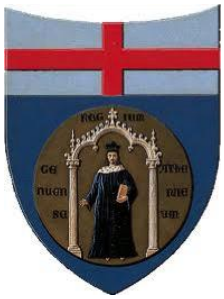

UNIVERSITY OF GENOA

MASTER'S PROGRAM IN BIOENGINEERING

Thesis submitted in partial fulfillment of the requirements for the title of

Master of Bioengineering

Design and preparation with 3D printing of implantable customized maxillofacial devices, in polymeric (PLA and PCL) and ceramic (hydroxyapatite and TCP) composite

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Abstract

Bone regeneration is a complex physiological process that involves a series of biological events which lead to the formation of new bone. Normally, this process occurs naturally, but in certain cases, the regeneration is impaired thus an artificial aid is necessary. Guided bone regeneration (GBR) is the commonly used technique, which involves the use of membranes for guiding osteocytes and osteblasts to form new bone. Different membranes can be used, from impermeable to permeable, synthetic natural. The current gold standard provides for the use of titanium meshes as supports for new bone formation. Unfortunately, this type of meshes must be removed when the process is completed, so new biodegradable materials have been studied to overcome this problem. Composite materials consisting of polymeric matrix and bioceramic reinforcement were the subject of this thesis work, mainly focused on the experimental verification of their mechanical properties.

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1. Introduction

The bone tissue is a biological tissue which stands out for its stiffness and flexibility. It is a particular type of connective tissue consisting of cells (osteocytes) dispersed in abundant calcified extracellular matrix (i.e. an extracellular matrix also constituted by minerals). The bone tissue then forms the bones, which are the building blocks of the vertebrate skeleton. The bone is the classic composite of natural origin. It is a strong system characterized by three main layers: *cortical bone*, *periosteum* and *cancellous bone*. Periosteum is the outermost connective membrane, responsible for bone development and the birth of calleus in case of fractures. Cortical bone (or compact bone), located under the periosteum, is an external dense layer which confers stiffness to the bone. Cancellous bone (or trabecular bone), located immediately under the cortical bone, is an internal layer full of empty spaces able of dissipating energy to cushion stresses generated during movement. In terms of composition, bone is constituted by a mineral part (about 60-70%) consisting of hydroxyapatite crystals, calcium carbonate and magnesium phosphate; and an organic part consisting of collagen, extracellular matrix and proteins. From mechanical point of view, the natural bone has an elastic behaviour for small deformations that became plastic for large deformations, so overall it is a stiff and ductile material. When a fracture occurs, the bone is able to regenerate itself, but in some cases (i.e. when the defect is large) the regeneration is impaired. In this thesis work the regeneration process in dentistry has been considered. Dental implantation is an effective solution to restored edentulous maxillae. However, an implant to be correctly placed and to successfully lead functional loads, must have an adequate alveolar bone volume, both horizontal and vertical. This is the most important prerequisite for a predictable, long-term prognosis in implant dentistry. Although the integrity of the jawbone is simply preserved doing everyday activities, like eating and chewing [1], tooth loss caused by pathological situations or traumas may results in alveolar bone resorption [2]. It must also be taken into consideration that dehiscence or fenestration defects may occur after the surgery and this frequently jeopardizes the successful outcome of an ideal implant placement [2]. In order to prevent such complications and to achieve an adequate position of the dental implants, several bone augmentation strategies have been developed with the purpose of promoting new bone's growth [3]. Some of these techniques include:

- Alveolar distraction osteogenesis
- Block bone graft
- Osteoinduction
- Guided bone regeneration (GBR)

This last is a procedure that, using an appropriate resorbable or non-resorbable barrier membrane, provides space and protection to the bone grafting material and the surrounding connective tissue, triggering an immune response that allows the formation of the new bone [4,5]. Nowadays the GBR is the standard clinical procedure used to increase the alveolar bone volume since it's reported as one of the most predictable and reliable methods and provides long-term stability to the newly augmented site.

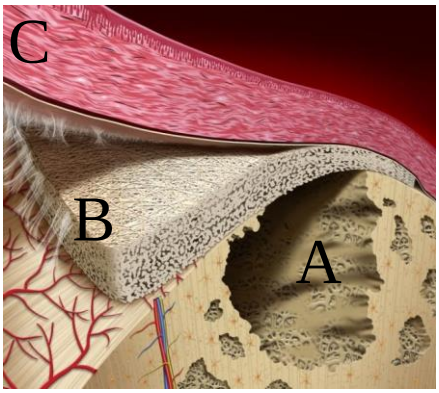

Fig.1. *Schematic illustration of the principle of guided bone regeneration (GBR). In this figure the bone defect (A) of the alveolar bone is covered by a porous barrier membrane (B). The membrane function is in fact to isolate the bone defect in order to enhance bone regeneration preventing soft tissues (C) infiltration [1].*

According to numerous studies and clinical trials, membranes' properties, materials and geometries are crucial to determinate the outcome of the procedure. Indeed, depending on the dimension of the bone defect and the quality of the remaining bone, one kind of barrier membrane might be chosen rather than another, especially considering the risk of collapse into the existent bone defect [6]. Beside the use of resorbable materials (ie. collagen or pericardium membranes) when the bone defect shows a complete horizontal loss or combined with a vertical atrophy, different techniques may be used, in order to maintain the space for the new cellular bone growth, such as Titanium meshes [7]. It has been demonstrated that the total achieved volume after the use of a titanium mesh is

comparable with all the other techniques [8] but with a statistically significant lower number of infections, especially in case of early or delayed exposure during the healing phase. Therefore, many clinicians addressed their practice toward this material rather than others with higher risks. One of the main problems related to conventional Ti mesh is that surgical insertion is time consuming and not always predictable because it is confined to the skills and the experience of the surgeon [9]. In order to solve this problem in the last decades several manufacturers started to employ 3D printing processes for the creation of customized devices, which may have the same rigidity of the traditional meshes but with a definite precision without any need of intra-surgical adjustments [10]. Unfortunately, once the bone has been regenerated, the titanium mesh must be removed, requiring a second surgery. To overcome this problem, the use of biodegradable materials was considered, so removal is not necessary. The materials studied in recent years are bioresorbable composites consisting of a polymeric matrix and a bioactive reinforcement, in order to improve the mechanical properties of the polymer and enhance cell adhesion and proliferation mechanisms. Usually, synthetic polyesters such as polylactic acid (PLA), polycaprolactone (PCL) or polyglicolic acid (PGA) are used as polymeric matrix, while bio-glasses or calcium-phospates are employed as reinforcements. The goal of this work has been the design, development and verification of composite materials based on synthetic polyesters (PLA and PCL) loaded with calcium phosphates (hydroxyapatite and β-tricalcium phosphate), for creating meshes, involved in the bone growth and preparatory to dental implants, able to allow adhesion and proliferation of cells, with mechanical properties similar to natural bone. This objective aims to avoid the removal of the mesh after the new bone is grown, since these composite materials have the property of being biocompatible, biodegradable and bioresorbable, maintaining the necessary mechanical properties to withstand load and stresses generated by surronding tissues. In particular, it is fundamental part of the goal the evaluation of mechanical behaviour of the designed materials and, on equals terms of cell adhesion and proliferation conditions, the comparison with the results obtained for titanium.

2. State of the art: use of titanium in biomedical field

Titanium and its alloys are excellent candidates for use as orthopedic and dental biomaterials. But first, it is important to define what a biomaterial is.

A *biomaterial*, according to the definition given by International Consensus Conference on Biomaterials in Chester, Great Britain, 1991, is "a material that interfaces with biological systems to treat, augment, or replace any tissue, organ, or function of the organism".

Titanium is a transition metal that can form solid solutions with elements with similarly sized atoms. In the solid state at room temperature, it has hexagonal close packed (HCP) geometry, known as the α structure. When the temperature rises above 880°, solid titanium changes to a body centred cubic (BCC) form known as the β structure, until it melts [11],[12].

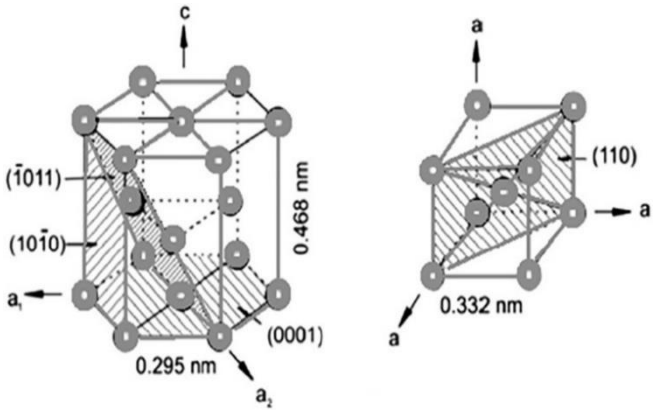

Fig.1. *Two crystallographic forms of titanium. At room temperature it exists in HCP (α) form on the left and as the temperature increases to 883°C, it changes to BCC (β) form on the right [2].*

Usually, in dentristry and orthopedic field the pure titanium is not used, a titanium alloy is preferable, because of its properties such as a better weight-to-resistance ratio.
In alloys, titanium occurs in a variety of forms, which can be pure α, pure β, or more common a combination of these two. Other elements present in the alloy are α-stabiliser, such as aluminum, or β-stabilisers, such as vanadium, iron, nickel, and cobalt [11]. In commerce, there are a lot of alloys containing at least three elements, but the most common alloy used in biomedical field is made up of Titanium, Aluminum and vanadium

(Ti-6Al-4V). In dental field, we deal with titanium alloys to produce meshes, used in the bone regeneration process.

A *mesh* is a support structure able to sustain cellular growth, migration and organization, thus serving as an extracellular matrix (ECM). It follows that the mesh must meet certain physical, chemical and biological requirements, described in the table below (Table 1). So it must maintain adequate 3D space that supports the regenerating tissue and temporarily make up for the functional characteristics of which the forming tissue is deficent [26].

Table 1. *Physical, chemical and biological requirements of a mesh [17].*

Physical requirements	Chemical requirements	Biological requirements
3D	Biocompatible	Biocompatible
Interconnected pores	Hydrophilic	Structure mimicking the ECM
Meccano-compatible	Biodegradable	Adhesion ligands, growth and proliferation

Listed here (Table 2) there are some of production center of titanium and its alloys in Europe.

Table 2. *List of production center of titanium and its alloys in Europe*.

Producers	Location
Hermit GmbH	Munchen, Germany
TiFast	Narni, Italy
L.C.M.A. S.A.	Luxembourg
Titalia	Monza, Italy
Harald Pihl	Sweden

2.1. Non-customized mesh

Starting from the second half of the 90s the first titanium meshes were introduced in dental field for the reconstruction of bone defects. Until recently, commonly used titanium meshes were standard, so they weren't customized for a single patient. Usually, these meshes, have a flat shape and defined thickness. It follows that, the meshes needs

to be manually bent and cut by the surgeon into a speficic shape that fits the missing part of bone. It's clear that this process has a lot of disadvanteges, such as the lost of time during the surgical procedure that could increase the probabilty of infection and patient's pain. But also, the bending could be very imprecise, altough the material has good plasticity and it's quite easy to model. Therefore, the mesh could not fit very well with the patient and this could lead to two main consequences: first, the poor contact between mesh and surronding area can affects the migration and proliferation of cells, and second, sharp edges of the mesh might cause a mucosal damage and consequently the excessive exposure of the mesh. Unitil now the pollution problem, due to the use of gloves during the bending procedure was not considered, but it could lead to a loss of mesh's biological properties [14].

2.2. Customized mesh

Therefore, with the development of avanced imaging technologies and compuer design techniques, such as Computed Tomography (CT), Computer Aided Design (CAD) and so on, customized-made titanium meshes have become one of the research trends in oral surgery. To create a custom device, the first step is a careful preoperative assessment of the patient, in which the bone to be reconstructed will be designed virtually with the help of a specific software. Up to now, two production methods of a titanium customized mesh are available: the first one includes a direct matching between custom-made titanium mesh on the reconstructed virtual model of the bone defect area, and the the use of CAD technology to print the device through a 3D printing procedure (Fig.2).

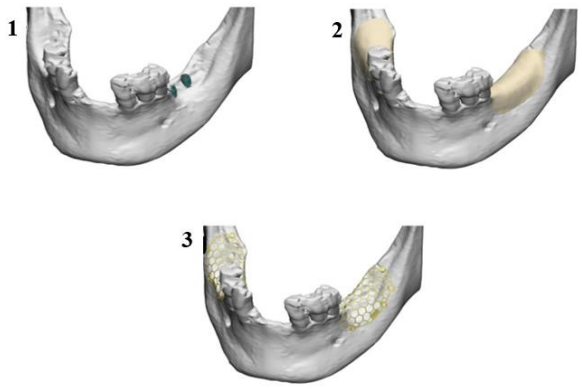

Fig.2. *Digital procedure for performing titanium mesh [3].*

While the second one, plans to create a custom-made mesh on the real 3D printed model of the reconstructed bone (Fig.3) [14].

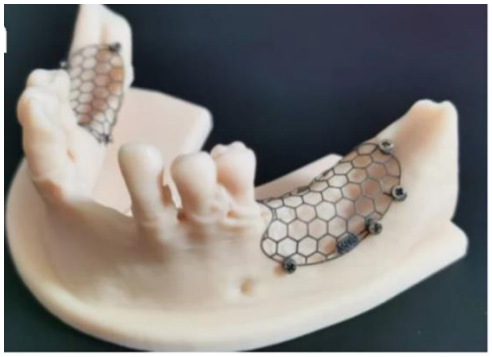

Fig.3. *Three-dimensional-printed titanium mesh [3].*

Compared to the non-customized titanium meshes, the customized ones have a lot of advanteges ranging from reduced operating times to improved fitability for the missing part of bone. In fact, regarding the latter, the shape of this mesh entirely fits the bone defect area and therefore the number of screws used to fix the mesh is also reduced. Furthermore, the problem of the mucosal damage is reduced, because with the 3D printing technique the edges of the mesh are more rounded and blunter. The tables below (Table 3, Table 4) show the profile and the results of a clinical study conducted by Faculty of Dental Science, Kyushu University, in which a comparison was made between the use of a conventional device (thickness equal to 0.1 mm) and a customized device [15].

Table 3. *Summary profile of patients who participated in the clinical trial. The data for parameters 2 and 5 were analyzed by the Wilcoxon signed rank test, and the data for parameters 3 and 4 were analyzed by the Chi-square test [4].*

	Custom-made device	Convetional device	*p*-value
1. Number of patient	13	13	-
2. Patient age (years)	34-68	35-67	$p = 0.85$
3. Patient sex (male:female)	4:9	3:10	$p = 0.66$
4. Simultaneous implantation case	13/13	11/13	$p = 0.14$
5. Total number of implants	21/13 cases	18/11 cases	$p = 0.91$

Table 4. *Comparison of results of guided bone regeneration (GBR) performed using the custom-made device and conventional method. The data for parameters 1 and 4 were analyzed by the Wilcoxon signed rank test, and the data for parameters 2 and 3 were analyzed by the Chi-square test [4].*

	Custom-made device	Conventional device	*p*-value
1. Operation time (min)	75.38 ± 11.6	111.9 ± 18.5	$p < 0.01$
2. Mucosal rupture	1/13	3/13	$p = 0.27$
3. Infection	1/13	3/13	$p = 0.27$
4. Number of screws for GBR	1.31 ± 0.48	3.23 ± 0.73	$p < 0.01$

2.3. Mesh features

Geometric matching of mesh with surronding space is achieved by the appropriate thickness, porosity, pore size and global architecture of the device. Let's start to analize the porosity of the mesh. Several studies have shown that the pore shape, pore number and pore size have a strong impact on cell response and rate of tissue regeneration, because the pores play an essential role in establishing blood supply and facilitating metabolic process of the grafts, since we have to consider that natural bone has a proper gradient in porosity (cortical bone: 5–10%, cancellous or trabecular bone: 50–90%). It was shown that the minimimal mesh porosity providing a sufficient cell colonization should be at least between 40% and 55%, to reach a degree of porosity of 65-85%, which is optimal for cells profileration process. It is important to point out that a consistent porosity can help in reducing the mismatch between Young's modulus of titanium alloy and bone, but if it is too excessive it can negatively influence mechanical properties [16]. Another issue to deal with is the choice of the pore size; according to some studies, pore size can vary from 50 to 500 μm up to a maximum of 1200 μm in particular cases. If the pore size is too small, clogging of the pores might occur and therefore the cells cannot penetrate the structure; on the other hand, if the pore size is too big leads to a smooth cells proliferation because they migrate to the target and then overcome the mesh due to the small adhesion surface [17]. Then we must consider the thickness of the mesh, that is another variable that strongly influences mechanical properties. Usually, the thickness of a mesh varies between 0.5 and 1 mm. Under 0.5 mm the mesh is more flexible, but the risk of surronding tissue collapse arises since the sufficient stiffness is not guaranteed, so 0.5 mm is the minimum thickness to ensure good mechanical properties. While over 1 mm it is possible that the tissues do not cover the mesh, and this leads to an exposure of the graft to the surronding environment and pollution [14]. In addition to porosity and

thickness, another important factor is the global architecure of the mesh, because it has a significant impact on cells migration. It has been ascertained that an architecture made up of square cross-section straight channels and straight connection between single pores, facilitates the growth of cells and allows a more efficient propagation of nutrients within the structure [17].

2.4. Mechanical properties of titanium and its alloys

Titanium is still widely used today in dentistry and orthopedics due to its excellent mechanical properties: it combines high strength, toughness, stiffness and low density [13]. Unfortunately, titanium has a high friction's coefficient which results in low wear resistance. Commercially used pure titanium (Cp-Ti) is classified into four grades according to the purity and the oxygen content, but also these grades differ in corrosion resistance, ductility, and strength [11]; normally in dental and orthopedic applications pure titanium is not used, a titanium alloy is used.

Titanium alloy meshes, have amazing mechanical properties such as high strength, high stiffness inherited from titanium, and good flexibility and plasticity due to the presence of aluminum. The high stiffness enables an efficient maintainance of the space required for bone regeneration, avoiding soft tissue collapse and displacement of bone graft. On the other hand, the good plasticity of the material, allows to easy bending and shaping of the mesh. However, it must be emphasized that mechanical properties strongly depend on some parameters such as thickness and porosity of the mesh, but they may also be influenced by manufacturing and sterilization process [13]. Biomaterials' mechanical properties of general importance to be evaluate are reported in the table below (Table 5).

