 4 presents a flow chart, which emphasizes the main steps used in the production of a personalized implant, starting with medical image acquisition and DICOM image analysis, followed by CAD design development, FEA and CFD analysis, and results interpretation, manufacturing of the implant based on different methods, and last but not least, post-processing supplementary steps in conjunction with material and biological investigations of the implant.

By analyzing all the methods described in Section 1.3 it was decided to adopt an innovative method of implant production, which was extensively described in the fourth doctoral paper and the Introduction section of the PhD thesis. Additionally, material and *in vitro* cytotoxicity tests were performed as suggested in Figure 4 to prove the suitability of the developed implant to be used in pre-clinical analyses.

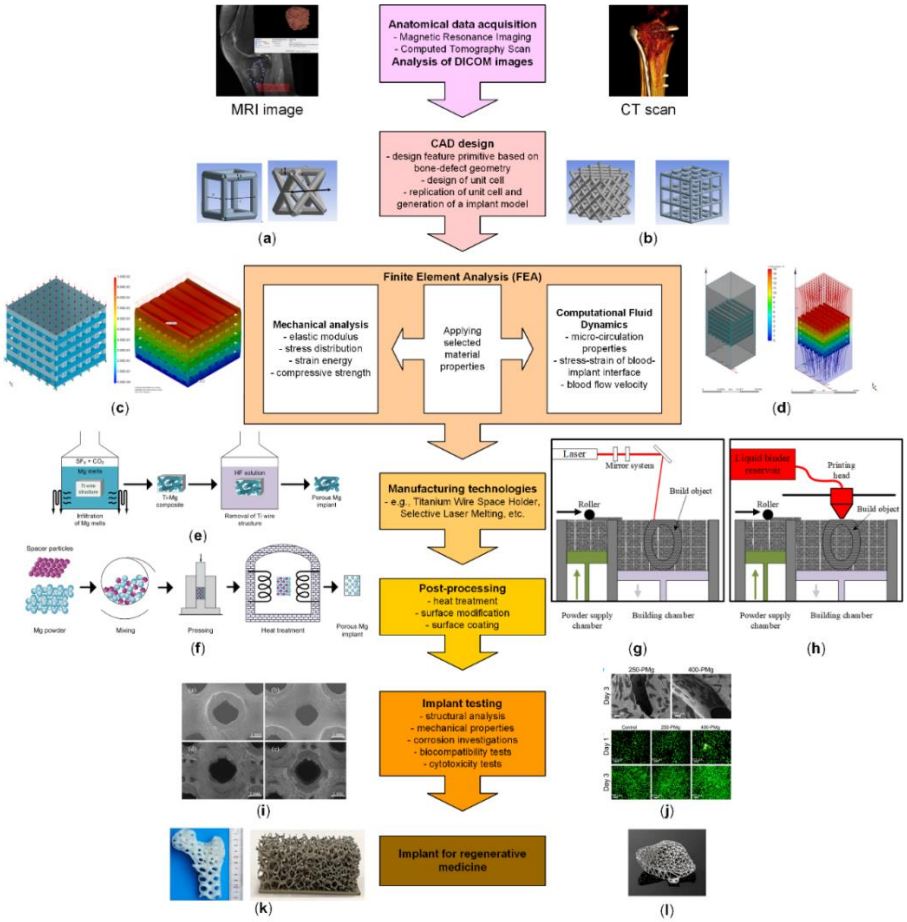


Figure 4. Flow-chart of personalized implant manufacturing main steps. (a) implant cells, (b) implant geometry, (c) mechanical FEA, (d) CFD test, (e) titanium wire space holder technology, (f) hydrogen injection method, (g) SLM, (h) binder jetting, (i) SEM images, (j) biological investigations, (k), (l) implants. Image was adapted from V. Manescu et al. [4] and V. Manescu et al. [8].

