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DEVELOPMENT OF BIOABSORBABLE MATERIALS BASED ON POLYCAPROLACTONE (PCL) WITH CONTROLLED DELIVERY OF ACTIVE COMPOUNDS

THESIS MASTER

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I. Summary

Within medical research, efforts are needed in the investigation of the materials employed in bond fracture applications. Metal prostheses currently used are heavy and expensive. These metal alloys are biocompatible and highly resistant but do not promote the bone regeneration and neither are degraded over time. Controlled delivery of osteogenic biomaterials is an alternative to traditional bone repair techniques.

Some applications such as fixing and screws would have huge advantages if they were biodegradable in physiologic environment and furthermore it promoted the bound regeneration. This effect could avoid a second surgical intervention in order to remove this fixing and screws. Moreover this would entail a faster recuperation of the involved joint. Such materials capable to fulfill these functions are the biodegradable polymers, which present also an easy processability and have a lower cost.

The bound regeneration would be accelerated if the fixing and screws were functionalized with bioactive molecules which promote the mesenchymal stem cell proliferation in the osteoblast and the calcium deposition.

In short, the treatment of complex bone fractures with fixing and screws is promising. Specifically if the materials used were osteoconductive and osteoinductive, which are able to entail the osteogenesis. This is the reason why the present project research focuses in the intention to study and develop fixings and screws based on polycaprolactone (PCL) strengthened with Halloysite nanotubes (HNTs) and hydroxyapatite (HA) in order to enhance the mechanical strength and also to obtain osteoconductive properties. At the same time, the second step of this project is to functionalize the Halloysite nanotubes with curcumin in order to give to these fixings and screws antioxidant, anti-inflammatory and potentially chemotherapeutic properties.

II.Introduction

1. Bone

Bone can be considered as man-made engineering materials. However, due to the nature of its synthesis as an organic material, it is likely to show more variation in measured properties than typical engineering materials.

1.1. Bone Tissue

The bone tissue is referred to specialize connective tissue which form the skeleton which has different functions, such as:

- Body support to provide a rigid structure to the muscles.
- Movement, muscles are inserted to the bone through the tendons in order to allow the movement.
- Protection, since the cavities formed by the skeleton protect different inters organs.
- Mineral homeostasis because bones has the function to store several ores such as Calcium (Ca) and Phosphorous (P). [1]

The main benefit of the bone tissue resides in bone regeneration property; this feature gives the peculiarity of being the only tissue which is able to repair itself without scarfs.

1.1.1. Composition

The bone is a composite material form of diverse cells and an extracellular matrix, where the principal components are collagen type I, Hydroxyapatite and water [2]. Although the bone composition depends of several factors (age, genetic variability, environmental effects and skeleton localization of the bone), on average of 70% of its components correspond to the mineral phase and the remainder corresponds to the organic phase besides the water (Figure I.1).