Table 5. *Mechanical properties of pure titanium, Ti6Al4V alloy and natural bone [5],[6].*

Properties/Materials	Cortical bone	Cancellous bone	Cp-Ti	Ti6Al4V
Young's modulus (GPa)	7-30	0.05-3.5	105	110-115
Flexural strength (MPa)	50-150	10-20	240-550	895-930
Yield strength (MPa)	105-222	1-12	170-485	825-870
Elongation at break (%)	1-3	5-7	15-24	6-10

After giving some information on mechanical behaviour of titanium and its alloys, it is important to emphasize that for the creation of implantable devices, titanium should have mechanical properties comparable to natural bone. By comparing Young's moduli of titanium alloy and natural bone, it turns out that the alloy's modulus is greater than that of the bone, as it can be seen in Table 5 [18]. This mismatch can lead to serious consequences such as a poor osseointegration and the so-called "stress shielding effect". Osseointegration involves a direct contact between the mesh and the bone mechanically stable, in the sense that the transmission of forces at the interface must not generate relative movements between the parties involved. But in this case, the great difference between Young's moduli may not allow for the creation of a stable interface. Instead, regarding the stress shielding effect, an uneven load distribution occurs, since the mesh (having a greater Young's modulus than natural bone) would bear almost the entire load and this may result in bone atrophy around the implant site. To overcome these drawbacks and thus ensure better load transmission, Additive Manufacturing (AM) techniques came into play to create highly porous devices with lower Young's modulus, as it is possible to see in the table below (Table 6).

Table 6. *Change of mechanical properties according to mesh porosity [7].*

Specimen	Mean porosity	Loading	E (GPa)	US (MPa)
Hot-rolled	0%	tension	117.2 ±	968 ± 29
		compression	3.3	1835 ± 23
Fully-dense	0.8%	tension	118.9 ±	989 ± 10
		compression	3.3	1842 ± 17
Porous surface	37.9%	tension	65.1 ±	589 ± 9
1 mm		compression	12.2	1072 ± 10
Porous core	48.4%	tension	47.6 ±	432 ± 11
		compression	11.2	579 ± 1
Porous surface	62.1%	tension	30.5 ± 2.0	230 ± 15
2 mm		compression		393 ± 22

2.5. Biocompatibility of titanium and its alloys

Titanium alloys have the highest biocompatibility than any other metal, since they are prone to perform electrochemical oxidation to create a passive and inert titanium oxide (TiO_2) layer between 4-6 μm thick. This process is called "passivation of the metal". This

oxide layer, which remains intact even at human pH, leads to improve wear resistance. An important preliminary evaluation of biocompatibility consists of an *in vitro* cytotoxicity test, namely a method of assessing acute biological damage casued by substances released from biomaterials by observing the effects they produce on cells grown in vitro [26]. In particular, it must be emphasized that most metals are initially non-toxic but wear over the time generating particles which can become so. For example, during the chewing process, a friction on titanium surface can erode TiO_2 layer, resulting in material loss. Therefore the corrosion behaviour is one of the most important factors influencing the biocompatibility of titanium meshes and consequently it becomes essential to evaluate the cytotoxicity of the released particles [11], [17].

3. Titanium pollution risk and limit thresholds

Another issue to deal when we talk about titanium devices is the pollution. There are numerous sources of pollution such as the corrosion of the device, the composition of the starting alloy, surface treatments or bacterial contamination.

Let's go in order, starting to analyze the problem of corrosion of the deivce that can cause the release of metal particles (dimension 1.8-20 μm) in surronding environment. This is perhaps one of the most controversial issues in literature, because there is no uniformity of view about titanium reactivity, diffusion and accumulation. Here it is reported a study contucted on 28 patients, from 6 to 24 months after surgery, in which grids, plates and surronding tissues have been investigated to evaluate the titanium release and accumulation. The following results came out: in the implant area, titanium particles have been found in the interfacial bone and in all surronding tissues, but it was not detected at more than 1 mm from the device's surface. But surprisingly, high titanium levels were detected in some blood cells inside the connective tissue. According to other studies, titanium was found in lymph nodes, urine, serum and in some organ such as spleen, liver and lungs. This event would not seem to be worrying because many authors believe titanium to be free from inflammatory or allergic reactions, the main issue might be its accumulation and concentration level. To conduct analysis on patients, X-rays were used, while for the devices were used both Scanning Electron Microscopy (SEM), and X-rays [19].

Let's take a look to the values of concentration of titanium particles in human body and its limit thresholds. In according with a study conducted by University College London on hip joint prosthesis (made of Ti-6Al-4V alloy) [20], it was found a median value of titanium concentration equal to 1.2 μg/L with the upper normal level (95th percentile) of 2.20 μg/L in blood, and 1.7 μg/L with the upper normal level (95th percentile) of 2.56 μg/L in plasma. The limit of detection was fixed on 0.77 μg/L (calculated as 3 x the standard deviation of the blank concentrations). So, in this study, limit thresholds were fixed on the upper normal level of titanium concentration (2.20 μg/L in blood and 2.56 μg/L in plasma). Instead, the current Mayo guidelines state that a well-functioning, unilateral hip implants should produce serum titanium levels of approximately 4 μg/L, while levels exceeding 8 μg/L should warrant further investigation [20].

Another important issue, related to pollution, is the contamination by carbon. This substance is mainly found on the surface of device, but also traces of carbon were detected during an experimental verification inside the devices, probably due to the composition of the alloy, even if the presence of carbon is not declared within it. In the table below (Table 1) it can be seen the chemical composition of Ti6Al4V alloy.

Table 1. *Chemical composition of Ti6AlV4 alloy [8].*

Chemical elements	Weight
Titanium	90 %
Aluminum	5.33%
Vanadium	4.22%
Oxygen	0.2% max
Iron	0.25%
Nitrogen	-
Hydrogen	-
Carbon	-

One last comment on the pollution caused by surface treatments. To produce desiderable properties, a surface treatment (sandblasting, chemical etching, anodization, laser treatment, and surface coatings) is frequently adopted in titanium-based devices. The problem of these treatments is mainly the pollution caused in the surronding environment once the device is implanted in human body. Consequntly to the pollution issue, a possible problem regards the damage of the tissues caused by infiltration of titanium nanoparticles (dimensions < 50 nm). This theme arouses controversial opinion among scientists because some studies have revealed that even higher concentrations of tianium nanoparticles do not cause adverse effects, since are biologically inactive and physiologically inertexhibiting relatively low toxicity. Other studies, and for example the Scientific Committee on Consumer Safety (SCCS) has described the genotoxic and carcinogenic effect of higher concentration of titanium particles. The apoptosis induced by titanium particles was found as results of these studies. Titanium particles induce apoptosis of cells in an intrinsic way: they enter in the cell, induce the reactive oxygen species (ROS) generation, and then enter the nucleous causing DNA damage. The damage of this vital molecule leads to cell death [21].

4. Production techniques of titanium devices

Up to now, many methods of preparation of porous metal devices have been introduced, but Additive Manufacturing (AM) technologies have elicited great interest in recent years. AM consist of an additive method to make a three-dimensional object of any shape starting from a computer-aided design (CAD) model or a model based on scans obtained using imaging systems such as magnetic resonance (MRI) or computer tomography (CT). Then the virtual 3D model is converted into an STL file and it is "sliced numerically" into the thickness chosen according to the desired resolution and supported by the printer, as it can be seen in the figure below (Fig.5). Unlike traditional techniques, AM allows us to manage the microstructure in terms of pore size, shape, distribution and porosity degree. Furthermore, with this method we can create complex customized device, with complex internal architecture, that fitting perfect the damage part anatomy.

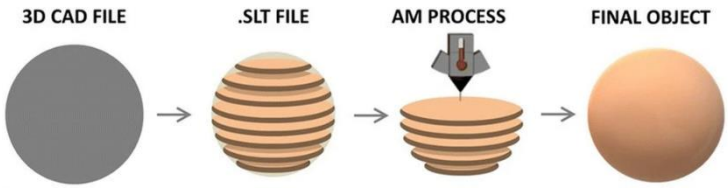

Fig.1. *Schematic illustration showing the AM process flow.*

4.1. Selective Laser Melting

Selective laser melting (SLM) is one of the most common AM techinques used to create titanium alloy (Ti-6Al-4V) devices. Starting from the 3D model and using thermal energy supplied by a focused laser beam, layers of metal powder are melted on top of the previous one to create an organized layered structure. Since we must guarantee certain mechanical and chemical properties to the material and a certain porosity degree, we have to carefully set some process parameters such as laser power, laser beam thickness, layer thickness and distance between scanning lines [18].

4.2. Selective Laser Sintering

Selective laser sintering (SLS) is another AM technique available to produce custom-made devices. In this case the device is always created by applying incremental layers of metallic powder, but unlike SLM, the sintering process do not fully melt the powder, it heats the powder to the point that it can fuse together on a molecular level. So, for each layer, the machine lays down a film of metal powder and then laser melts selected areas, so they conform to the previous layer. Then the platform moves down, and a new film of metal powder is laid down; this process continues layer by layer until the pattern is completed. Some studies were conducted to evaluate biologic behaviour of osteoblasts grown on surface of devices manufactured with SLS technique, and it was found that the the cells produced osteogenic and angiogenic factors, together with a considerable amount of new bone matrix [22].

4.3. Electron Beam Melting

Electron beam melting (EBM) is one of the additive manufacturing techniques mainly used for metallic biomaterials. This technique works as follows: the device is manufactured by melting the metal powder layer by layer using a directed electron beam under a high vacuum atmosphere. In this technique the challenging part is to optimize the surface finish of the device to ensure the cell attachment, differentiation, proliferation, and obviusly biocompatibility. It's clear that the final result depends on the choice of processing parameters such as beam current or powder particle size. Test *in vitro* and *in vivo* were carried out and biocompatibilities were evaluated for EMB devices: the results were positive, so EBM may be another promising manufacturing technique that can be used in biomedical field [23].

4.4. Directed Energy Deposition

Directed energy deposition (DED) process can be employed to create net devices starting from powder or wires, through a layer-by-layer process. Using this technique, it is possible to create complex shaped devices that can be used in different engineering applications. The functioning is as follows: a focused laser beam is used as heat source to

melt in-situ delivered powder or wire-shaped raw materials, a melt pool is formed on the surface of the last deposited layer and at the same time powder is blown through the laser beam and into the melt pool. So, DED is carried out by simultaneously feeding of powder or wire and energy source or feeding different powders at the same time [24].

4.5. Laser Powder Bed Fusion

Laser powder bed fusion (L-PBF) is a used process for producing titanium implants in need of enhanced mechanical properties and feature complexity. The aim of L-PBF is to create devices with lower elastic modulus maintainig good mechanical properties. In this technique a focused laser beam melts the metal powder, which is located on a plane powder bed, in order to create a layer, and then it goes on until the end. Also, in this case to achieve a good final result, processing parameters such as laser power, scanning speed, layer thickness, must be chosen carefully [25].

4.6. Laser Metal Deposition

Laser Metal Deposition (LMD) is a representative synchronous powder feeding forming AM method. LMD can used to create titanium devices from a 3D model as well as SLM or SLS. The focused laser beam melts the metal powder fed and creates a molten pool on the substrate, the molten metal powder solidifies to form a final object. In this technique, compared to SLS/SLM we have a synchronous powder feeding, which can be coaxial (i.e. laser beam and powder have the same central axis) or side. This technique presents some other differences respect to the previous ones (SLM and SL) such as spot diameter, LMD is in millimeter level while SLM/SLS is in micrometer level, and furthermore the manufacturing accuracy, which is definetly lower compared to SLM or SLS [22].

4.7. Post-production processing

The most common post-production processing are mechanical and chemical cleaning ones, which involve roughening of surfaces through blasting or acid treatment. The roughening process permits to altere the device’s surface energy to enhance the attachment of cells and osseintegration. This treatment can be carried out by different

methods, one of these involves blasting with particulates, for example sand or albumina. Another method consists of etching with mineral acids such as hydrochloric acid (HCl) or sulfuric acid (H_2SO_4) of appropriate concentrations. Acid treatment can be used alone or in combination with sandblasting, to produce surfaces with a higher degree of roughness [11].

As soon as the surface modification process is completed, some surface treatments using bioactive coatings may be carried out, in order to accelerate cell adhesion and proliferation. Studies conducted on rats shown that: compared to the untreated titanium mesh, in the treated one with bioactive coatings there are no soft tissues under the mesh and the bone density is significantly higher [14]. The most common bioactive coating is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), which is a calcium-phospate compound already used in orthopaedic field [11]. Another material used as coating, at least for experimental studies, is diamond-like-carbon (DLC). It is an amorphous material that shows typical characteristics of diamond with a high inhert biocompatibility with bone. This material is applied to the surface by chemical vapour deposition (CVD) or by electrodeposition; in both cases it would be ideal to include an intermediate layer, such as amorphous silicon, to promote adhesion between DLC and the substrate. The purpose of this treatment would be to improve corrosion resistance and enhance biocompatibility, but this approach has not yet had any clinical results because it is a treatment that is currently only performed *in vitro* [11]. Furthermore, it is possible to improve the hydrophilicity of titanium through a technique called photofunctionalisation, using UV rays.

5. Laboratory tests on titanium and explants

Now let's analize some data from laboratory tests, previously performed by a colleague of mine, Lorenzo Alvito [27] on titanium samples and explants. In particular experimental activity was conducted on samples of titanium from two companies: BIOTEC S.R.L (BTK), Vicenza, Italia and BONE-EASY (BE), Lisbona, Portogallo, and furthemore on explants from patients. This activity involved mechanical test and compositional analysis of both specimens and explants.

5.1. Mechanical tests

Three-points flexural bending tests were conducted on two types of samples: *full* and *with holes* from both companies (5 BE full, 5 BTK full, 5 BE with holes and 5 BTK with holes). BE specimens have large ellipctical perforations with 1 mm of distance between each hole, while BTK specimens have circular holes with 2.4 mm of distance between each hole. Here a stress-strain curve for each tested specimen is reported, from which it was possible to extract important parameters such as elastic modulus in bending and flexural strength.

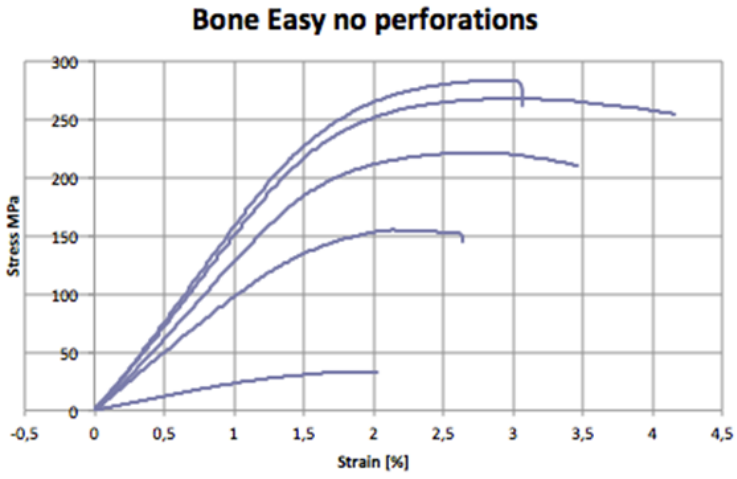

Fig.1. *Stress-strain curve of BE full specimens.*

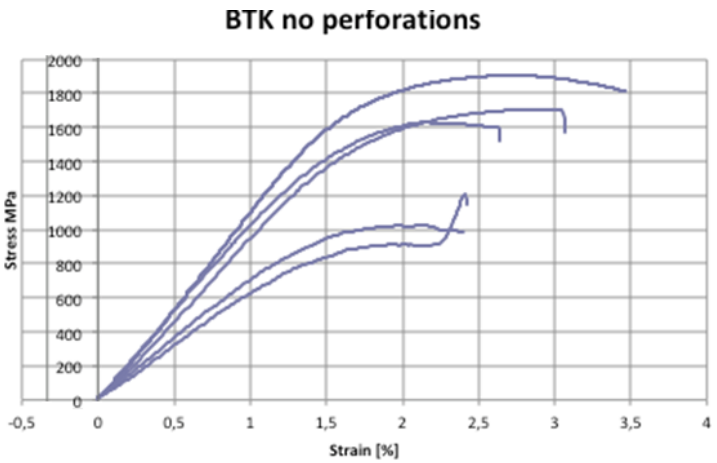

Fig.2. *Stress-strain curve of BTK full specimens.*

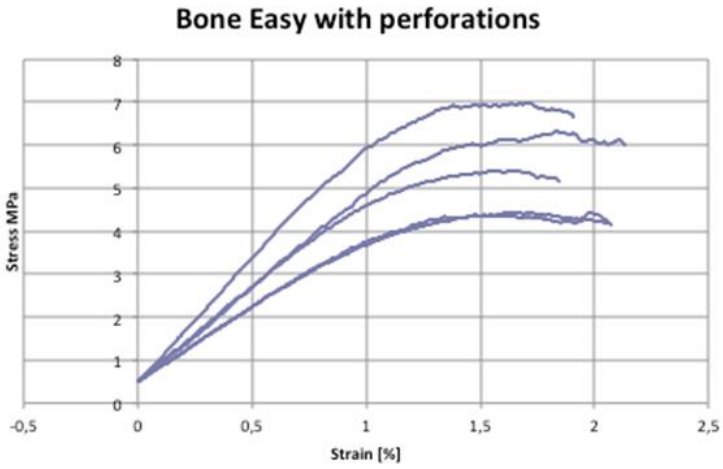

Fig.3. *Stress-strain curve of BE with holes specimens.*

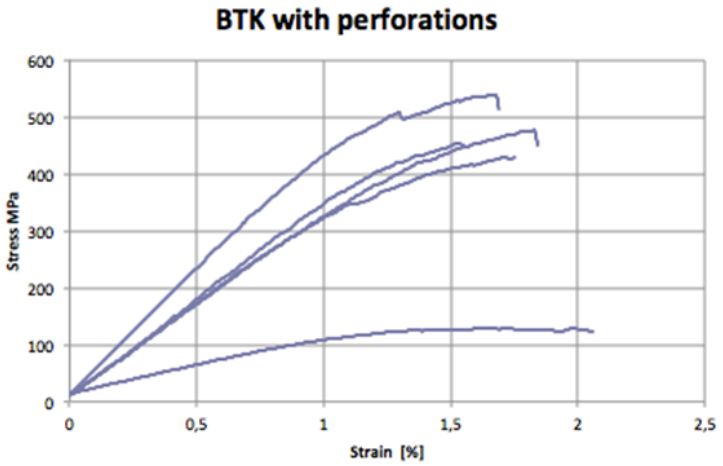

Fig.4. *Stress-strain curve of BTK with holes specimens.*

It is possible to observe that full samples have higher Young's modulus than those with holes. Furthermore BE full samples exhibits slightly higher Young's moduli respect to BTK full samples. Regarding flexural strength, in any cases, BE samples show values of maximum load lower that BTK ones; this difference may be caused by a different printing process between the two companies. Therefore BE samples show a greater ductility than BTK samples [27].