1.4. Numerical modelling of an innovative Mg-based structure for the treatment of bone defects

Nowadays, mechanical FEA is used to estimate the Young's modulus and other mechanical properties of designed Mg implants to determine whether they can be used in bone defect treatment. On the other hand, the CFD Analysis is important in computing the implant wall shear stress and permeability, two physical quantities that are directly linked to cell viability, multiplication, and adhesion. According to the literature, the implant porosity must be between 25% and 90%, while the pore size should be comprised between 10 μm and 1000 μm [52]. However, if porosity is too high, lower mechanical properties are expected, and as a direct consequence, the implant may not be suitable for load-bearing applications. Besides porosity, other important properties are the mechanical ones. Young's modulus should not exceed 20 GPa [53]. To make it easier, the FEA test, the elastic region of the stress-strain curve is considered, the applied load is constant, and the designed implant models are placed between two rigid bodies for compression simulation [8].

Improved fluid transport properties and good wettability must characterize an ideally designed implant. The interactions, which occur at the boundary between implant and fluid are analyzed based on wall shear stress (WSS) that proved to have a positive impact on cell viability when it was kept below 30 mPa [54]. Another parameter that must be investigated is the implant permeability, which is usually computed under the assumption of Newtonian fluid behavior [55] incompressible fluids [56], rigid implants [57], and the entire system satisfying the Navier-Stokes equation [9]. Based on [9], the implant permeability is computed with the Darcy relationship, while the viability of this calculus is checked with the help of the Reynolds number that must be lower than 8.6 as stated in [58].

In the third doctoral paper, a detailed numerical analysis was performed to choose the most adequate design that will be practically used to manufacture the channeled Mg-based implants via a new and innovative method. Figure 5 presents an example of the results regarding mechanical and computational fluid dynamics' analyses obtained in the third doctoral study.

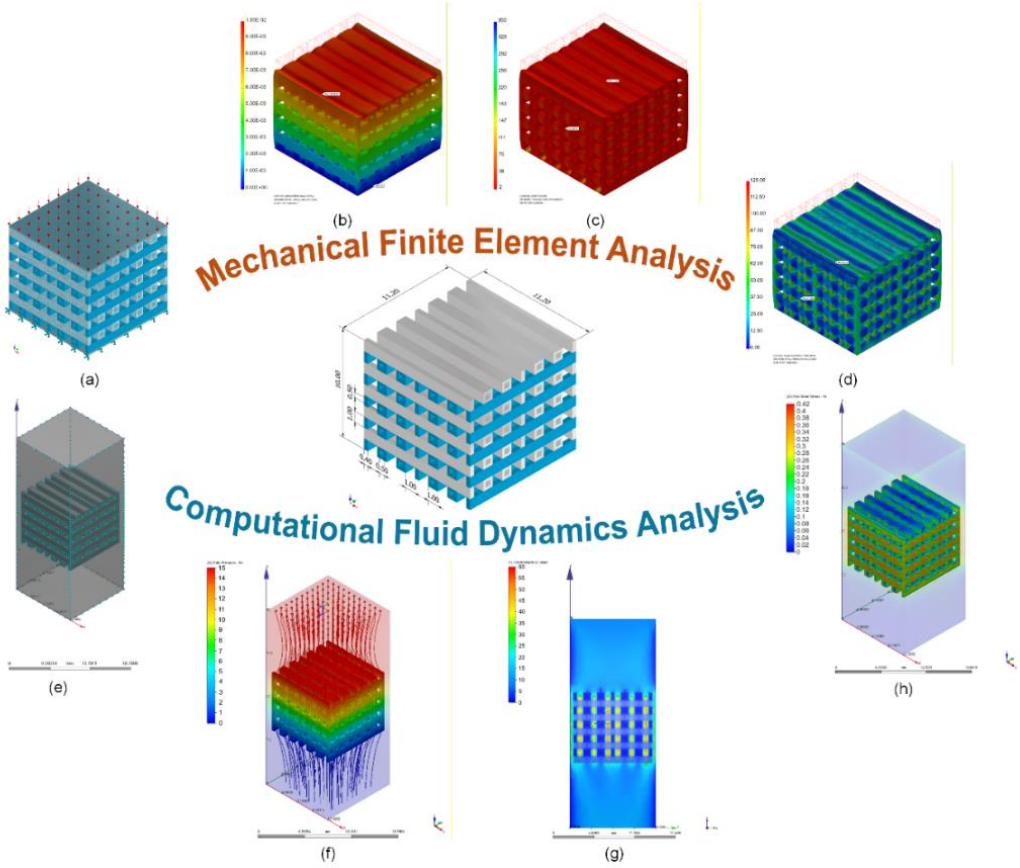


Figure 5. Results of mechanical FEA: (a) boundary conditions, (b) displacement map, (c) safety factor map, (d) von Mises stress map. Results of CFD simulations: (e) volume elements mesh, (f) pressure drop, (g) fluid flow velocity, (h) wall shear stress. Image was adapted from V. Manescu et al. [8].

1.5. Biological mechanisms at the interface between Mg-based alloys and bone

Mg-based channeled implants are known to have a beneficial effect on bone defect regeneration through dedicated signaling pathways, diverse cell-type actions, and key regulators. The biological properties, *in vitro* cytotoxicity, and *in vivo* analysis of two of the most commonly used alloys in practice, Mg-Nd and Mg-Zn, were investigated in the first, second, and fourth doctoral papers, respectively.

Human bone can be defined as an open-porous composite structure made of osteogenic cells, proteins, hydroxyapatite, and an intricate blood vessel system [59]. The main osteogenic cells are the osteoblasts that help in the new bone construction and are derived in some special conditions from the mesenchymal stem cells (MSCs), osteoclasts, and osteocytes, with an important action mechanism on the bone resorption process. These last-mentioned cells are obtained from hematopoietic stem cells (HSCs) [60]. The first stage in the bone healing process is the inflammatory response of the body, based on immune system cells. After that, in the repair stage, vascular precursors and fibroblasts help with bone healing, and the defect environment permits the formation of a neo-vascularization network, leading to a callus. In the last stage of the bone repair process, under the action of osteogenic cells, the callus structure changes into a mature lamellar bone.

Regarding the host immune system modulation, one of the important findings consists of Mg^{2+} ions' effect on macrophage modulation from a pro-inflammatory state (M1) to an anti-inflammatory state (M2) at the bone defect site [61]. It was noticed that in the first 3 days, it is necessary for the macrophage to be in the M1 state to clear the debris, and in the following 4

days, the PI3K/Akt signaling pathway is activated and the macrophage shifts to the M2 state, releasing growth factors necessary for new bone and blood vessel formation [4], [61]. An interesting study performed by Zhi et al. [62] proved that Mg^{2+} ions stimulate the release of the so-called calcitonin gene-related peptide (CGRP). The scholars [63], [64] found that a good bone healing process should always be associated with an increase in plasmatic concentration of IL-10 that had an anti-inflammatory effect in conjunction with the decrease of FG23.

By reviewing the literature [65], [66], it was found that Mg^{2+} ions that act as secondary messengers for endothelial cells (ECs) are characterized by nitric oxide (NO) production, known for an important vasodilation effect, and to upregulate the expression of vascular endothelial growth factor (VEGF). The VEGF/VEGFR2 signaling axis drives dormant blood vessel cells to form a neovascular network at the defect site, enabling the flow of oxygen and nutrients, as well as waste elimination. Some recently published studies [67], [66] proved that ECs are of two categories as it follows: tip cells, which investigate the medium with the help of filopodia, and their differentiation from ECs is mediated by Mg^{2+} ions via the Notch1 signaling pathway, and stalk cells that proliferate and generate new blood vessels. Another outstanding aspect is that Mg-based alloys exhibit the property to simulate the conditions of a low oxygen environment, stabilizing in this way the HIF-1α [68]. The M2-state macrophage enhances the transforming growth factor-beta (TGF-β) and basic fibroblast growth factor (FGF-2) to stabilize the newly formed blood vessels [69].

As stated before, during the Mg degradation, Mg^{2+} ions are released, behaving as secondary key factors that stimulate the osteoblast production from MSCs [70]. Transport mechanism is ensured through channel-kinase transient receptor potential melastatin 7 (TRPM7) and selective transporter magnesium transporter 1 (MAGT1), which provide the insertion of Mg^{2+} ions into cells [71]. Then, intracellular signaling phenomena after the entrance of Mg^{2+} ions, such as the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway, which determines good cell proliferation and viability [72]; Wnt/β-catenin pathway linked to nuclear translocation process of β-catenin, which switches "on" the osteogenic genes [73]; and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase (ERK1/2 (MAPK)) pathway that triggers the well-known osteogenic markers osterix and runt-related transcription factor 2 (RUNX2) [74], occur. The genetic upregulation is characterized by measuring the alkaline phosphatase (ALP), osteocalcin (OCN), and type I collagen [75].