5.2. Compositional analysis

A compositional analysis with a probe electron microscope, was carried out both on the samples and the explants to evaluate the materials' structure and composition. In particular, in this analysis the focus was placed on the search of impurities such as carbon, that is a dangerous substance for human body. Here the chemical composition of samples provided by BTK and BE (Table 1).

Table 2. *Chemical composition in percentage of BE and BTK samples.*

Material	**Minimum %**	**Maximum %**	**Minimum %**	**Maximum %**
	BTK		**BE**	
Ti	Balance	Balance	Balance	Balance
Al	5.5	6.5	4.5	7.5
V	3.5	4.5	2.5	4.0
Fe	-	0.25	-	0.18
O	-	0.13	-	0.13
C	-	0.08	-	0.06
N	-	0.05	-	0.04
H	-	0.012	-	0.015
Y	/	/	/	/

First, a surface analysis was conducted on both samples and explants, and the results are reported here.

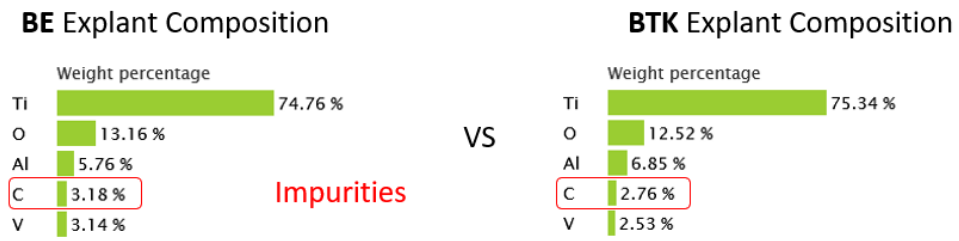

Fig.5. *Surface chemical composition of BE and BTK explants.*

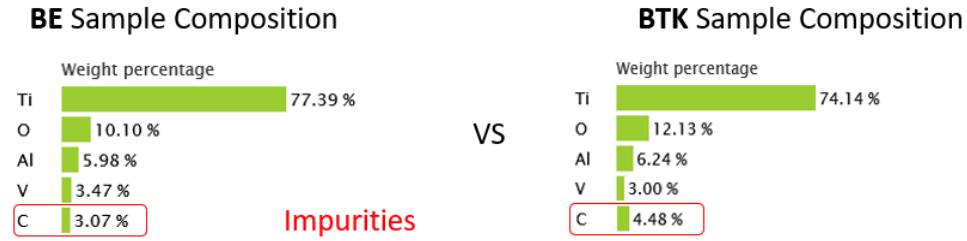

Fig.6. *Surface chemical composition of BE and BTK samples.*

A further analysis was conducted after breaking the sample, therefore in thi case a bulk analysis was conducted to evaluate the composition inside the sample, and here are reported the results on samples.

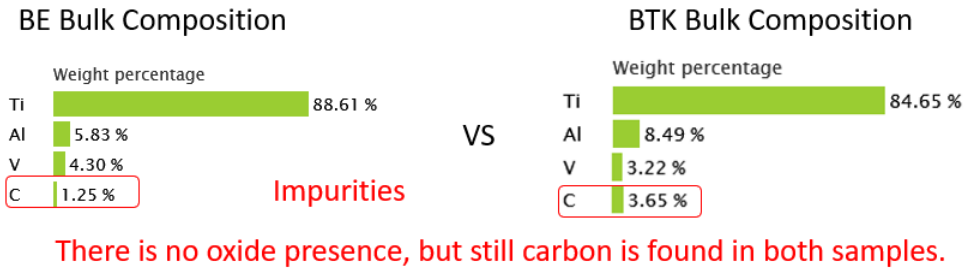

Fig.7. *Bulk chemical composition of BE and BTK samples.*

There is a consistent presence of carbon both in intact samples (on the surface) and in broken samples, althought the companies declared a maximum carbon percentage of 0.08% for BTK and 0.06% for BE. Instead, in this analysis, an average carbon presence inside the samples was estimated about 3% in BTK samples and about 1% in BE samples. To explain this difference between literature and experimental results, some hypotetical reason were found, such as the carbon contamination is due to samples machining and manipulation, or it is in the productive flowchart. However, the presence of carbon leads to some important consequences on the material such as a degradation of mechanical properties (tensile strength, ductility…) of the titanium alloy, making the alloy stronger but less ductile since carbon is an α stabilizer [27].

6. Polymers in biomedical field and their use

In the last few years, the use of polymers in biomedical applications has had a great success and it has strongly impacted on modern medicine, especially in the field of bone regeneration. Surely, one of the advantages of using biodegradable polymers over titanium is the property of *biodegradability*, that is the polymer's ability to degrade when it comes into contact with biological fluids [28]. This means not having to remove the material when it has completed its function and having fewer post-operative complications for patients. Clearly biopolymers have different properties and behaviour than metals, such as lower mechanical strength and stiffness. But, in the case of bone regeneration, if you choose to use biodegradable materials to create scaffolds, it is essential to achieve certain mechanical properties in order to ensure the capability to withstand stresses and loads of surrounding tissues, and to work as a natural body component [29]. So, using a biodegradable material is not as easy as it sounds, because it is required a careful analysis on some important issues such as: the guarantee not to evoke adverse reactions from the organism, the correct esteem of degradation time or the choice of appropriate mechanical properties. In particular, from a functional perspective, the degradation time is an important issue to consider, because the biomaterial must completely degrade only after having performed its function. Therefore, one must also be careful on the erosion mechanism: bulk erosion or surface erosion. Bulk erosion is characterized by a molecular mass degradation of the material, which cause a rapid loss of mechanical properties, although the physical structure is manteined. While, surface erosion is characterized by a surface degradation, that leads to a shrinking of the material over time, maintaining the same structure; in this case mechanical properties are loss more slowly over time. Usually, whe we deal with devices like scaffolds, the best degradation is the surface one, because it allows to maintain an adeguate mechanical behaviour [30].

Polymers used in biomedical field are classified in two main categories: natural and synthetic polymers. Let's begin with natural ones.

6.1. Natural polymers

Polymers of natural origin, mainly, belong to the family of proteins and polysaccharides. Proteins are high molecular weigth polymers, consisting of the repetition of amino acids

monomers linked by a particular type of covalent bond called amine bond. While polysaccharides are polymers constituted by the repetions of monosaccharides joined together by a covalent bond called glycosidic bond. The difference between these two types of polymers, is mainly in term of degradation, because the proteic ones are more delicate, as they undergo denaturation when there is an increase in temperature or under the action of particular enzymes [30].

Among the main *proteic polymers* there are:

Collagen

Collagen is a fibrous protein rich of α-helix structures. It is one of the major components of extracellular matrix (ECM), ligament, cartilage, and tendon. There are 28 types of collagens depending on the tissue considered and the function it must perform. In general, collagen has a particular structure: it is constituted by triple helix microfibrils which are organized parallel to each other to form fibrils, to have fibers of collagen. From biomedical point of view, collagen, has a great biocompatibilty and good mechanical properties such as the ability to bear quite high tensile load (92.5 Mpa ultimate tensile strength), due to its fiborus nature; but it is always a protein, so it can undergo denaturation losing both its structure and function [18]. The main applications of collagen are in the field of drug delivery as shields in ophthalmology, sponges for wounds, tablets for protein delivery; while in the branch of tissue engineering, collagen is used for skin replacement, artificial blood vessel or valves [31].

Elastin

Elastin is a fibrous protein, consituted by heavily cross-linked tropoelastin molecules. It is a natural polymer that provides a great resistance and with a high elasticity, in fact on the mechanical point of view it acts like a spring. Due to its behaviour, it is the major component of vascular and lung tissue, and it is responsible for the contraction of them after applying a stress. The main disadvantage of elastin is its high hydrophobicity, that sometimes limits its use. In biomedical field, elastin is used for a wide range of tissue engineering applications, for example split-skin autografts for burn wounds or autologous blood vessels allografts for coronary graft reconstruction and so on. Furthermore, with the process of decellularization, it is possible to obtain natural three-dimensional

scaffolds. This process involves the removal of cells from a natural tissue to obtain a natural matrix, which can be repopulated with host cells [32].

Fibrin

Fibrin is a large, cross-linked biopolymer constituted by fibronectin. It is biocompatible, biodegradable, and capable of promoting cell proliferation. Being a polymer with a high cross-linking aptitude, it can be easily modified to suit the desired application. Fibrin is widely used in the field of drugs, proteins, and genes delivery, for therapeutic biomedical applications [32].

Instead, regard to *polysaccharide polymers* we have:

Hyaluronic acid

Hyaluronic acid is a natural polysaccharide polymer of human origin, consisting of alternating units of N-acetyl-D-glucosamine and glucoronic acid. Usually, it comes from rooster combs and bovine vitreous humor. It is a negative charged and high molecular weight polymer, and it has a great hydrophilicity, in fact in 6 months it is degraded by a particular class of enzymes present in human body, called hyaluronidase. Due to its hydrophilic behaviour, it is one of the major components of extracellular matrix but also, it plays an important structural role in articular cartilage and skin. The hyaluronic acid is used in many biomedical applications, that vary from regenerative medicine for realizing hydrogel, tissue engineering (vascular, cartilage and skin), to drug delivery especially in oncology field [33].

Chitosan

Chitosan is a semi-synthetic polysaccharide, obtained by a process of alkaline hydrolysis at high temperatures. Chitosan is a bioactive polymer and it is riched of amino groups that make it the only cationic polysaccharide. And it is thanks to its positve charge that chitosan has great antibacterial properties and an amazing ability to heal wounds. In fact, chitosan is widely used for realizing bioadhesives films, that create a seal over a wound to act as an antibacterial barrier and to reduce hemostasis [28].

Chitin

Chitin is a linear glucose polymer, with an acetyl-amine group on carbon two of the carbonaceous skeleton. Structurally, it is like hyaluronic acid, and from a functional point of view, it is proved its ability to accelerate wound healing. Unfortunately, chitin is insoluble in main used solvents, so it becomes difficult to work with and this limitates its use in biomedical field [32].

Alginate

Alginate is a linear polysaccharide, taken from the cell wall of brown algae. It has a great ability to form spontaneous gelation when exposed to divalent cations (like Ca^{++}) so, this property make alginate a good candidate for biomedical application, especially in field of hydrogels. Unfortunately, this material has poor mechanical properties, it is too weak on structural point of view so, it is unlikely that alginate be used alone [32].

6.2. Synthetic polymers

The limitation of natural sources has brougth to creating synthetic polymers, to overcome the problem of reproducibility and controllability. Furthermore, synthetic polymers have more significant mechanical properties respect to natural ones, and they are easier to process. The most famous class of synthetic polymers are polyesters which include for example polyglycolic acid (PGA), polylactic acid (PLA), polycaprolactone (PCL). But then there are polyethers, such as polyethylene glycol (PEG), polycarbonates and polyurethanes [30].

Let's start with the *polyesters*' family:

Polyglycolic acid (PGA)

Polyglycolic acid was one of the first degradable polymers studied in biomedical field. From mechanical point of view, PGA has great properties like a high tensile strength (12.5 GPa) and high toughness but, due to its rapid degradation (that leads to a loss of these mechanical properties) and insolubility in many common solvents, it is better to use

PGA in combination with other polymers. It is emplyed to produce scaffolds for bone, tendon, or cartilage regeneration [30].

Polylactic acid (PLA)

Polylactic acid is a biodegradable e bioresorbable polymer, very used in biomedical field. It posseses chiral molecules and, the two common forms of PLA are: poly-L-lactic acid (PLLA) and poly-D-lactic acid (PDLA). Since it has interesting mechanical properties and controllable degradation times, it has widely used in tissue engineering applications like scaffolds for bone regeneration, produced with 3D printing technique [30].

Polycaprolactone (PCL)

Polycaprolactone is a semicrystalline polyester with a very low degradation rate and great solubility in organic solvents. From mechanical point of view, PCL has low tensile strenght, but it presents a high elongation at break. These characteristics make PCL a great biomaterial for tissue engineering applications [30].

Polymers such as *PLA* and *PCL* will be dealt with in more detail in the following chapter.

From *polyethers'* family it is important to mention this polymer:

Polyethylene glycol (PEG)

Polyethylene glycol is a hydrophilic polymer resulting from ethylene oxid, used in many medical and biological applications such as bioconjugation, drug delivery, biosensing or tissue engineering [30]. Often, to enhance its biomedical potencial, PEG is usually used in combination with other polymers to ensure steric stabilization limiting interactions between the device and the host.

7. Bioresorbable and medical grade polymers

In the previous chapter were briefly shown some biodegradable polymers, natural and synthetic, used in biomedical field. Now, in this section the attention will focus on bioresorbable polymers, in particular on the two most commonly synthetic polymers used for tissue engineering (TE) applications (e.g. scaffolds): polylactic acid (PLA) and polycaprolactone (PCL) as it can be seen in the figure (Fig.1). It is important to specify that all polymers used for biomedical applications must be *medical grade* polymers, namely materials certified for biocompatibility through different tests and standards to ensure the absence of cytotoxicity, irritation or inflammation. Furthermore, the manufacturing process must also be medical grade, this means that the producer has exceeded quality and safety requirements for design, producing, installing and serving of medical devices [34], [35].

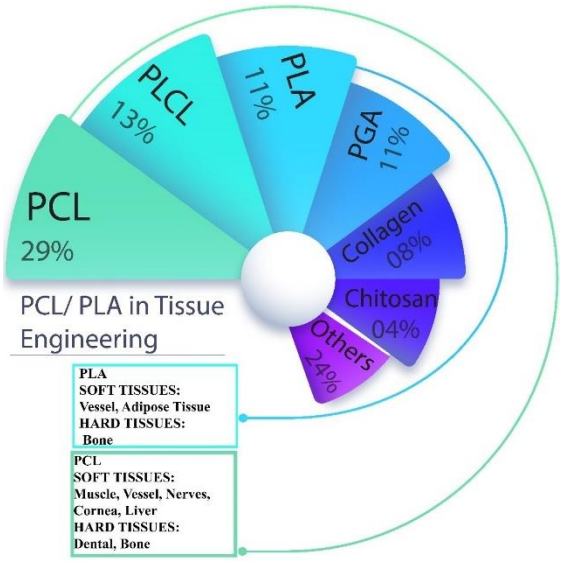

Fig.1. *Different biopolymers used to fabricate scaffolds for tissue regenerations applications [9].*

But before starting, it is good to give a definition of what bioresorbability is.

Bioresorbability is the ability of a material to undergoes a progressive and slow degradation, whose degradation products are not toxic for the organism [28].

7.1. Polylactic acid

In the last few years, polylactic acid (PLA) has become one of the leading biomaterials FDA (*Food and Drug Administration*) approved in biomedical field, thanks to its interesting properties such as being a thermoplastic, bioresorbable polymer with good mechanical behaviour. From a chemical point of view, PLA is a linear aliphatic polyester, constituted by monomers of lactic acid. Since lactic acid is a chiaral molecule, exists in two enantiomers: L- and D-lacitc acid (Fig.2). Therefore, it is possible to distinguish two stereoisomers of PLA, that are poly(L-lactic) acid (PLLA) and poly(D-lactic) acid (PDLA). The crystalline nature of both PLLA and PDLA gives them similar physico-chemical characteristics, the main difference between them is in terms of degradation and mechanical properties: PDLA present a faster degradation rate, which is preferable as the degradation time of PLA is quite long; while PLLA has better mechanical properties than PDLA, which therefore makes it more usable for load-bearing applications.

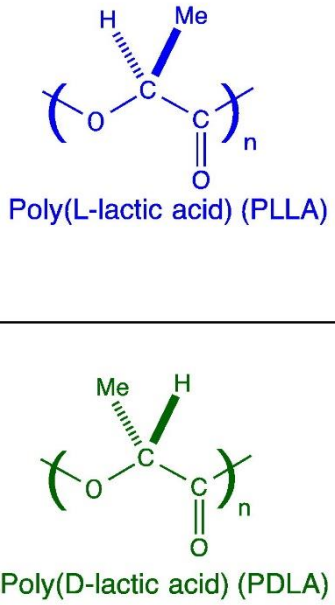

Fig.2. *Two stereoisomers of PLA: poly(L-lactic) acid (PLLA) and poly(D-lactic) acid (PDLA) [10].*

Since PLA constituent unit (lactic acid) can be obtained by fermentation of sugars from sugarcane or corn starch, that are renewable resources, it is possible to affirm that PLA is an eco-friendly polymer. Synthesis methods for PLA starting from lactic acid, are

numerous, but the two most used techniques are: direct polymerization (such as azeotropic dehydratation polycondensation and enzymatic polymerization) and ring-opening polymerization [36]. Briefly, the direct polymerization is a reaction involving the direct incorporation of functional groups into the polymer backbone eliminating water; while the ring-opening polymerization is a reaction in which one polymer chain with a reactive center on its terminal end reacts with another cyclic monomer, hence opening its ring system to form a longer polymer chain [37].

Cleary PLA has numerous applications outside the medical field, it is used in a large variety of consumer products such as disposable tableware, cutley, kitchen appliances or compost bags. It is also used for automotive parts such as panels, covers and floor mats; or it is employed in agriculture to create sandbags, planting pots and ropes.

In the table below (Table 1) there is a list of the major PLA producers.