From the biological point of view, one can immediately notice the outstanding effect provided by Mg-based alloys on bone regeneration and their great potential in bone defect treatment. To shortly summarize, by ensuring the basis of angiogenesis and osteogenesis, new bone formation could start and be finished by obtaining a high-quality mineralized new bone as presented in the second doctoral paper. However, as mentioned before, attention should be devoted to limiting the hydrogen emissions, to reducing the corrosion speed, and to preventing the hypermagnesemia effect, which occurs at local Mg^{2+} ion concentrations higher than 10 mM [76].

Nowadays, channeled Mg-based implants are investigated based on adequate testing protocols because, in their case, the methods used for bulk material do not match, since these types of medical devices exhibit an increased surface area, which is flooded by body fluids. It was noticed in many research studies [43] [77] that there was a difference between the results obtained in the laboratory and in the living tissue.

Firstly, the so-called immersion test could not entirely sustain the real *in vivo* behavior, due to the fact that H_2 gas could be trapped inside the inner channels, and a direct consequence is a diminished corrosion process in association with a high pH that could decrease the cell viability [78]. Some studies [79], [80] suggested that micro-perfusion bioreactors should be involved.

Regarding the *in vivo* analyses, some ethical issues must be considered because animal models are still the only way that exactly mimics the degradation profile of the implant and the ability of blood vessels and bone cells to get deep into the center of the implants [7]. Almost always, the number of involved animals must be minimal, and in some cases, where it is considered unnecessary, Sham groups or negative control should be avoided. The channel diameter has to be carefully chosen due to the fact that bone grows from the periphery inward, and an unwanted effect of a hollow, weak center could be present [81].

Considering all the aspects mentioned in Chapter 1 of the PhD thesis, a channeled implant model was developed and investigated as a suitable treatment for bone defect treatment. A detailed analysis of the theoretical and experimental program is provided in Chapter 2 of the PhD thesis, which includes the published version of the four doctoral papers.

Chapter 2 – *In extenso* published scientific papers related to the PhD thesis scientific outputs

The PhD thesis objectives were developed based on four extensive scientific papers, as follows: a comprehensive review and three scientific research papers. All of these studies were published in journals ranked in the Web of Science database (WOS). The Q1 or Q2 classification of the respective journals endorses the novelty and innovative character of the research. A cumulative Impact Factor (IF) of 16.20 was reached.

1. **V. Manescu (Paltanea)**, I. Antoniac, A. Antoniac, D. Laptoiu, G. Paltanea, R. Ciocoiu, I.V. Nemoianu, L.G. Gruionu, H. Dura, *Bone Regeneration Induced by Patient-Adapted Mg Alloy-Based Scaffolds for Bone Defects: Present and Future Perspectives*, **Biomimetics**, vol. 8, iss. 8, 618, 43 pag., 2023, doi: 10.3390/biomimetics8080618, eISSN 2313-7673, WOS: 001131224100001, IDS Number DH8F8, **IF 3.9 (Q1)**

2. **V. Manescu (Paltanea)**, A. Antoniac, M.C. Moraru, I. Antoniac, C.M. Cotrut, S. Gradinaru, A.I. Dreanca, B. Sevastre, R. Pop, F.A. Tabaran, G.M. Vlasceanu, M. Ionita, M. Manole, *Suitability of Mg-Nd and Mg-Zn Alloys to Obtain Biodegradable Structures for Bone Defects*, **J. Funct. Biomaterials**, vol. 16, issue 11, 423, 32 pag., 2025, DOI 10.3390/jfb16110423, WOS: 001623847400001, eISSN 2079-4983, IDS Number F7439, **IF 5.2 (Q1)**

3. **V. Manescu (Paltanea)**, G. Paltanea, A. Antoniac, L.G. Gruionu, A. Robu, M. Vasilescu, S.A. Laptoiu, A.I. Bita, G.M. Popa, A.L. Cocosila, V. Silviu, A. Porumb, *Mechanical and Computational Fluid Dynamic Models for Magnesium-Based Implants*, **Materials**, vol. 17, iss. 4, 830, 2024, eISSN 1996-1944, doi: 10.3390/ma17040830, WOS: 001172420600001, IDS Number JI0P0, **IF 3.2 (Q2)**

4. **V. Manescu (Paltanea)**, A. Antoniac, G. Paltanea, I. Antoniac, E. Páll, M.C. Moraru, A.I. Dreanca, B. Sevastre, R. Stefanoiu, R. Ciocoiu, S. Gradinaru, J.V. Rau, M. Manole, *Biofunctional Testing of a Degradable Implant Made by Mg-Nd and Mg-Zn Alloys Used for Bone Defects,* **Biomimetics**, vol. 11, iss. 3, 169, 29 pag., 2026, doi: 10.3390/biomimetics11030169, eISSN 2313-7673, WOS: - , IDS Number -, **IF 3.9 (Q1)**

Chapter 3 – PhD thesis conclusions

As stated already in the Introduction section, the bone defect treatment based on different types of biomaterials is a vital domain for investigations due to the increased number of clinical cases, as well as the limited number of autografts, allografts, or xenografts.

Biodegradable metals represent the future for bone defect treatment compared to their counterparts, traditional permanent implants such as Ti and its alloys, and stainless steel. For iron, zinc, or magnesium-based alloys, the imminent necessity of a second surgery is prohibited by gradual implant degradation. This phenomenon occurs simultaneously with the new bone formation, being entirely replaced in a certain amount of time with the host's own hard tissue. The class of biodegradable metals is considered today a biological stimulant, exhibiting improved bioactivity due to the emission of ions or biocompatible by-products, which behave as localized "drugs" and help natural tissue regeneration. Within this important biomaterial class, one is considered outstanding in science: Mg-based alloys. These materials are characterized by a Young's modulus with values similar to those of the human bone, a mass density almost identical to that of the hard tissue, and last but not least, their adequate fracture toughness that permits Mg-based alloys to withstand the snapping forces and twisting that occur in the case of a normal gait. This last-mentioned property is essential for bone fixation. Other advanced features selected from the recent scientific papers underlined the fact that rare-earth-free systems, such as Mg-Zn, exhibit increased corrosion resistance and eliminate the previous concerns in relation to Nd and Y toxicity and the antibacterial alkalinization of the medium placed in the implant vicinity that significantly reduces the risk of post-surgery infections.

Taking into account all the presented aspects, the PhD thesis experimental design was gradually followed in accordance with the plan presented in Figure 1. From the **first doctoral paper**, which offers a comprehensive roadmap for Mg-based alloys orthopedic implants, one should conclude that a shift from "passive" to "active" implants is necessary to reduce the health insurance burden and the patient hospitalization time in orthopedics. As stated before, the so-called biological "triple synchronized processes" (osteogenesis, angiogenesis, modulation of the host immune response) are met by Mg-based alloys. In addition, there is a continuous need for successful approaches to sustain the present necessity of patient-adapted geometry, by considering an interconnected channel system, as well as an anatomical match with the patient's bone defect. A significant shift in implant production strategy was identified by presenting different techniques in association with their advantages and drawbacks. It was found that the additive manufacturing technologies led to biomimetic, complex, and highly biocompatible implants, but these methods require expensive and challenging equipment. On the other hand, other technologies that are much easier and environmentally friendly are presented, in order to develop a new innovative route in the PhD thesis dedicated to Mg-based implants for osteointegration. Another important finding in this review paper is that today, artificial intelligence (AI) is no longer an optional criterion, being essential for predictive models that present the implant degradation together with new bone formation and should be undoubtedly incorporated into medium or large quantity production of implants. However, the main general conclusion was the necessity of developing a new innovative route for channeled Mg-based implants. The paper was considered as a link between material sciences and orthopedics, highlighting the fact that the future of bone defect treatments lies in personalized, biodegradable structures.