Table 3. *List of the major PLA producers.*

Producers	**Locations**
NatureWorks	Minnesota, United States
WeforYou	Austria
Evonik	Germany

Physical and chemical properties

When we talk about physical properties of PLA, we are dealing with molecular weight (M_w), density, thermal conductivity, and rate of cristallinity; and it is important to remember that all these properties will affect the performance and mechanical behaviour of the material in some way.

Let's start talking about molecular weight, which has a strong impact especially on degradation kinetic, solubility and mechanical strength. High M_w PLA has been shown to be completely reabsorbed in a time ranging from 2 to 8 years; it is a long existence in human body, and this may cause infections and inflammatory reactions. So, some research has been conducted on low M_w PLA, finding that it has a shorter degradation rate but such as to ensure mechanical properties for the time necessary to the new bone growth.

Densitiy is also an important parameter to consider when we deal with PLA, in particular for the calculation of 'specific properties' (i.e. dividing mechanical properties by density), which are helpful to evaluate the intrinsic strength of the device to be created [36].

Now let's consider the rate of cristallinity, a very important property that strongly affects the mechanical behaviour of PLA. In this case, cristallinity is defined as the amount of cristalline region in the material respect to amorphous region. It follows that we can have both semi-crystalline and amorphous polymers, while it is rare to find a completely cristalline material. A semicrystalline polymer is consituted by regular repetion of units that leads to a folding of the chains into dense regions called crystallites, which act as cross-links, conferring to the polymer a high tensile strength and high modulus. Instead, an amorphous polymer is made up of chains arranged in a disordered way in space, as shown in the figure below (Fig.3).

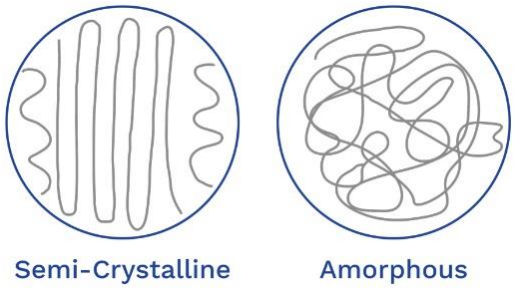

Fig.3. *Rate of crystallinity: on the left a semy-crystalline structure, while on the right an amorphous structure [11].*

Directly linked to the rate of cristallinity there are two important parameters for predicting PLA's behaviour: melting temperature (T_m) and glass transition temperature (T_g), let's consider them in detail. Normally, mechanical properties of a material are evaluated in standard conditions (e.g. room temperature), but it would be good to know the material's behaviour at varying temperatures. So, the parameters mentioned above come into play: T_g can be considered a limit above which mechanical properties decay dramatically, while T_m is the temperature above which the whole polymer chain mobility occurs, and mechanical properties go directly to zero. So, for a semicrystalline PLA, T_g (of approximately 60°C) and T_m (ranging from 160 to 180°C) are important parameters for determining the process temperatures across different applications, while for an

amorphous one only T_g can be considered [36]. In the table below are summarized some physical properties of PLA (Table 2).

Table 4. *Physical properties of PLA [12].*

Physical characteristics	Amount
Molecular weight (g/mol)	66 000
Solid density (g/cm^3)	1.252
Melt density (g/cm^3)	1.073
Glass transition temperature (°C)	55-60
Melting temperature (°C)	160-180

Mechanical properties

Now, let's talk about mechanical behaviour of PLA, that as mentioned above, is highly dependent on their physical properties. In biomedical applications, in particular in the field of bone regeneration, high mechanical properties are required to bear stresses from surronding tissues, so a semi-crystalline PLA is preferred. Young's modulus of PLA is around 3 Gpa, and tensile strength ranging from 30 to 100 Mpa. Due to its low elongation at break and a T_g close to 60°C, PLA is considered a very brittle material so, this limits its use in applications requiring high plastic deformations at higher stress levels. So, PLA can be combined with other materials to reduce its fragility. The M_w is a parameter that greatly influences mechanical properties; in fact, it has been shown that a raise in M_w from 50 to 100 kDa leads to an increase in tensile modulus by a factor of 2 and a doubling of tensile strength. Furthermore, the mechanical behaviour of PLA varies according to the stereoisomer being considered [36]. Main mechanical parameters are shown in the table below (Table 3).

Table 5. *Main mechanical properties of PLA [12].*

Mechanical characteristics	Amount
Flexural strength (MPa)	30-105
Elongation at break (%)	7
Young's modulus (Mpa)	1000-3500

Biocompatibility and degradation

Many researches have been conducted on PLA to evaluate the effective degree of biocompatibilty, here are the results obtained from a study conducted by the Department of Biomedical Engineering, Stevens Institute of Technology, Hoboken (United States). A cell colture of human fetal osteoblast was carried out on a PLA scaffolds and then an evaluation of adhesion and proliferation was conducted using an MTT assay. Over the culturing time (28 days) an increase in the number of cells adhered to the scaffold has been shown, as well as a rise of the cell proliferation without having damaged or dead cells. So, it is possible to conclude that PLA has a good biocompatibility, since it does not produce toxic effects on cells and induces a specific host response. Furthermore, as mentioned above, PLA is also a biodegradable and especially a bioresorbable polymer, considering that its degradation products do not interfere with bone regeneration process and are non-toxic for organism. PLA is degraded by hydrolysis during a two-stage process, involving a first part (physical degradation) characterized by a reduction in M_w resulting from a non-enzymatic chain scission of ester groups; and a second part in which M_w is further reduced until the lactic acid is naturally metabolized to produce carbon dioxide (CO_2) and water (H_2O) [36].

7.2. Polycaprolactone

Polycaprolactone (PCL) is another aliphatic polymer, FDA-approved, belonging to the polyesters' family. PCL is one of the most preferred polymers in biomedical field, for its excellent properties such as high biocompatibility, bioresorbability and mechanical stability; but also, from a reproducibility point of view, because it is cheap and truly versatile since it can be fabricated by different 3D printing techniques and in a great variety of structures and forms. Even if, it is important to note that due to its low melting temperature, the most commonly used technique for printing PCL is the Fused Deposition Modeling (FDM), which consists of extrusion of a filament in a series of layers on a plate to create a three-dimensional object. It is possible to synthesize PCL in numerous ways but, the ring-opening polymerization of Ɛ-caprolactone monomers, using stannous octate as catalyst (Fig.4), is the most used [39]. It is clear that by changing parameters and conditions under which polymerization takes place, it is possible to obtain PCLs with

different properties such as different molecular weight, degradation rate and mechanical behaviour [38].

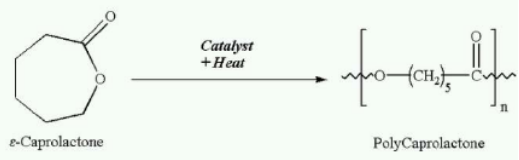

Fig.4. *Ring opening polymerization of Ɛ-polycaprolactone to polycaprolactone [13].*

Outside the medical field, PCL is often used as an additive to improve the processing characteristics and properties of resins or in the hobby field to develop any invention and to make repairs.

In the table below (Table 4) there is a list of the major PCL producers.

Table 4. *List of the major PCL producers.*

Producers	Locations
BASF	Germany
Perstorp	Sweden

Physical and chemical properties

Physical properties of PCL such as molecular weight, density, degree of cristallinity or chemical structure strictly depend on the method used to synthesize the polymer. The repetion unit of PCL consists of five non-polar methylene groups and an ester group relatively polar. PCL, at room temperature is in an amorphous state, but, thanks to its uniform structure, it crystallizes easily, therefore it is normally considered a semicrystalline polymer, with a T_g approximately equal to -60°C and a T_m of about 60°C (ranging from 59 to 64°C). It has a good resistance to water since it has an hydrofobic behaviour, but it is soluble in non-polar organic solvents such as chloroform, dichloromethane, toluene or benzene, at room temperature [39]. Typical values of the properties mentioned above, are shown in the table below (Table 5).

Table 5. *Physical properties of PCL [13].*

Physical characteristics	Amount
Molecular weight (g/mol)	3000-80 000
Density (g/cm^3)	1.10-1.15
Glass transition temperature (°C)	-60
Melting temperature (°C)	59-64

Mechanical properties

With regard to mechanical behaviour, PCL has somewhat different properties than PLA. It has been shown that PCL has low tensile strength (about 23 MPa), but a high elongation at break, which gives it a highly elastic behaviour. About elasticity, Young's modulus of PCL varies from 0.2 to 0.4 Gpa, that is about hundred times smaller than that of the PLA. Clearly, having a lower elastic modulus means a lower stiffness so, the material can deform more easily before failure, but this also implies a collapse of the device before it has completed its function [40]. Some data about mechanical feature are shown in the table below (Table 6).

Table 6. *Main mechanical properties of PCL [14].*

Mechanical characteristics	Amount
Flexural strength (MPa)	14-23
Elongation at break (%)	>1000
Young's modulus (Mpa)	200-400

Biocompatibility and degradation

It has been shown that PCL has good biocompatibility, even if a little lower than polylactides but, despite this, it is still widely used in biomedical field due to its more stable degradation process, since its ester bonds per monomer are less frequent. Many studies have been conducted to evaluate the biocompatibility, here for example are the results obtained from a research carried out by a group of Department of Plastic and Hand Surgery, Faculty of Medicine, University of Freiburg, Germany. It has been evaluated the

viability and proliferation of cells after 28 days of incubation, finding that also in presence of PCL, a continous cell proliferation on the plates and so an excellent cell viability, without reporting any damage. About the degradation of PCL, the process is based on enzymatic hydrolysis, carried on by a particular class of enzymes called lipase (contained in the interstitial fluid secreted by cells), whose function is to cleave the ester bonds of PCL. The first degradation product is 6-hydroxycaproic acid, which later undergoes a series of oxidations, to form 3-acetyl CoA molecules, and the metabolization into citric acid cycle. The complete degradation of the polymer takes 2-3 years, that is a relatively long time, but it is possible to reduce it combining PCL with other elements such as hydroxyapatite (HA) or tricalcium phosphates (TCP). It is important to note that some physiological parameters affect the degradation rate, one of these is pH of the medium. It has been demonstrated that in an alkaline environment, the degradation of PCL is faster than in an acid environment, so it is an important parameter to keep under control [41].

8. Composite ceramic polymers

The continuous and constant development of biomedical technologies, especially in the field of tissue engineering and regenerative medicine, has led to the need for materials with specific characteristics and increasingly performances, such as the ability to perform multiple advanced functions simultaneously. The aim is to obtain a multifunctional material, namely a material in which all basic requirements, such as mechanical properties, biodegradability, bioactivity, osteogenicity etc, ect… meet. For example, in the bone regeneration process, a material is defined as multifunctional when it induces the formation of new bone tissue and new blood vessels, progressively deteriorates at a rate coinciding with bone growth, and has an anti-inflammatory and antibacterial activity. From a practical point of view, the requirements described above, can be achieved by design and development of multi-component materials such as *composite ceramic polymers*, namely materials constituted by a polymeric matrix containing ceramic reinforcements. Having such a material for creating customized scaffolds, with characteristics as similar as possible to natural bone, allows the problem linked to allografts and autografts (i.e. the potential risks of disease transmission) to be overcome and the problem associated with titanium removal. In the previous chapter, only pure biodegradable polymers have been considered as materials for use in biomedical applications; but it is important to point out that polymers used alone may not fulfill the functions mentioned above. The main disadvantages of synthetic biodegradable polymers are surely the loss of mechanical strength with the increase in implantation time (due to degradation and resorption) and the lack of cell-recognition (in terms of affinity with cells) as a result of their hydrophobicity. Therefore, ceramics modifiers come into play to enhance mehcanical properties of polymers and to increase osteogenic activity [42], [43].

First, however, it is important to define what bioceramic materials are.

8.1. Bioceramics

Bioceramics are biocompatible ceramic materials, considered as an alternative solution for natural bone; even if used alone, they still have some drawbacks: their primary ionic or covalent bonds make them very brittle materials with low ductility, and therefore with a scarce ability to withstand stresses and loads. It is possible to classify bioceramics in

two large families: bioinert and bioactive ceramics. Bioinert ceramics were the first developed and used, they came from zirconia (ZrO_2) and alumina (Al_2O_3) and they were mainly employed for the production of femur heads of total hip prostheses. Being a *bioinert* material means being a biocompatible material which, however, does not induce the tissue to interact whit it. *Bioactivity* is an important property in the field of bone regeneration, as it is the ability of a scaffold to induce a positive biological response (such as the promotion of cell adhesion and protein production) and to interact actively with surronding tissues without damaging them. Bioactive ceramics include bioactive glasses, calcium phosphates or silicates; this paper will mainly deal with calcium phosphates, as they were the main focus of the experimental work [43], [44].

8.1.1. Calcium phosphate ceramics

Calcium phosphates (CaP) are perhaps one of the most widely used ceramics in the area of bone regeneration, especially thanks to their similar chemical composition with the mineral phase of natural bone, which allows them to bind easily to bone tissue and to promote cell adhesion. They show some good characteristics, that goes from high biocompatibility, biodegradability and bioactivity, but it is important to rembember that all these properties strongly depend on the Ca/P ratio, crystallinity and phase composition. There are various types of CaP ceramics that clearly show different properties and biological effects in vivo, this paper will deal with hydroxyapatite and tricalcium phosphate.

Hydroxyapatite

Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) is the most stable CaP ceramic, bioactive but not resorbable (Fig.1). However, non-resorbability is not considered as an obstacle that might limit its use, because HAp is one of the major mineral components of the natural bone. HAp is osteoconductive but not osteoinductive, and it can be of both natural and synthetic origin. The natural one can be obtained from animal sources (mammalian bone, mussel and crab shells) eliminating the organic part; while the synthetic one, can be prepared trough different methods, for example the hydrotermal method, solid-state reactions, chemical precipitation and so on, but this type of HAp lacks some important

ions such as magnesium, potassium, strontium, silicon, natrium and iron, which are responsible for noumerous positive bilogical effects. The ready availability, low processing costs and better biodegradability make natural HAp the preferred option for biomedical applications. Unfortunately, HAp has poor mechanical properties, like high brittleness and low fracture toughness, and this limits its use for load-bearing bone applications [44],[42].

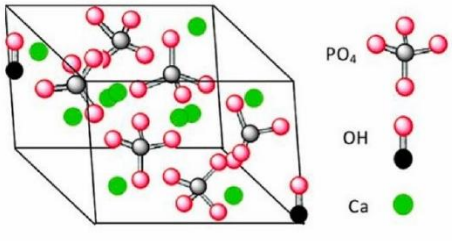

Fig.1. *Crystal structure of a HAp unit cell [15].*

Tricalcium phosphate

Tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) is another member of CaPs' group with a similar chemical composition to mineral phase of bone tissue. TCP occurs in two different crystalline forms: α-TCP and β-TCP (Fig.2); even if β-TCP (β-$Ca_3(PO_4)_2$) is the most used in bone regeneration field due to its major biocompatibility, resorbability and better osteoconductivity. β-TCP is synthesized through a thermal conversion, above 650-750°C, of amorphous calcium phosphate, by solid-state reactions or by precipitation. According to the method used, it is possible to obtain β-TCP in powder or granules, of different size and shape. In terms of biodegradability, β-TCP degrades quicker than HAp under in vivo conditions, and furthermore unlike HAp it is reabsorbed over a span of 10 months to 2 years. Also, β-TCP has poor mechanical properties, it is brittle and it shows a higher fracture toughness than HAp, so this limits its use alone in load-bearing applications [45],[43].

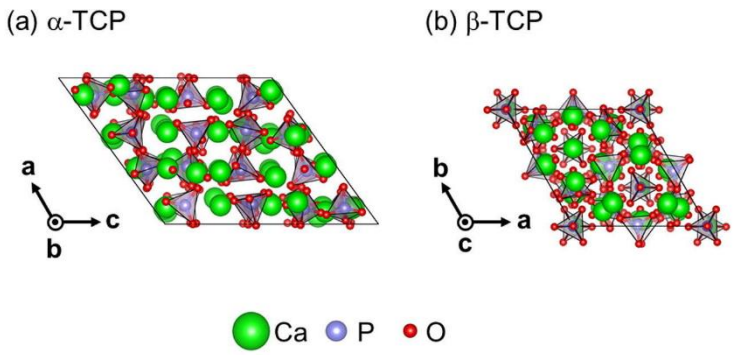

Fig.2. *Crystal structure of α-TCP (a) and β-TCP (b) [16].*

Now, it is good to deepen the discussion on ceramic composite polymers, going to see what possible combinations can be and what effects they have on scaffolds' properties. To maximize the desiderable properties of both ceramics and polymers, CaPs are combined with various biopolymers to produce 3D printed scaffolds for bone regeneration. Numerous researches were conducted on the combination of CaPs and polymers, for example, considering HAp, it was tested a combination with PLA: PLA/HAp at a mass fraction of 25%, and a study was published on *European Journal of Orthopaedic Surgery & Traumatology*. Mechanical tests revealed a more than twofold increase in Young's modulus, therefore this amount of HAp is too much because it leads to an increase in PLA brittleness. An *in vitro* evaluation test was carried out after removing the scaffold from a SBF solution, which shown an increase in calcium absorption by the sample after 28 days and in apatite formation [46]. Furthermore, the addition of HAp has reduced the degradation time of PLA. Another combination examined by a work group of the *Department of Oral and Maxillofacial Surgery, Massachussetts General Hospital and Harvard School of Dental Medicine, Boston*, is between PCL and β-TCP: PCL/ β-TCP at a mass fraction of 50%. Simulations were carried out, and what was found is an increase in Young's modulus as the new bone grews; this does not allow the scaffold to collapse before it has performed its function. Therefore, scaffolds with this combination, given their high stiffness, turn out to be suitable for large defects reparing. Furthermore, due to the presence of calcium phosphates, an increase in bioactivity and cell adhesion has been shown [47].

8.2. 3D printing techniques for composite materials

The method of choice for creating customized devices, starting from composite materials, is 3D printing, since it achieves an excellent combination of flexibility and high performances. As mentioned in the previous chapters, 3D printing is a process in which objects are created layer-by-layer starting from a 3D model built with computer-aided design (CAD) or obtained through imaging systems (MRI, CT…). Following the commonly used 3D printing techniques are reported.

8.2.1. Fused deposition modeling

Fused deposition modeling (FDM) is one of the most used 3D printing techniques when dealing with thermoplastic polymers such as PLA, PCL, ABS etc, etc. In FDM the starting material is in the form of filament and the working principle is extrusion and deposition. The filament is loaded into the printer, then at nozzle level it melts into a semi-liquid state in order to be extruded layer-by-layer onto the bed platform, where the layers are fused together to create the final object. Clearly the quality of printed objects varies according to the printing parameters chosen. This technique has a lot of advantages such as low cost, high speed, semplicity of use or the deposition of different materials simultaneously. But, however, it has some drawbacks linked to the state of starting materials which must be in the form of a filament: it is difficult to produce composite materials with homogeneously disperse reinforcements and to remove the voids created during manufacturing process [48].

8.2.2. Powder-liquid 3D printing

Powder-liquid 3D printing (3DP) technique was developed at the Massachusetts Institute of Technology (MIT) in 1993. 3DP involves a starting material in the form of powder. At first, powders are placed on the bed platform and then using a liquid binder, deposited by an inkjet printhead able to move in X-Y direction, it is possible to create a patterned layer. Then after creating the first 2D layer, the bed goes down and, in the same way, another layer is formed, until to obtain the final product. The quality of the final object and its internal structure can be controlled varying the amount of deposited binder, as well as its

deposition speed and viscosity, or powder size. The advantage of this technique is surely the flexibility of usable materials since any polymer in powder state can be printed [48].

8.2.3. Selective laser sintering

Selective laser sintering (SLS) is a technique similar to 3DP, as both use a powder as starting material, but there is a difference in the method employed for joining powder particles, in fact, the binder used in 3DP is replaced by a laser beam which scans the powders to sinter them by heating. Using high laser power and by exploiting molecular diffusion, the powder particles are fused together to form the first patterned layer, then the bed goes down and another layer is created. Theoretically, any polymer in powder state can be used, but due to the complex consolidation behaviour and molecular diffusion process, the choice of materials is limited (usually polycaprolactone and polyamide are used) [48].

9. Sterilization process

After discussing about what ceramic composite materials are, their properties and use in biomedical field, it is important to talk about the sterilization process, since all biomaterials involved in cellular and animal experiments or clinical applications, must be sterilized before being used. Sterilization is any physical or chemical process in which all pathogenic organisms such as viri, viroids or bacterial spores that lie on the surface of an object are destroyed, in order to minimize the risk of infection. The EN 556 standard establishes the sterilization safety level, namely SAL (Sterility Assurance Level), which specifies the probability of finding a living microorganism within a sterilization batch: a SAL limit of 10^{-6} is considered acceptable. According to FDA sterilization methods are divided into two categories: Category A and Category B. Category A methods are dry heat, ethylene oxide (EO), steam and radiation sterilization, while Category B methods include hydrogen peroxide and ozone. The difference between the two is: methods belonging to Category A have a long story of safety and efficacy, having standards for validation and process control recognized by FDA, while methods of Category B have no FDA-recognized standards, although there is a dense literature on development, validation and process control [49]. Beyond the chosen method, implantable devices are always labeled 'sterile', and the sponsor has to submit all documents as listed in the guidance of 'Submission and Review of Sterility Information in Premarket Notification (510(k)) Submissions for Devices Labeled as Sterile' [50]. After explaining what sterilization process is, it is important to point out that it is a process thay may affect physical and mechanical properties of the material.

9.1. Traditional sterilization methods

Let's talk about sterilization techniques for polymers, starting from methods of Category A:

Dry heat sterilization

Dry heat sterilization is one of the most common sterilization techniques used for heat-resistant polymers (such as polypropylene, silicone, polyurethane), heat-sensitive polymers cannot be sterilizied in this way. The sterilization is performed using an oven at high temperature: the duration and temperature are chosen depending on the targeted

microorganisms, which are eliminated by coagulation of proteins. It is important to point out that the chosen temperature must be lower than the melting temperature of the polymer. A typical dry heat sterilization lasts about 2 h at a temperature of 160°C [49].

Steam sterilization

Steam sterilization is a technique used for polymers that are heat and moisture resistant. Sterilization occurs using a combination of heat, moisture and high pressure, and takes place in an autoclave. Increasing the pressure, the high temperature steam impacts on the polymer surface destroying all microorganism by coagulation and denaturation of structural proteins. After this process, the sterilized object is removed from the autoclave. The adavantage of this method, respect to dry heat technique, lies in being able to perform a complete sterilization at lower temperatures and shorter times due to the presence of moisture which favours heat penetration. A typical steam sterilization lasts about 20-60 min at a temperature of 121°C [49].

Ethylene oxide (EO) sterilization

Ethylene oxide (EO) sterilization is one of the most used techniques for all polymer that are not resistance to heat or moisture, since in this method lower temperatures (lower than glass transition temperature) are achieved. EO gas is used, a colorless gas with a boiling point of 10.4°C, and its alkylating power (i.e. the transfer of an alkyl group from one molecule to another) is exploited. Sterilization is divided into several phases: the first stage involves the removal of about 97% of oxygen from the sterilization chamber making a deep vacuum, then the sterilization chamber is heated and humified to regain the lost moisture. At this point EO gas is introduced in the chamber at a pre-determined pressure and for a certain amount of time (about 3h). Then the EO is removed, and a series of nitrogen washes are made. To complete the cycle, the sterilization chamber is brought to atmospheric pressure. The sterilization efficiency depends upon the concentration of EO gas, temperature, humidity and gas exposure duration. A typical EO sterilization lasts about 10 h at temperatures ranging from 30°C to 60°C. A limitation of this methods is the toxicity of possible EO residual post sterilization [49].

Radiation sterilization

Radiation sterilization is a technique that involves the use of ionizing radiations to sterilize the material. Radiations destroys microorganisms by breaking down their DNA and thus inhibiting their division. It is possible to have two sources of radiation and thus to have two possible methods of sterilization: Gamma sterilization, that uses high-energy gamma rays, and Electron beam sterilization, which utilizes a constant stream of high-energy electrons. The process involves the exposure of the material to one dose of radiation between 15-45 kGy for a certain period of time. The sterilization efficiency depends on radiation dose, temperature and duration of the process. This sterilization method has the advantage of being a relatively fast process, but it has some drawbacks such as the possibility of the degradation of polymer (i.e. breaking of molecular bonds) caused by radiation beam [49].

Now let's discuss about the methods of Category B:

Hydrogen peroxide (H_2O_2) sterilization

H_2O_2 is a chemical compound with a microbicidal function: it destroys microorganisms by generating oxidative stress, since it produces reactive oxygen species that attack specific targets such as nucleic acids, enzymes and lipids. Depending on how H_2O_2 is used, it is possible to have two types of sterilization: vaporized hydrogen peroxide (VHP) sterilization and hydrogen peroxide plasma (HPP) sterilization. VHP sterilization consists of three phases: vacuum generation, H_2O_2 injection and areation. It can be used for all those materials that do not withstand high temperatures and moisture since the range of process temperatures is between 25-50°C. A typical VHP sterilization lasts about 1.5 h. While HPP sterilization consist of a cycle of four phases: vacuum generation, H_2O_2 injection, diffusion and plasma discharge. This technique bases on the action of the combination between H_2O_2 and generation of free radicals during the plasma stage of the cycle. In this process the temperatures varies between 40-65°C and the duration takes 1-3 h [49].

Ozone sterilization

This technique involves ozone as sterilizing agent since it is a strong oxidative gas able to inactivate numerous pathogen organisms. This process consist of three stages: vacuum

creation, humidification of materials and generation of ozone. As mentioned above, since ozone has a strong oxidative power, it is important to point out that the polymers subjected to this type of sterilization must be resistant to oxidation. Compared to the other techniques ozone has a greater penetration power than H_2O_2 and unlike the EO, it leaves no toxic residual on the surface of the sterilized material [49].

9.2. Novel sterilization techniques

In addition to traditional techniques, new methods have been experimented to sterilize biomaterial, and here are some of them.

Vaporized peracetic acid (VPA) sterilization

This technique involves peracetic acid (PAA) to carry out the sterilization. Peracetic acid ($C_2H_4O_3$) is a peroxide, namely a chemical compound containing the functional group O-O. It is produced by the reaction between acetic acid and hydrogen peroxid and it has a remarkable microbiocidal function, since it is able to oxidate some cellular components such as enzymes and proteins. PAA has low stability and decomposes easily, but fortunately decomposing products (acetic acid, water and oxygen) are not toxic. Despite this, PAA has a great oxydating power and acidity, which can be affect pysico-chemical and mechanical properties of the material. VPA sterilization consist of four phases: chamber evacuation, chemical injection *via* vaporization of PAA, chamber dehumidification and chamber ventilation, all performed at room temperature. Since it does not require high temperatures, it is suitable for all those heat resistant polymers. A typical VPA sterilization is completed in 2-4 h [49].

Ultraviolet light (UV) sterilization

In this method, UV rays, with a wavelength between 328 and 210 nm, are used to kill all pathogenic organisms. As source of UV light are usually employed mercury lamps. Surely, UV rays do not have the same penetrating power as ionizing radiations (used in traditional methods), but neither do they have all their drawbacks [49].

High intensity or pulse light (PL) sterilization

Pulse light sterilization is an emerging technique to sterilize materials. Short duration pulses of broad-spectrum light are used to sterilize the surfaces destroying all microorganisms present, but since it is a novel method, the specific mechanism is already unclear [49].

Microwave sterilization

Microwave sterilization involves the use of nonionizing radiation combined with low-pressure steam to produced localized heat that interfere with cell membranes of microorganisms and it kill them. Temperatures achieved with this technique are lower than those of the traditional steam sterilization methods, so it can be used for all those biomaterials that are not heat resistant. Unfortunately, being an emerging method there are still few pubblication on it [49].

9.3. Regulations

After explaining the various methods for sterilizing a material, it is important to highlight the regulatory aspect behind the sterilization process. It is fundamental to adapt the sterilization procedure to current European regulations to be able to define a device as sterile. That means to achieve certain technical characteristics and to carry out specific tests, reported in the harmonized standards made available by CEN, in order to verify the matching of the product with minimal requirements of Directive 93/42. It is important to remember that harmonized standards are mandatory since all community directives are mandatory for the member states of the European Community. Among all various standards laid down by CEN, UNI EN 556 and UNI EN 285 deserv particular attention.

"UNI EN 556 is the standard which reaffirms that the safety of a sterilized product devoid of viable microorganisms cannot be stablished except in terms of the probability of survival of the microorganisms. It is generally considered that a product can be qualified as sterile when the probability that a single viable microorganism is present is equal to or less than 10^{-6}". [50]

“UNI EN 285 is the standard referred to ‘large’ sterilizers (sterilization chamber with a capacity of a volume of 30x30x60 cm) and it describes in detail:

- design principles related to dimensions, materials and safety
- the characteristics of the regulation / monitoring / recording devices
- the tests to which they must be subjected, divided into prototype tests, factory tests, installation tests
- the methods for carrying out these tests, the reference loads to be used, the minimum results to be obtained.” [50]

10. Materials and methods

10.1. Design of materials

The first part of the project involved the design of biodegradable and bioresorbable composite materials usable for creating implantable customized devices for bone regeneration process. The choice fell on polyester-type polymer matrix composite materials, loaded with calcium phosphate-type ceramic modifiers. The polyesters used are polylactic acid (PLA) in its enantiomeric form poly(D-lactic) acid (PDLA) and polycaprolactone (PCL); while the ceramic modifiers employed are hydroxyapatite (HAp) and tricalcium phosphate (TCP) in its β-form (β-TCP). Two combinations were created: PDLA/Hap with HAp at a mass fraction of 10% and PCL/β-TCP with β-TCP at a mass fraction of 20%. PLA and PCL have been chosen as matrix because are considered the most biocompatible, bioresorbable medical grade polymers FDA-approved, with mechanical properties such as to withstand loads and stresses generated by surronding tissues. Furthermore, from the point of view of processing, PLA and PCL are the easiest polymers to use for 3D printing. While HAp and β-TCP have been chosen as reinforcement since are the ceramic materials with the most similar composition to natural bone (HAp is a component of natural bone and β-TCP is bioresorbable), and which make up for the lacks of the polymer in an optimal way. The properties of HAp and β-TCP have been widely discussed in the previous chapter. The percentage of calcium phosphates (CaP) in the composite was calculated in order to make the material manageable during the printing process, and to maximize the properties of the filament: an excessive amount of CaP would have made the material too brittle, conversely too low a percentage would not have made it possible to meet the requirements. From a technical point of view, the designed materials have been produced by Nadir S.r.l (Venezia), in the form of filaments (diameter equal to 1.75 mm) (Fig.1). Other two filaments of neutral PLLA (provided by Ultimaker, diameter equal to 2.85 mm) and neutral PCL (provided by Nadir S.r.l, diameter equal to 1.75 mm) were used to make a comparison with the designed materials. Regarding production process of the different filaments, the polymer has been printend by extrusion after mixing with ceramic powders. Details of the process were not provided due to the company secrecy.

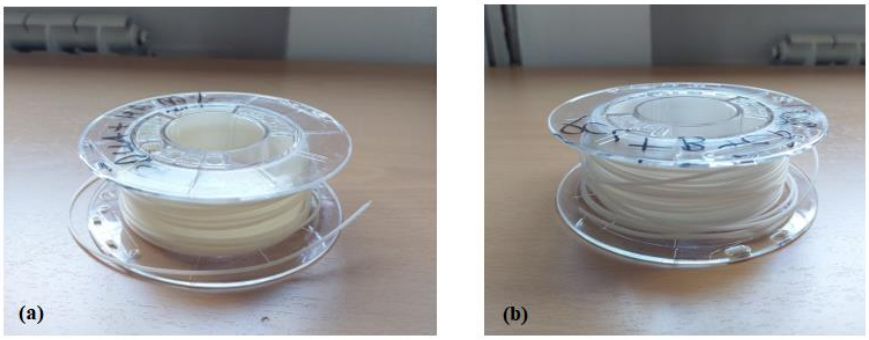

Fig.1. *Filament of PDLA/HAp 10% (a) and filament of PCL/ β-TCP 20% (b).*

10.2. 3D printing

After the design of the materials, the development phase took place, in which the 3D printing technique, in particular the Fused Deposition Modeling, (described in the previous chapters) was used to create specimens of regular shape. Two different 3D printers were used depending on the filament employed: “Original Prusa MINI+” and “Ultimaker S3”, as shown in the figure (Fig.2); but the same software, called Cura, was used to create the printing project. The “Original Prusa MINI+” supports filament with a diameter of 1.75 mm, so it has been used to print neutral PCL, PCL/β-TCP and PDLA/HAp samples; while to print neutral PLLA specimen, it has been used the “Ultimaker S3” because the PLLA filament was only available with a diameter of 2.85 mm. Eight samples were printed for each type of material, with dimensions in compliance with UNI EN ISO 178 standard: 70 mm length, 30 mm width and 8 mm height, as it is possible to see in the figure below (Fig.3). Since the materials have different physical characteristics, numerous tests were conducted before the optimal printing parameters were found. For example, a problem that initially occured, was the detachment of the sample from the bed, before printing was completed. In particular, this has been encounterd for PCL, which is more difficult to manage than PDLA. Therefore the bed temperature has been adjusted: for neutral PCL it has been set on 30°C because was the optimal value without melting the polymer; while for PCL/β-TCP it has been set on 60°C, since PCL is more susceptible by ceramic modifiers, which change the melting

temperature of the polymer and they make possible the increase of bed temperature. Furthermore, a special adhesive, called *Magigoo 3D printing adhesive*, has been spread on the bed to make the specimens adhere better during printing. Instead, for PDLA, these problems have not occured, since it has a better thermal stability and thus the bed temperature was kept constant on 60°C for both neutral PLLA and PDLA/HAp specimens. The chosen parameters are reported in the table below (Table 1). The printing time of each specimen was about 11 min with "Original Prusa MINI+" and about 8 min with "Ultimaker".

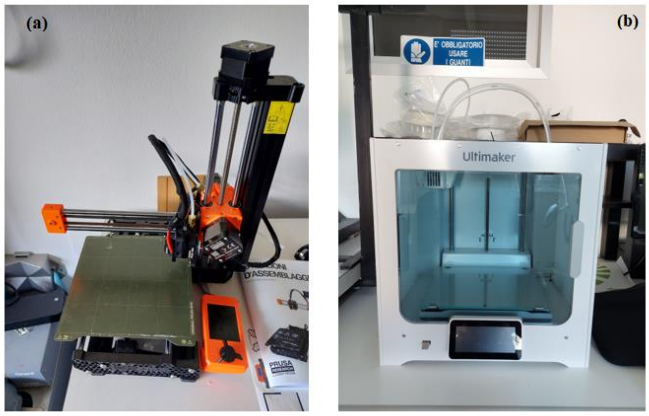

Fig.2. *"Original Prusa MINI+" printer (a) and "Ultimaker S3" printer (b).*

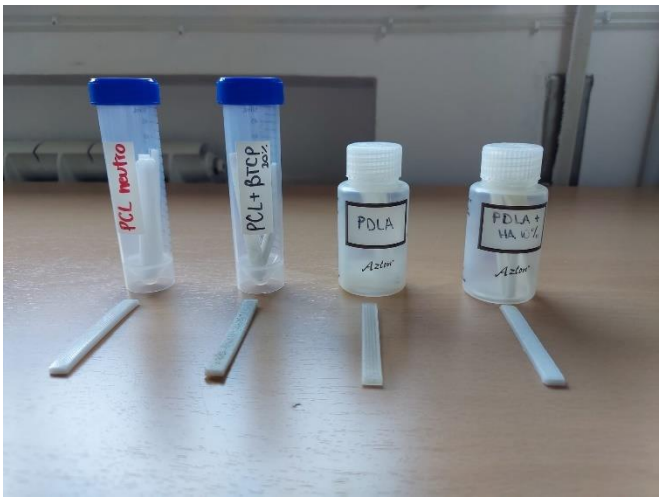

Fig.3. *Specimens printed following ISO 178 standard, in order: netural PCL, PCL/ β-TCP, neutral PLLA and PDLA/HAp.*

Table 1. *Printing parameters of neutral PLLA, PDLA/HAp, neutral PCL and PCL/ β-TCP.*

Material	Filament diameter (mm)	Printing velocity (mm/s)	Bed temperature (°C)	Nozzle temperature (°C)
PLLA	2.85	100	60	210
PDLA/HAp 10%	1.75	100	60	210
PCL	1.75	100	30	174
PCL/β-TCP 20%	1.75	100	60	215

10.3. Sterilization

After printing the specimens, the sterilization phase occurred, since all biomaterials, for the reasons described in the previous chapters, must be sterilized. Since the specimens are made of non-heat resistant polymers, it has not been possible to use a traditional sterilization method, so it has been developed a novel method based on the immersion of the specimens in a solution of peracetic acid (PAA). In particular, it has been employed a sterilizing solution of 10% PAA in physiological solution. First, this solution has been placed in a megnetic stirrer for about 30 min without heating, in order to eliminate any type of particles present inside the solution itself. Then, four of the printed samples of each type (4 of neutral PLLA, 4 of neutral PCL, 4 of PDLA/HAp and 4 of PCL/β-TCP) have been placed in four different containers, and they have been covered with the prepared solution, as it is possible to see in the figure below (Fig.4). Specimens have been left in the solution for 12 min, then they have been extracted through the use of steel tweezers (so as not to contamine them with the hands) and placed in four different sterile plastic test tubes without using any drying method (Fig.5).