In the **second doctoral paper**, a critical comparison between a rare-earth-containing and a rare-earth-free Mg alloy, Mg-Nd and Mg-Zn, respectively, is presented. The main goal of this published work was to determine the biomaterials' clinical viability in bone defect treatment. The Mg-Nd alloy exhibited superior mechanical behavior, while the Mg-Zn binary

system showed good stability, evidenced by the reduced corrosion rate determined based on electrochemical analysis. Another general finding consists of the validation of *in vivo* biocompatibility for both Mg alloys. No important foreign body reactions were reported, accompanied by reduced toxicity of Nd and Zn effects. It was concluded that both alloys are suitable for regenerative use in medicine. The main conclusion of this study was that both binary systems exhibited good properties and are viable candidates in bone defect treatment for future implant design.

From the **third doctoral paper**, one can conclude that this study moves beyond simple material selection, highlighting that internal structure and geometry are also key factors in achieving successful bone healing. The goals followed in these analyses are the obtaining of an implant with a similar Young's modulus as the human bone, in conjunction with a permeability located in the biological window, as well as the fact that its channels offer the possibility for nutrients and oxygen transport and removal of the metabolic waste. It was concluded that the developed implant geometries are characterized by a uniform distribution of von Mises stresses, and the safety factors confirmed that the structural integrity will be maintained even during the Mg alloy gradual degradation. The main goal of this paper was to choose the best candidate for an implant geometry, which will be further implemented via an innovative manufacturing route based on polymeric matrix 3D printing, Ti wire weaving procedure, elimination of additional helping structures (polymer and Ti wires) and obtaining of the final Mg-based implants, as will be presented in the fourth doctoral paper.

The geometrical architecture developed in the third doctoral paper was practically manufactured based on a new proposed production route, which does not need a magnesium powder 3D printing device (**fourth doctoral paper**). The model was 3D printed in PLA polymer. After that, Ti6Al4V medical-grade wires were woven into the structure's pores, and the polymeric reinforcement matrix was dissolved using a dedicated solvent. The Ti6Al4V woven geometry was introduced together with Mg-Nd and Mg-Zn pieces in an induction oven, and a metallic hybrid was achieved. Lastly, the samples were inserted into hydrofluoric acid, and the Ti6Al4V wires were eliminated, generating intricate channeled implants. To investigate and understand the results correctly, reference samples were prepared from bulk cast alloys and compared with implant samples. The surface properties were analyzed based on a combined strategy between wettability and roughness. Compression mechanical analysis and immersion tests in SBF proved the superiority of the implant samples. Regarding the surface properties based on X-ray diffraction tests, a protective conversion coating consisting of MgF_2 was put in evidence. This fact increases the roughness parameters and leads to highly hydrophilic behavior. *In vitro* cytotoxicity tests were conducted to support the research, using a human patella-derived osteoblastic cell line. The main conclusion of this last study was that both implant samples exhibited adequate properties to be used as a possible candidate for pre-clinical trials.

In the near future, it is expected that the developed manufacturing route will be used in implant manufacturing in an environmentally friendly way, with no need for expensive equipment. The method and implant geometries could be adapted in a direct relationship with patient requirements, and the biomaterials could be accordingly chosen.

Chapter 4 – Original contributions

The scientific investigation described in this PhD thesis has various original contributions to the field of biomaterials science. The final goal was the design, manufacturing, and testing of a biodegradable structure with special geometry featuring channels, dedicated to being the support for a multimaterial system used for a specific orthopedic application, respectively for the treatment of bone defects. The proposed technology is a completely new and innovative one that was never used in practice until now.

Firstly, a theoretical study containing more than 200 scientific references was conducted. The most important contribution is providing a synthesis that starts with patients' digital information and ends with biological integration of the orthopedic implant. Firstly, the microenvironment placed in the vicinity of the bone defect could be programmed based on the immune system modulation, angiogenesis induction, and direct osteogenesis process. Another original core contribution is the personalized morphogenesis approach that could be defined as patient-adapted geometry implants. The well-known rationale "one-size-fits-all" is no longer valid nowadays, so a design derived from CT/MRI medical images of a patient that perfectly matches from a mechanical point of view the bone anisotropies should be followed. Last but not least, the digital twin and AI integration is a unique scientific contribution that shows that the importance of machine learning algorithms, and AI is a critical part of biomaterials science.