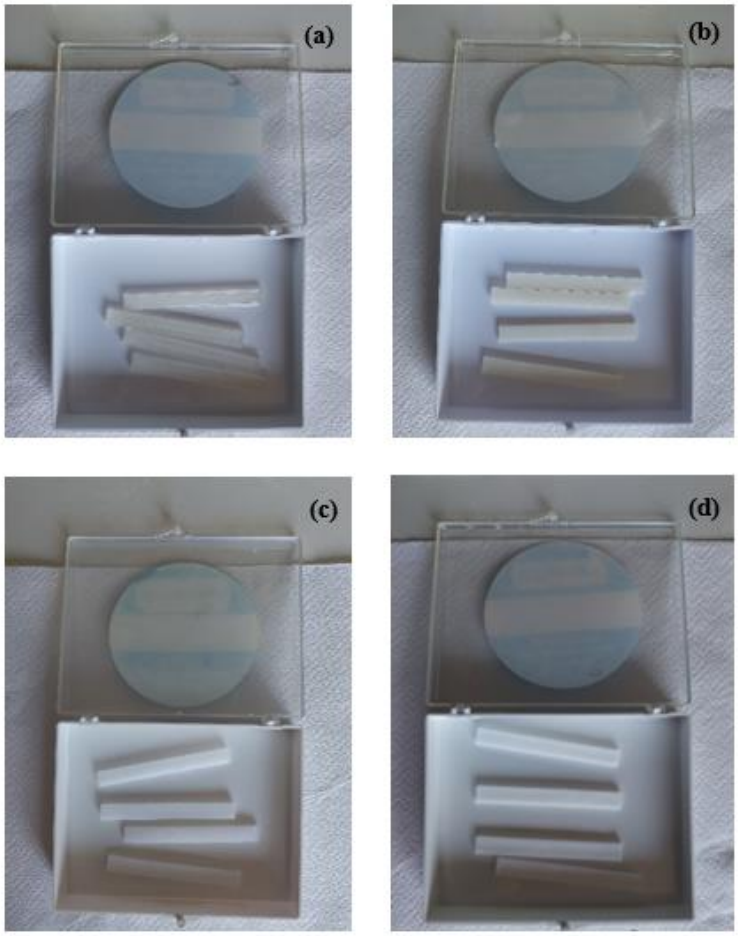

Fig.4. *Sterilization of specimens: neutral PLLA (a), PDLA/HAp (b), neutral PCL (c) and PCL/ β-TCP (d).*

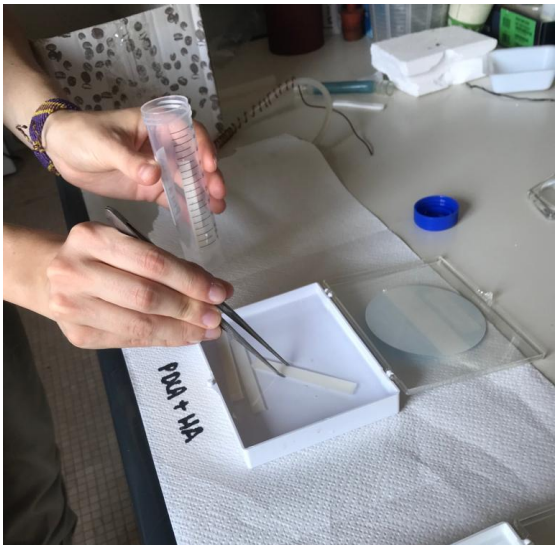

Fig.5. *Extraction of the specimen and placing in sterile plastic test stubes.*

10.4. Mechanical tests

After the sterilization phase, mechanical bending test have been carried out both on sterilized and non-sterilized specimens, in order to evaluate their mechanical behaviour and to understand if the sterilization process have modified the properties of the materials. Mechanical tests on sterilized specimens have been made 96 h (4 days) after the sterilization process, because the samples were still wet; otherwise if they had dried up it would not have been possible to tell if sterilization was affecting the mechanical properties in some way. Static and dynamic mechanical bending tests have been performed on the specimens, as required by UNI EN ISO 178 standard. Bending tests comprise both tensile and compression tests since the upper part of the specimen is subjected to compression while the lower part is subjected to traction.

10.4.1. Dynamic mechanical bending tests

First, dynamic mechanical bending tests have been carried out on two sterilized and two non-sterilized specimens per type (2 of neutral PLLA, 2 of neutral PCL, 2 of PDLA/HAp and 2 of PCL/β-TCP), since they are non-destructive tests. Dynamic Mechanical analysis (DMA) is a technique used to obtain information on mechanical properties of a material subjected to sinusoidal oscillating force. With DMA, it is possible to measure stiffness and damping (in terms of energy disspation) of a material, reportered as modulus and phase shift. In this case, the modulus obtained is expressed as a complex number: the real part is called *storage modulus* (i.e. the energy stored by the material and released when the sollecitation is finished) while the imaginary part is called *loss modulus* (i.e. the energy dissipated by the material due to its permanent deformation). So, with DMA through the distinction of the two moduli, it is possible to discriminate elastic and viscous behaviour of the material. A prototype for DMA has been developed by Dipartimento di Ingegneria delle Costruzioni, dell'Ambiente e del Territorio (DICAT) at Università degli studi di Genova, to perform dynamic mechanical tests. The machinery (Fig.6) consists of:

- *shaker*: that permits dynamic stimulation of the sample
- *amplifier*: that is linked to the shaker and it generates variable-frequency sinusoidal waves
- *load cell*: that transforms the applied stress into an electric signal
- *accelerometer*

- *sensor signal conditioner*: that recieves the signal from load cell and transform it in tension
- *laser vibrometer*: that is orientated perpendicularly to the plate (on which the sample is placed) and linked to the shaker. It notes the position of the sample through the vertical displacement of the plate.
- *software*: developed using LabView

Fig.6. *Prototype for DMA used to conduct analysis on specimens.*

A number of parameters have been set before using the machinery, which are reported in the table below (Table 2).

Table 2. *Parameters set for dynamic mechanical tests.*

Distance between supports (mm)	Oscillation frequency (Hz)	Proportionality constant of laser (µm/V)
30	1-100	80

Then, after correctly positioning the specimen on the supports, calibration must be carried out: an *indenter* (metal bar) must be brought closer to the sample and the zero must be set when the metal bar touches the specimen. Meanwhile the samples have been measured with a centesimal precision digital gauge and the correct dimensions have been entered

into the software. At this point a pre-load, expressed as a percentage of displacement with respect to specimen thickness, is chosen. In particular, three different values of pre-load have been set: -10%, -20%, -30%. As outcomes, there are two frequency output signals: stress and deformation. These signals, through the capture card, are sent to the computer and elaborated by LabView software. The tests carried out thus make it possible to assess the elastic and viscous moduli (of the studied specimens) as a function of frequency. Therefore, by evaluating these trends it is possible to understand the nature of the designed materials: if the *storage modulus* is greater than *loss modulus* the sample has mainly elastic behaviour (with typical features of an elastic solid), otherwise the sample has a viscous behaviour (typical of fluids).

10.4.2. Static mechanical bending tests

Static mechanical tests have been performed after dynamic ones since they are destructive tests. In particular, three-points bending tests have been carried out, that is to say tests in which there are two lateral supports with symmetrical central load (Fig.7). The typical outcome of a static mechanical test is the stress-strain curve. Whenever a load acts on a body, it produces stress as well as strain in the material: the increasing force applied and the strain produced are recorded until a fracture occurs, and then they are plotted on an X-Y graph, on X-axis the strain in percentage and on Y-axis the stress in MPa (the unit of measurement have been chosen in accordance with the ISO 178 standard). So, the stress-strain curve is a graph that shows the change in stress as strain increases. For a ductile material the typical form is the following: a first straight section defines the elastic region of the material where the relantioship between stress and strain is linear; then there is the yield point, that is the transition zone from elastic to plastic region, which is characterized by a flattening of the curve where the strain increases at a faster rate than stress, up to a possible fracture of the material. From a stress-strain curve it is possible to evaluate elastic modulus in bending, flexural strength etc, etc… of the material in order to assess its elasticity, ductility or reproducibility. Tests have been performed on three sterilized and three non-sterilized specimens per type (3 of neutral PLLA, 3 of neutral PCL, 3 of PDLA/HAp and 3 of PCL/β-TCP). A universal machine for mechanical tests (UTM) has been used to carry out static mechanical tests. This machine is defined as universal because it can be used to execute multiple static tests including tensile and

compression tests, as well as bending tests. A traditional UTM consist of load cell, crosshead, strain gauge, grip, electronic and drive system; and it is managed by a software used to establish machine and store test parameters defined by international standards. In particular, the laboratory has the machine Zwick/Roell Z050: a double column machine, with a single moving screw that transfers the force to the sample (Fig.8). It supports maximum test force of 50 kN and it is equipped with load cell of 1 kN. The machine has two sensors: the load cell to measure the applied force and another sensor to measure the vertical displacement of the moving crosshead, from which the deformation of the specimen is then obtained. Through the testXpert test software, it has been possible to enter the dimensions of the specimens after having measured them with a digital gauge. Test have been conducted following the UNI EN ISO 178 standard, which specifies a method for determining the flexural properties of rigid and semi-rigid plastics under defined conditions. The static mechanical tests parameters, chosen according with this standard, are summarized in the table below (Table 3). Let's explain the meaning of some parameters reported in the table: *pre-load* is the minimum load above which the machine starts acquiring data. *Pre-load velocity* is the velocity of the machine during the pre-load phase. *Modulus velocity in bending* that is the bending velocity in the region in which the material behaves as an elastic solid, this value of velocity is kept between 0.05% and 0.25% of the deformation (*modulus range in bending*). In this region the velocity of the test must be less than the velocity of the whole test because it is necessary to acquire more points to easily calculate the elastic modulus.

Table 3. *Parameters set for static mechanical tests.*

Pre-load (N)	Pre-load velocity (mm/min)	Modulus velocity in bending (mm/min)	Test velocity (mm/min)	Modulus range in bending (%)	Distance between supports (mm)
0.5	5	1	5	0.05-0.25	32.5

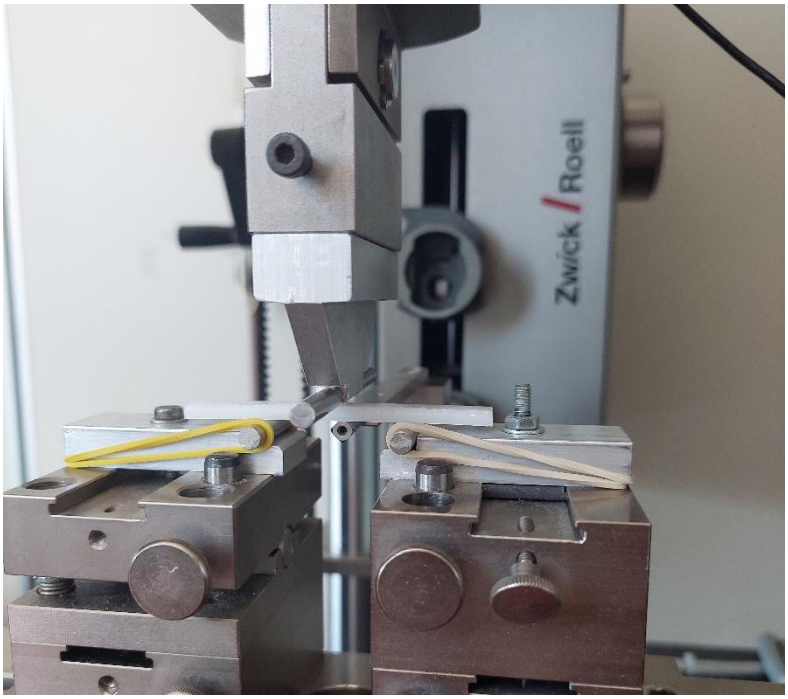

Fig.7. *Example of a three-points bending test conducted on printed samples.*

Fig.8. *Zwick/Roell Z50 universal machine for mechanical test used to conduct mechanical bending tests.*

11. Results and discussion

From *static mechanical tests* it has been possible to obtain the following results: stress-strain curves for each type of material before and after sterilization, useful to evaluate their mechanical properties. From stress-strain curves of non-sterilized and sterilized specimens, it has been possible to obtain flexural modulus (E_f) in MPa, flexural strength (σ_{fM}) in Mpa and flexural strain at flexural strength (ε_{fM}) in percentage. In general, the flexural strength is represented by the value of the maximum load that the material can bear before breaking, and therefore the flexural strain is the value of strain corresponding to maximum load. It is important to note that the value of flexural modulus (E_f) or Young's modulus shown in the table, is not the value returned by the machine, but it has been recalculated starting from the stress-strain curve, so as to have a more precise result. To calculate E_f, a linear regression, between stress and strain variables, has been performed and the maximum value has been taken as Young's modulus.

Let'analyze results before and after the sterilization process.

Before sterilization

Let's start talking about results obtained from the tests conducted on neutral PLLA and PDLA/HAp composite.

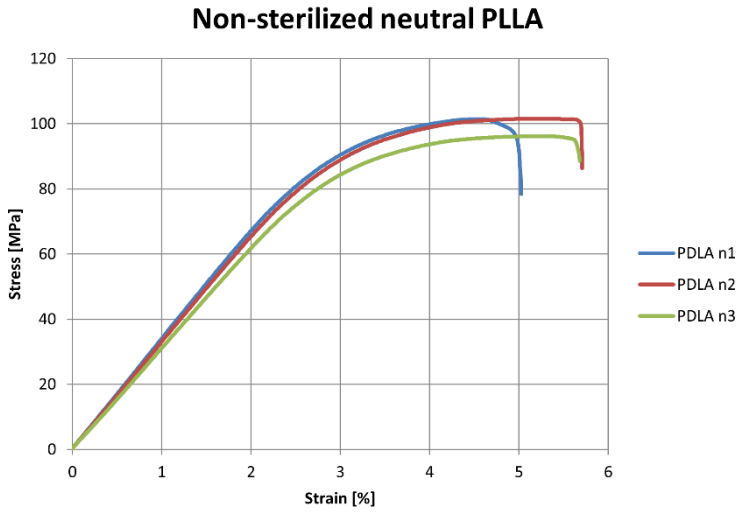

Fig.1. *Stress-strain curve of non-sterilized neutral PLLA specimens.*

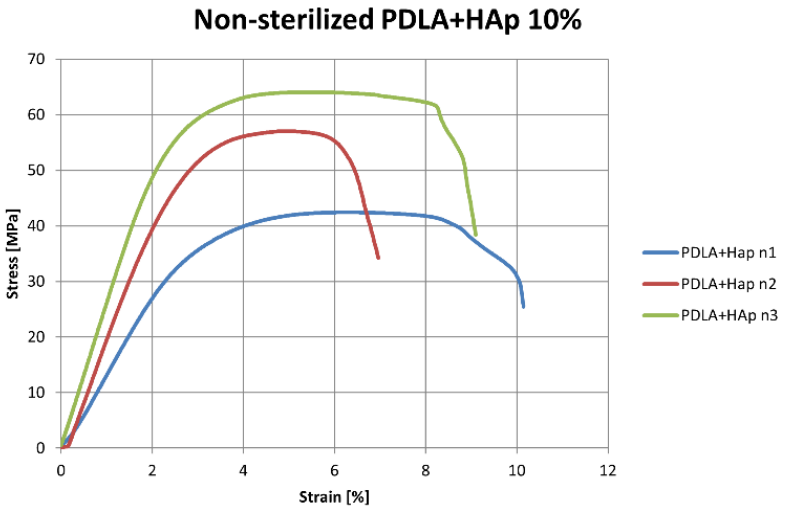

Fig.2. *Stress-strain curve of non-sterilized PDLA/HAp specimens.*

In the tables below (Table 1, Table 2) are summarized the quantities and their average values, extracted from the stress-strain curves of non-sterilized neutral PLLA and non-sterilized PDLA/HAp 10%.

Table 1. *Results for non-sterilized neutral PLLA specimens and average values.*

	E_f (MPa)	**σ_{fM} (MPa)**	**ε_{fM} (%)**
PLLA n1	3468,9	101,40	4,55
PLLA n2	3361,1	101,52	5,09
PLLA n3	3167,9	96,19	5,24
Average values	3332,6	99,70	4,96

Table 2. *Results for non-sterilized PDLA/HAp specimens and average values.*

	E_f (MPa)	**σ_{fM} (MPa)**	**ε_{fM} (%)**
PDLA + HAp n1	1496,9	42,44	6,37
PDLA + HAp n2	2463,7	57,03	4,94
PDLA + HAp n3	2654,2	64,07	5,46
Average values	2204,9	54,51	5,59

By observing graphs and tables, it is possible to note that neutral PLLA has a stress-strain curve typical of a low ductile and low tough material, with a high flexural strength.

Young's moduli are high (of the order of 3000 MPa) and similar for the three specimens. Having a high modulus means being a very rigid material, since the greater the Young's modulus greater the stiffness. Neutral PLLA has a good elastic behaviour up to 3% of strain, then there is the yield point and the plastic region. The maximum load, similar for all three specimens, is quite high, so the material will have good flexural strength as was assumed at the beginning. The plateau area is rather short, which means that the material does not undergo large deformations for a constant stress, so it does not have a high elongation at break, as confirm by the literature. These characteristics of the curve indicate a brittle behaviour of the material, i.e. not very ductile and not very plastic. Furthermore, it can be seen from the curves that all three specimens suffered a clean break before the end of the test (set at 20% strain) that is typical of a low ductuile material. It can be seen that the material has broken, because immediately after the plateau zone, the curve decreases istantaneously with a drastic drop in the load bearing capacity. The curves are similar and roughly overlapping, which indicates good reproducibility.

By observing graphs and tables of PDLA/HAp, it is possible to note that the curve is very steep at the beginning and then there is a flattening. Young's moduli are similar for specimens 2 and 3, the specimen 1 has a bit lower modulus, but still they are all high, so the material is quite rigid with a good elasticity. The plateau area is quite large especially for specimens 2 and 3, while it is a little shorter for specimen 1. Anyway, this means that the specimens deform much under costant stress before breaking; this is the typical behaviour of a ductile material. There is some discordance with the maximum load: the value for specimen 1 is lower than that for specimens 2 and 3, so, the specimen 1 is less resistant to bending (it deforms more) that the other two, in fact is also the one with the longest plateau zone. Also in this case, the fracture of specimens occurs before the end of the test (set at 20% strain), but it was not a clean break, there was a gradual subsidence and then a crack. Curves have similar shapes, but there are some differences described above that affect reproducibility somewhat.

By comparing the two tested materials, it can be seen that the addition of HAp has brought few changes in the values of mechanical properties of PLA. Looking at the average values of E_f and σ_{fM}, it can be seen that: the average Young's modulus of the composite material is about thousand times lower than that of the neutral PLLA, which means that the composite material is less rigid than neutral PLLA; furthermore the average value of maximum load of composite is about half of that of the pure PLLA, this means that the

composite will be less resistant to bending but more ductile, so it will deform more before breaking. Hence, the PDLA/HAp composite, having no net breaks, will be more manageable than the pure PLLA, as a clean fracture is never desiderable because it is difficult to control. So the combination between PDLA and 10% HAp actually gave the results we expected: a reduction in the brittleness of PLA, making it more ductile and deformable before breaking.

Now let's discuss results obtained from the tests conducted on neutral PCL and PCL/β-TCP composite.

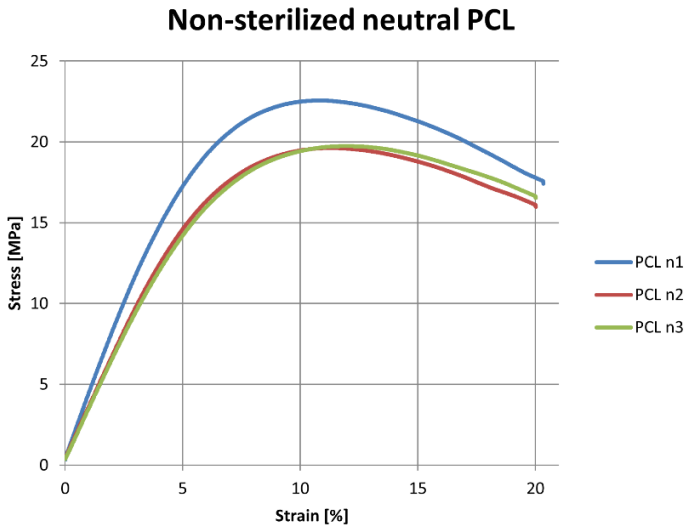

Fig.3. *Stress-strain curve of non-sterilized neutral PCL specimens.*

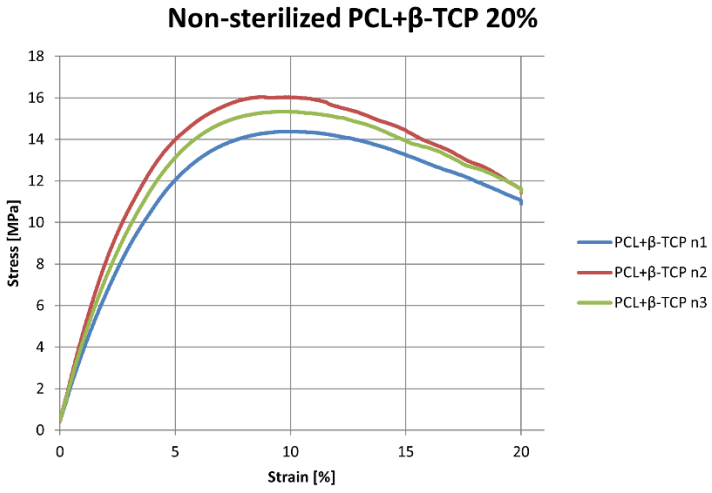

Fig.4. *Stress-strain curve of non-sterilized PCL/β-TCP specimens.*

In the tables below (Table 3, Table 4) are summarized the quantities and their average values, extracted from the stress-strain curves of non-sterilized neutral PCL and non-sterilized PCL/β-TCP 20%.

Table 3. *Results for non-sterilized neutral PCL specimens and average values.*

	E_f (MPa)	σ_{fM} (MPa)	ε_{fM} (%)
PCL n1	429,0	22,56	10,83
PCL n2	355,2	19,62	11,40
PCL n3	322,5	19,75	11,96
Average values	368,9	20,64	11,40

Table 4. *Results for non-sterilized PCL/β-TCP specimens and average values.*

	E_f (MPa)	σ_{fM} (MPa)	ε_{fM} (%)
PCL + β-TCP n1	398,0	14,38	10,04
PCL + β-TCP n2	488,0	16,04	8,76
PCL + β-TCP n3	420,6	15,34	9,71
Average values	435,5	15,25	9,50

By observing graphs and tables of non-sterilized neutral PCL the curves are typical of a ductile material. Young's moduli are similar each other, only the specimen 1 has a slightly higher modulus. The average value of neutral PCL modulus is much lower than that of the neutral PLLA, therefore PCL is a much less rigid material, as confirmed by the literature. The plateau zone is not very pronunced, in fact, after the yield point, the curves reach their maximum value and then decrease slightly. However, during this progressive decrease in stress, there are quite large deformations; in particular, the elongation at break is high and therefore a very ductile material. In this case, the test reached its end (set at 20% strain) and fracture did not occure, since there is no sharp decrease in the curve after the plastic zone. The maximum loads of the three specimens are similar and the average value is of the order of 20 MPa, which is much lower than the neutral PLLA, so the pure PCL is not as resistant to bending as pure PLLA. The curves are roughly overlapping, so neutral PCL has a good reproducibility.

By observing the behaviour of the non-sterilized PCL/β-TCP composite, it is possible to see that the average value of Young's modulus is of the order of 400 Mpa, and the moduli of the three samples are a slightly different. After the elastic region, again there is no real

plateau zone, in fact the curves reach their maximum and then gradually decrease up to 20% strain that corresponding to the end of the test. The specimens have not broken since there are no sharp decreases in the stress-strain curve after the plastic region. So, also in this case the material is very ductile and highly deformable.

By comparing the two tested materials, it can be seen that the addition of β-TCP has brought few changes in the values of mechanical properties of PCL. This reinforcement led to an increase in the Young's modulus of neutral PCL, so as to make it more rigid and improve its ability to whitstand stresses generated during bone regeneration process, thus avoiding possible collapse. The addition of β-TCP has led to a slight decrease in the average value of the maximum load and thus in the flexural strenght, but results in greater ductility.

After sterilization

Now let's see what the effect of sterilization was on the tested specimens.

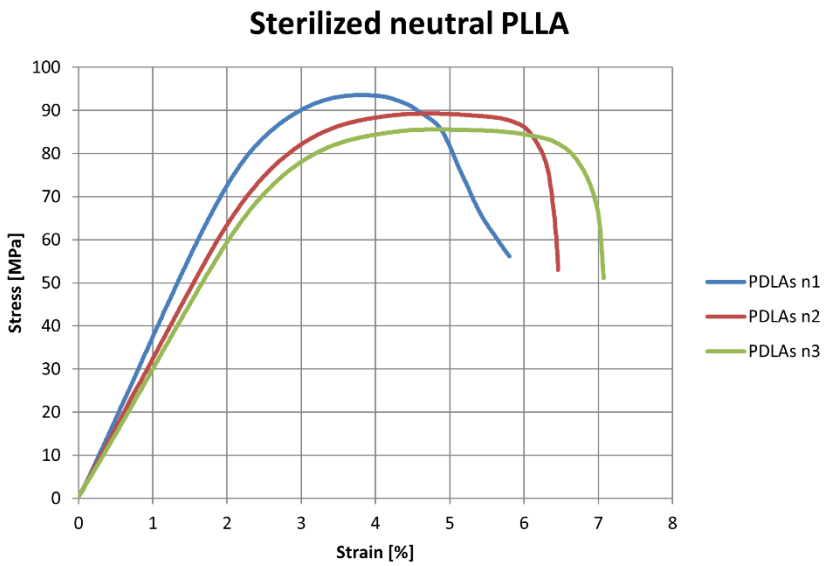

Fig.5. *Stress-strain curve of sterilized neutral PDLA specimens.*

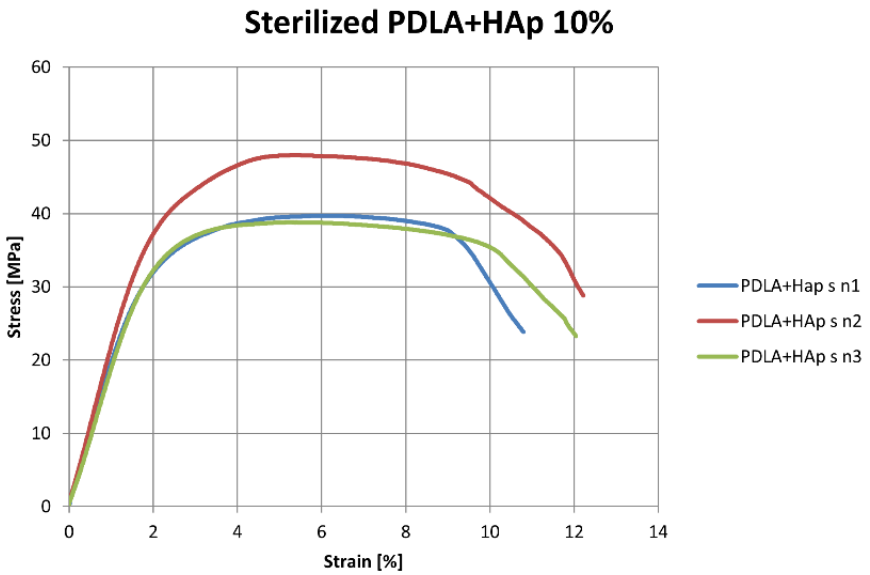

Fig.6. *Stress-strain curve of sterilized PDLA/HAp specimens.*

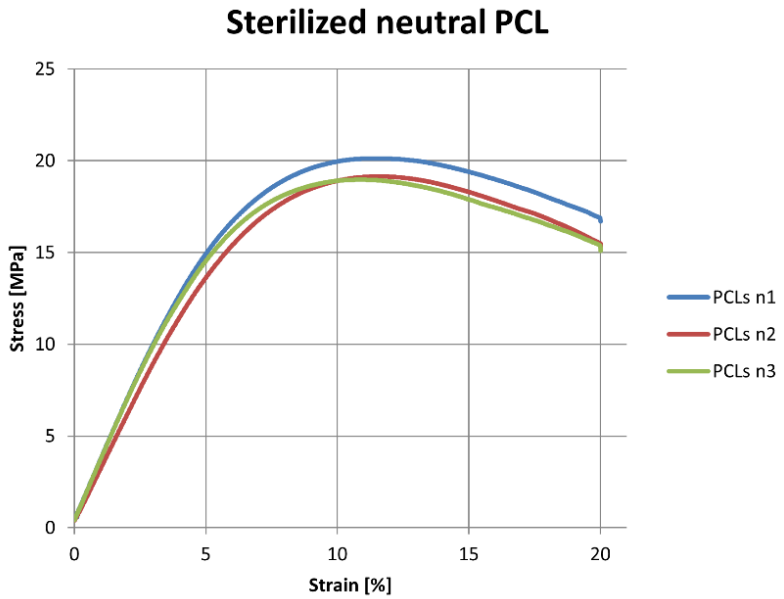

Fig.7. *Stress-strain curve of sterilized neutral PCL specimens.*

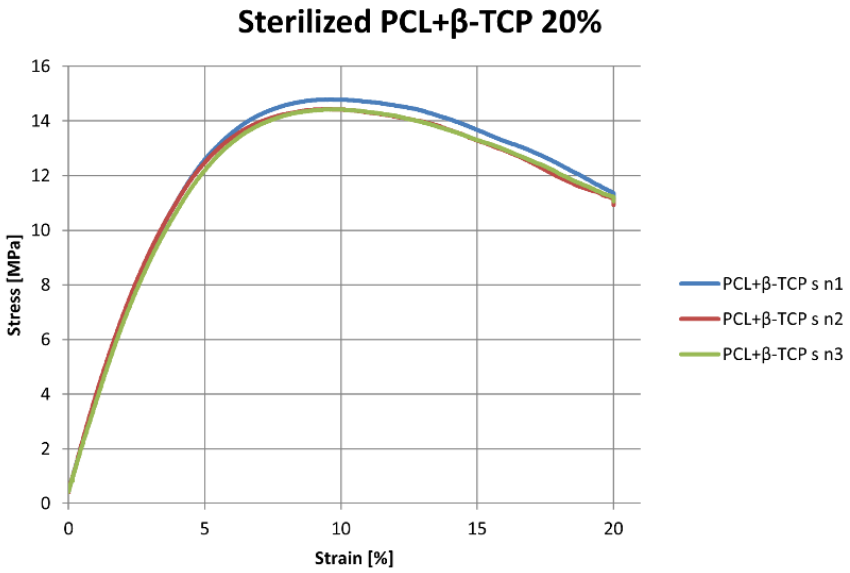

Fig.8. *Stress-strain curve of sterilized PCL/β-TCP specimens.*

In the tables below (Table 5, Table 6, Table 7, Table 8) are summarized the quantities and their average values extracted from the stress-strain curves of all sterilized specimens.

Table 5. *Results for sterilized neutral PLLA specimens and average values.*

	E_f (MPa)	σ_{fM} (MPa)	ε_{fM} (%)
PLLAs n1	3854,2	93,57	3,82
PLLAs n2	3440,0	89,25	4,70
PLLAs n3	3069,7	85,58	4,79
Average values	3454,6	89,47	4,44

Table 6. *Results for sterilized PDLA/HAp specimens and average values.*

	E_f (MPa)	σ_{fM} (MPa)	ε_{fM} (%)
PDLA + HAp s n1	2004,3	39,73	6,13
PDLA + HAp s n2	2263,3	47,98	5,36
PDLA + HAp s n3	1975,4	38,81	5,23
Average values	2081,0	42,17	5,57

Table 7. *Results for sterilized neutral PCL specimens and average values.*

	E_f (MPa)	σ_{fM} (MPa)	ε_{fM} (%)
PCLs n1	365,2	20,13	11,38
PCLs n2	297,3	19,15	11,68
PCLs n3	357,6	18,97	10,89
Average values	340,0	19,42	11,31

Table 8. *Results for sterilized PCL/β-TCP specimens and average values.*

	E_f (MPa)	σ_{fM} (MPa)	ε_{fM} (%)
PCL + β-TCP s n1	393,1	14,79	9,59
PCL + β-TCP s n2	398,1	14,43	9,42
PCL + β-TCP s n3	399,5	14,43	9,72
Average values	396,9	14,55	9,58

By observing graphs and tables of sterilized neutral PLLA specimens and comparing them with those of non-sterilized samples, it is possible to note that there is a small variation in terms of flexural modulus and flexural strangth: the average value of E_f of sterilized samples has undergone a small increase, while the average σ_{fM} decrease slightly. All three specimens in any case underwent breakage, even if it is less clear than in the non-sterilized case. The curves are similar and roughly overlapping, only the one of specimen 1 is slightly different, so the reproducibility was not affected by sterilization. In the case of PDLA/HAp composite, after sterilization the average Young's modulus decreased slightly and so is the maximum load. The plateau area is somewhat larger than that of the non-sterilized samples, so ductility is slightly increased.

By observing graphs and tables of sterilized neutral PCL specimens and comparing them with those of non-sterilized samples, it is possible to ascertain a slight decrease in the average Young's modulus and maximum load. The sterilized specimens did not break. So, the sterilization process did not affect the mechanical behaviour of the material and either its riproducibility since the curves are similar and roughly overlapping. By analyzing PCL/β-TCP composite, it is possible to note that the sterilization process has led to a slight decrease in Young'modulus and maximum load. Even in this case the

specimens did not break, since after the plateau zone the curves do not have a sharp decrease.

Beyond these small differences, it is possible to conclude that sterilization process did not affect either the mechanical properties or reproducibility.

From *dynamic mechanical tests* it has been possible to obtain the following results: the trend of storage (E') and loss modulus (E") as a function of the frequency (frequency of solicitation) in order to evaluate the degree of viscoelasticity of the tested materials. Here only the results for one sterilized and non-sterilized specimen per type are reported, the choice fell on the samples with the most regular curve.