Another main scientific contribution of the PhD thesis is represented by the comparative clinical validation of two important binary Mg-based alloys to clearly find the specific use-place in orthopedics. It was concluded that the high-load candidate material for implant manufacture is the Mg-Nd alloy, which could be applied for bone defects requiring an improved mechanical strength, while the high-stability candidate is the Mg-Zn alloy, which could be involved in non-load bearing implants dedicated to applications in which a long-time presence of the healing properties of the implant is a necessity. Another core contribution is linked to the *in vivo* validation of the two proposed materials exhibiting a given chemical composition, which proved that Nd and Zn could be safely used in bone defect alleviation. A possible clinical translation is foreseen for the proposed alloys. Defining a strategy that clearly explains which alloy should be chosen for each patient in relation to the necessary healing window is another core contribution of the thesis.

Description and quantitative validation of a new weave-inspired 3D channel architecture is also a main scientific contribution to the field. This multi-physics proof of concept demonstrates that the woven-derived structures can be similar to the human bone trabecular anatomy and mimics well the biological transport properties. This "titanium wires-inspired" method is characterized by a scientific breakthrough: it adapts a traditional method (classical weaving procedure) to the additive manufacturing of 3D implant shapes, with no need for an expensive 3D printer or inert working conditions. Another core scientific contribution consists of the multi-physics validation framework (CAD-FEA-CFD), which shows the importance of digital validation as a first step in the implant manufacturing domain. Based on this approach, it was foreseen that no stress shielding effect could occur in addition to biological matching, as proved by implant permeability computation based on Darcy's Law and wall shear stress numerical estimation. The scientific impact of this analysis consists of a proposed standardization of an analysis, which touches three directions: mechanically matching, fluidically matching, and the possible application of a new innovative manufacturing route.

As stated before, the main scientific contribution of the PhD thesis is the development of a new manufacturing route inspired by the Ti wire weaving and the Ti wire space holder methods. Based on this proposed and practically applied technology, 3D channeled implants for bone defect treatment were developed. After the theoretical study, Mg alloy material and

biocompatibility tests, and CAD-FEA-CFD validation of the best candidate numerical model were conducted. The use of a polymeric matrix in combination with Ti wire weaving represents an important original contribution by considering its high novelty grade. Then, important core scientific contributions could be attributed to the fact that the implant produced exhibited adequate mechanical and surface properties, as well as good cytotoxicity. The research conducted in the PhD thesis could be further used to manufacture dedicated bone implants that could enhance the anatomical shape of the patient's defect.

An important original contribution is the practical implementation of a new manufacturing route combining 3D wire weaving with casting-infiltration procedures. This manufacturing method provides a safer, non-pyrophoric, and scalable alternative. It eliminates the "staircase effect" of 3D printing, providing smooth, cylindrical internal channels that can be modelled as laminar flow zones. Another original contribution is represented by the biomimetic mechanical balancing of the implant structure to achieve a Young's modulus and a compressive strength, whose values are very close to those of the human bone. In addition, proving that the HF conversion coating does more than just stop corrosion, it "bio-functionalizes" the surface by simultaneously increasing surface free energy and creating specific R_q-driven nanoniches of about 0.2 μm is an important contribution to the field. Last but not least, the *in vitro* studies on human patella-derived osteoblasts proved that Mg-Nd extracts not only are highly biocompatible with the cells but also actively increase osteoblast viability by up to 25% and reduce necrosis by nearly half.

The innovative character and novelty of the proposed manufacturing route are also highlighted in a national patent application (A/00592/04.12.2025) on December 2025: "*Metodă de elaborare a unui implant poros din aliaj de magneziu cu acoperire bioactivă*" by Vasile-Iulian Antoniac, Veronica Mănescu (Păltânea), Augustin Semenescu, Mihnea-Cosmin Costoiu, Ileana-Mariana Mateș, Ioan-Ștefan Voicu, Aurora Antoniac.

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