Before sterilization

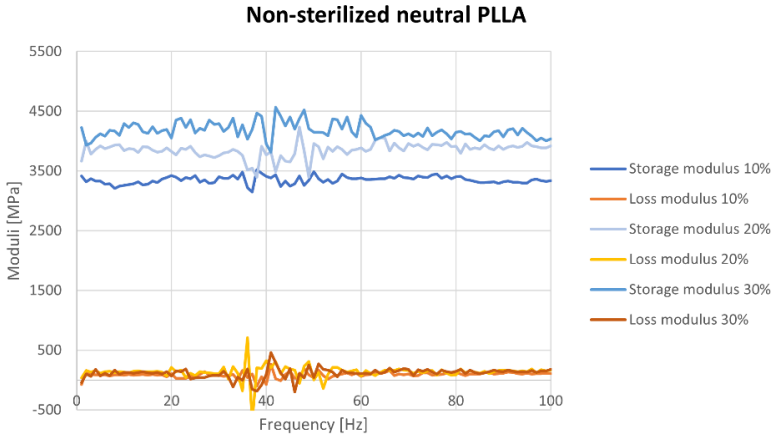

Fig.9. *Storage and loss modulus as function of frequency of non-sterilized neutral PLLA specimens.*

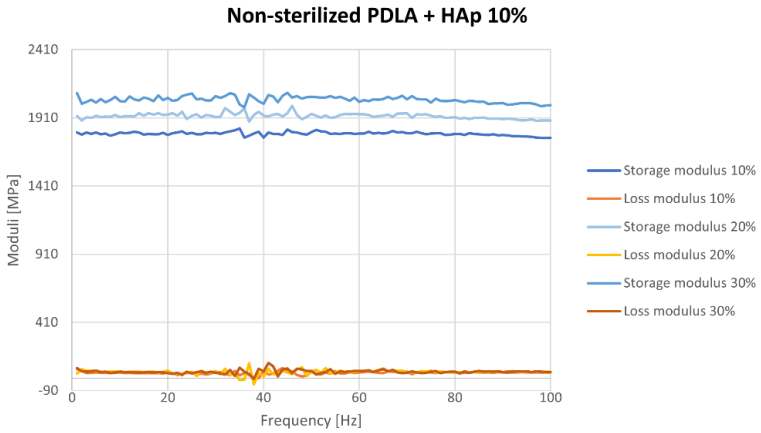

Fig.10. *Storage and loss modulus as function of frequency of non-sterilized PDLA/HAp specimens.*

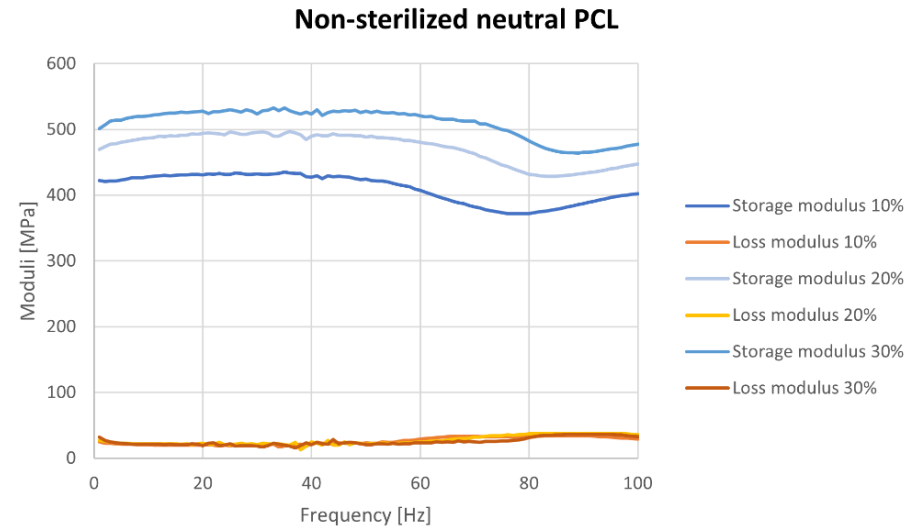

Fig.11. *Storage and loss modulus as function of frequency of non-sterilized neutral PCL specimens.*

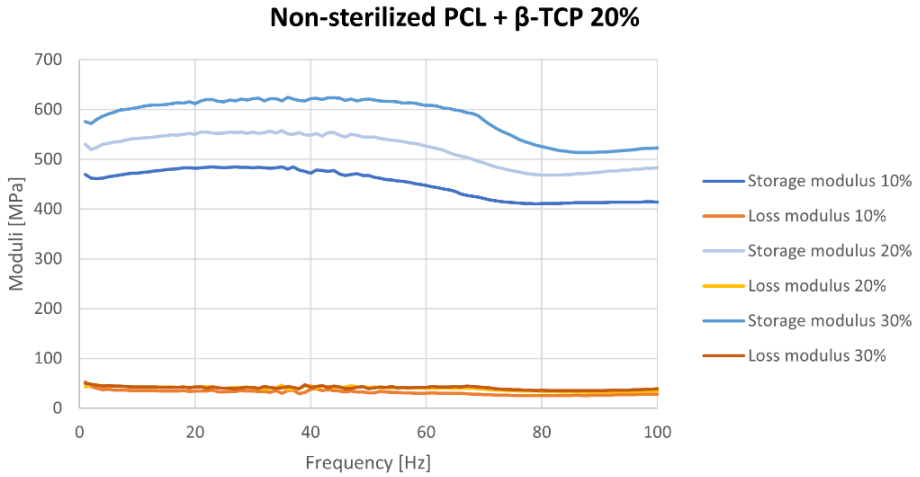

Fig.12. *Storage and loss modulus as function of frequency of non-sterilized PCL/β-TCP specimens.*

On X-axis is reported the frequency in Hz, while on Y-axis are reported the values of storage and loss modulus in MPa.

For all non-sterilized neutral PLLA specimens, the trends of storage and loss modulus are quite regular. From the graph it can be seen that the two moduli never meet, and furthermore the storage modulus is greater than the loss modulus, so the material will have a mainly elastic behaviour since there is no change of state from solid to liquid. It can be seen that the storage modulus increases as the value of pre-load increases, which

is a confirmation of the linear relationship between E' and load. Dynamic mechanical tests confirms what has been found in static mechanical tests since the values of storage modulus and flexural modulus are quite similar. In the case of non-sterilized PDLA/HAp composite the trends of E' and E" are quite regular. Also here, E' and E" never meet and E' is greater and E", so the composite has a mainly elastic behaviour. The storage modulus values are simile to the flexural modulus values found in the static tests. For non-sterilized neutral PCL, the storage modulus is always greater than loss modulus, thus they never meet. Therefore also PCL has an elastic behaviour. The same conclusion can be drawn for the PCL/β-TCP composite, since E' is greater than E". Also in the case of neutral PCL and the composite it can be seen the linear relationship between storage modulus and load. It can be see that storage and loss modulus trends for PCL smaples (both neutral and composite) are more regular than for PLLA samples.

After sterilization

Now, let's analyze the sterilized specimens, evaluating if the sterilization process has changed anything.

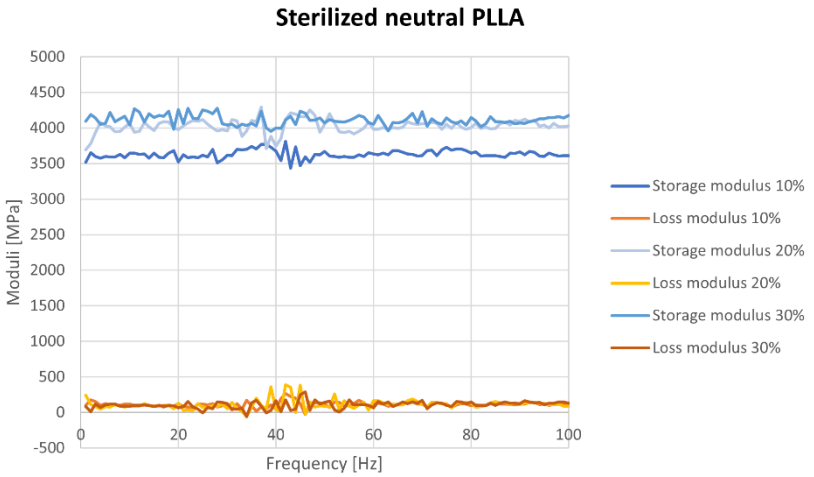

Fig.13. *Storage and loss modulus as function of frequency of sterilized neutral PLLA specimens.*

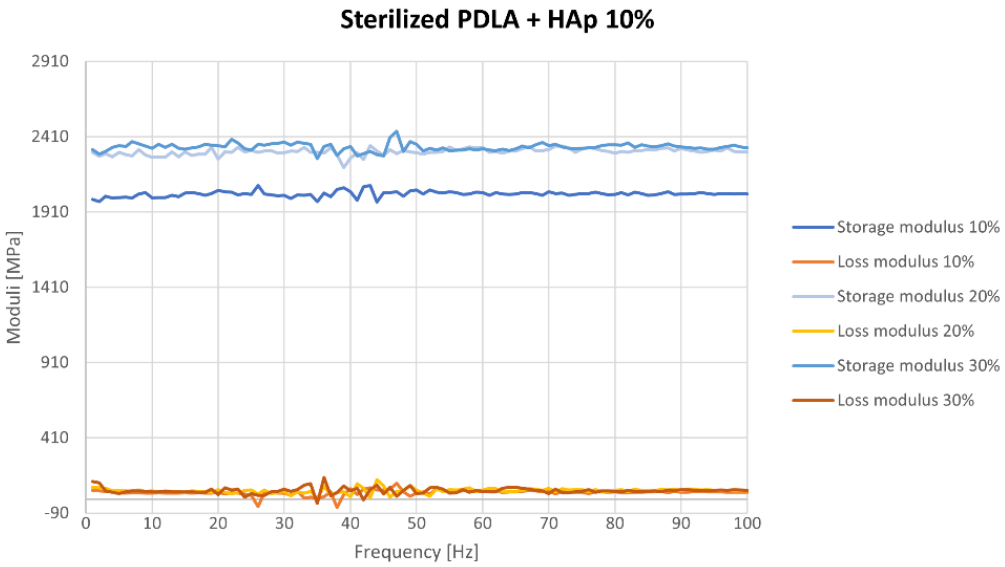

Fig.14. *Storage and loss modulus as function of frequency of sterilized PDLA/HAp specimens.*

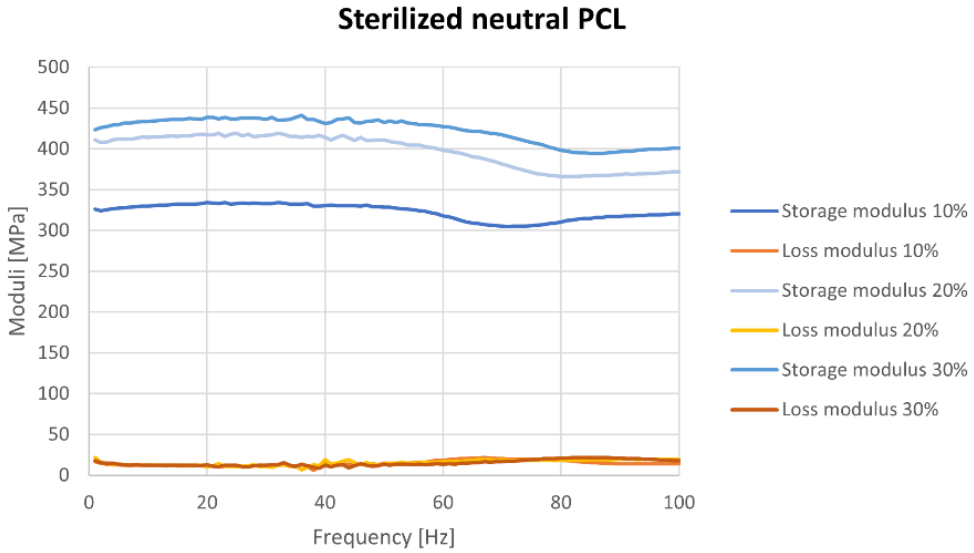

Fig.15. *Storage and loss modulus as function of frequency of sterilized neutral PCL specimens.*

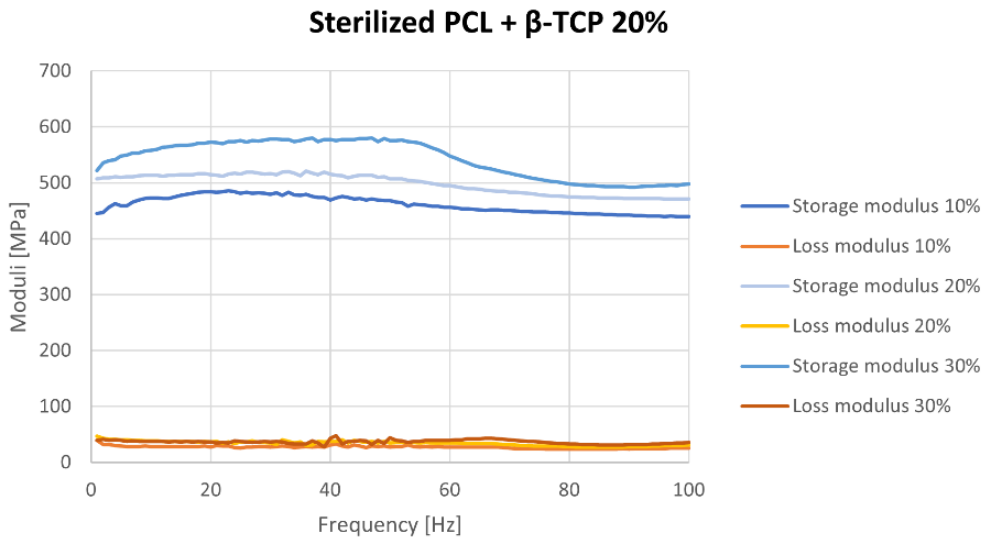

Fig.16. *Storage and loss modulus as function of frequency of sterilized PCL/β-TCP specimens.*

For sterilized neutral PDLA, a slight increase in the modulus values can be noted, especially in storage, as found for flexural modulus in static tests. The trends of storage and loss modulus are quite regular. For PDLA/HAp composite, the sterilization has led to a slight decrease in storage modulus. By observing graphs for sterilzed neutral PCL, the trends have not changed much compared to non-sterilized neutral PCL. Storage modulus is always greater than loss modulus, so the degree of elasticity remained unchanged. No change occured in the case of sterilized of PCL/β-TCP. So, it is possible to conclude that the sterilization process did not bring great changes to the mechanical behaviour of the various tested materials. It can be deduced that all specimens showed mainly an elastic behaviour, since in all cases the storage modulus in grater than the loss modulus. This characteristic is in agreement with polymers' properties since both PCL and PLA are in a solid state at room temperature. The dynamic mechanical characterization has been a confirmation of the static mechanical characterization.

Comparison of the two designed materials

Now, let's discuss the general behaviour of the designed biodegradable materials, starting with a comparison between sterilized PDLA/HAp and PCL/β-TCP. It has been possible to note that the sterilization process not only make the materials safe and usable for

biomedical applications, but also maintains their mechanical properties. Looking at the Young's moduli of sterilized specimens, it can be seen that PDLA/HAp has an average value of the modulus much greater than that of PCL/β-TCP, so PDLA/HAp is a stiffer material than PCL composite. Being a rather rigid material means better load-bearing capacity and less risk of collapse when the new bone is growing. Regarding maximum load, PDLA/HAp has an average value greater than PCL/β-TCP, therefore it will be more resistant to bending, but also more brittle respect to PCL/β-TCP. The latter, for its part, will be a more ductile material with a high elongation at break, demonstrated by the fact that it shows large deformations before the end of the tests. In both cases the addition of calcium-phospates (HAp and β-TCP) did not lead to major changes in the mechanical properties of the polymers, if not small changes in the stiffness: the HAp has made the PLA a little less brittle and the β-TCP has made the PCL a little stiffer. The reproducibility is good for both.

Comparison of the designed materials and natural bone

Let's now compare the mechanical properties of the designed materials and with those of natural bone. It can be seen that the Young's modulus of PDLA/HAp is similar to the upper limit of cancellous bone modulus (which is between 50-3,500 Mpa), while it results a bit lower that cortical bone modulus (7,000-30,000 MPa). Thus PDLA/HAp is slightly less stiff than natural bone. Conversely, Young's modulus of PCL/β-TCP is more similar to that of cancellous bone, so it is more less rigid than cortical part of the bone. Regarding the ductility: the average maximum load of PDLA/HAp falls within the range of that of natural bone, while the average maximum load of PCL/β-TCP is lower, so this composite will be more ductile. Therefore, the presence of calcium-phospates has made it possible to obtain materials with characteristics similar to natural bone, thus a good osseointegration.

Comparison of the designed materials and titanium

Now let's see the differences between composites and titanium alloy, on equal terms of biocompatibility. As mentioned in the previous chapters, there is a large mismatch between Young's moduli of titanium and natural bone, which generates the so called

“stress shielding effect”. In the case of composites this effect is reduced as the average elastic modulus of both composites is much lower than that of the titanium alloy (about 100,000 MPa) and more similar to that of bone. So, both composites will be less rigid than titanium, but they will have a greater elastic deformation. Looking at values of maximum load, the average value for both composites is much lower than that of titanium, so the designed composites will be less resistant to bending and more ductile, thus they will deform more before the fracture or in laboratory conditions before the end of the test.

12. Conclusions and future developments

The thesis work was primarily focused on revising current gold standards, such as titanium meshes and their production in the bone regeneration field. Then the focus shifted to the analysis of biodegradable and bioresorbable composite materials consisting of a polymeric matrix and a bioactive reinforcement, as alternatives to the titanium for which a removal intervention is required. Therefore, the main part of this thesis has been concentrated on the design of two type of bioresorbable composite materials (PDLA/HAp and PCL/β-TCP) and in particular on the experimental verification of their mechanical properties before and after sterilization process. The results showed materials with good mechanical properties, characterized by a relative high stiffness (even if lower than titanium) such to withstand loads and stresses generated during the bone regeneration process, but also a high ductility in order to ensure a high degree of deformation before fracture. It is important to point out that the sterilization process of specimens did not affect their mechanical behaviour. Compared to titanium, certainly the mechanical strength of composites is lower, but their properties are more similar to natural bone, thanks to the presence of calcium-phosphates. The addition of HAp and β-TCP led to improved interface with natural bone, thus a better osseointegration, since these reinforcements show a high osteconductive capacity and an internal porosity such to interact positively with surronding tissues. Therefore they allow to overcome the problem of the "stress shielding effect" caused by the mismatch between Young's moduli of titanium and bone. The use of the engineered materials avoid the risk of metal particle pollution caused by the wear of titanium mesh or possible carbon contamination. Also, since these composites are bioresorbable, removal surgery is not required. *In vitro* biocompatibility tests on designed materials conducted by Università di Camerino are ongoing, but preliminary data provide promising results, so it is possible to consider these bioresorbable composite as a future replacement of the current gold standards. Unfortunately, for now, there is still not much *in vivo* data on animals and humans to evaluate the actual biodegradability and bioresorbability of these materials, so further clinical trials will be useful.

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A chi ha sempre creduto in me…

Grazie