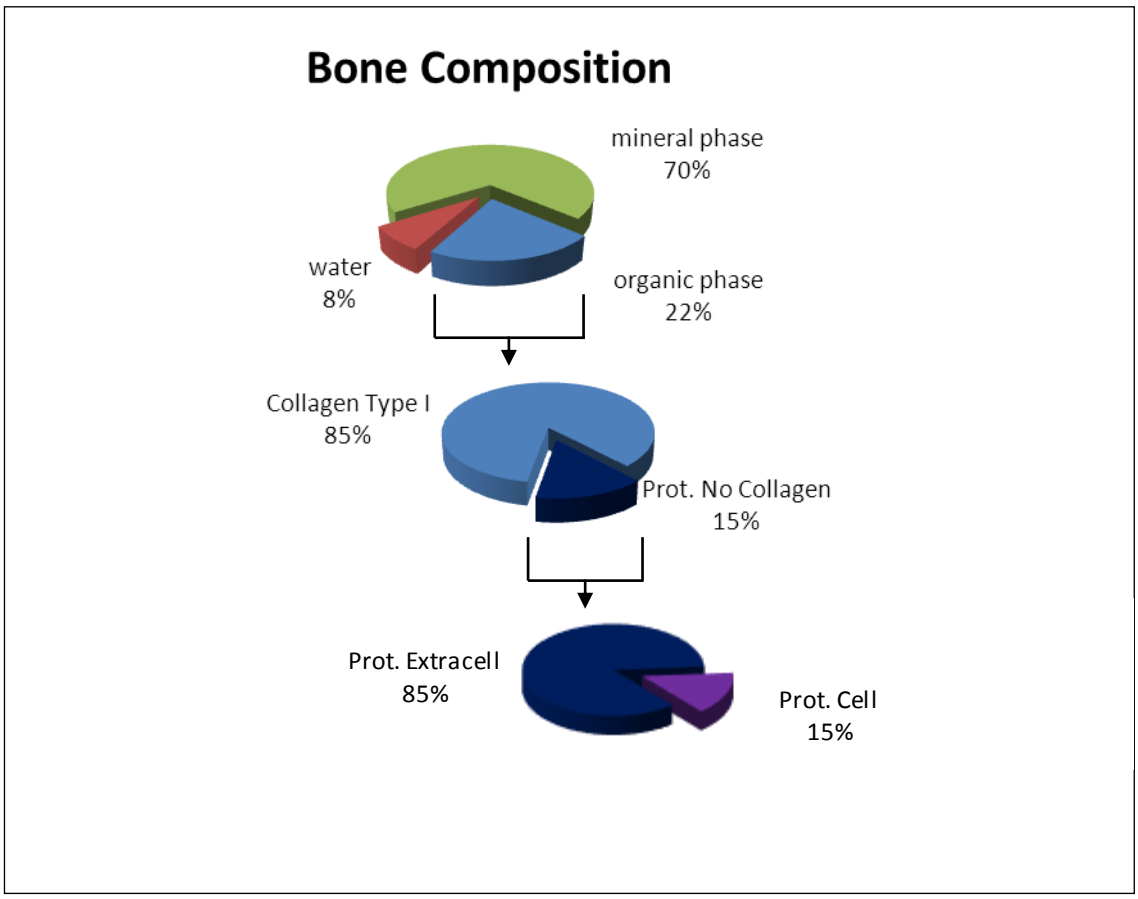


Figure II.1 Bone composition

Inorganic components

The inorganic component is the most abundant component of the bone, composed of a 95% of apatite crystals, which is analogous to Hidroxyapatite ore present in the nature (HAP, $(PO_4)_6(OH)_2$ Ca_{10}). This bone apatite (hydroxyapatite) is a deficient in calcium and hydroxide, containing 5% of impurities such as sodium, potassium, fluorides, citrates, carbonates, pyrophosphates and chlorides among others [3-6] . These

crystals, produced by osteoblasts, have approximately 30nm in size and form an imperfect crystalline network, facilitating the absorption of ions and their dissolution by the osteoclast. These apatite crystals are deposited between the collagen fibers, giving stiffness to the bone.

Organic components

Collagen Type I is the major organic component of the bone. Collagen is synthetized by osteoblast; this protein gives tensile strength, elasticity and flexibility to the bone. Moreover, it offers a perfect place to produce nucleation and hydroxiapatite crystals growth [7-9]. There are different collagen types, but the most abundant is collagen type I with an average of 95%, while the remaining 5% corresponds to collagen type V.

Collagen molecules are composed by a triplex, with a diameter of approximately 1.5 nm in size with a twist to the right, integrated for three polypeptide chains namely α-chain.

Every α-chain is composed by a repetitive characteristic structure through all the three amino acids chain, “glycine-X-Y, where X and Y mainly are proline and hydroxyproline respectively [7].

In addition to collagen type I and HPA, others proteins form the extracellular bone matrix, such as proteoglycans, osteocalcin, osteonectin, osteopotina, sialoproteins, fibronectin, thrombospondin, vitronectin and growth factors [10-12].

Frotein (Chromosome)		Function
Collagen	type I (17q21.23, 7q22.1)	The most abundant protein in the extracellular bone matrix.
	type X (6q21)	Found in hypertrophic cartilage
	type III (2q31)	Small amounts in bone, can regulate the diameter of fibrillar collagen
	type V (9q34.2-34.3; 2q24.3-31; 19q13.2)	Small amounts in bone, can regulate the diameter of fibrillar collagen
Whey proteins in the bone matrix (4q11-13)		Decrease the growth of HAP crystals
Proteins which contain glicoaminoglicano and proteins which contain leucine rich regions	Aggrecan (15q26.1)	Matrix organization; retention of calcium and phosphorus
	Versican (5q14.3)	Defines the space destined to become bone
	Decorin (12q21.3)	regulates the diameter of collagen fibers; union to TGF-β
	Biglycan (Xq28)	Union to collagen, union to TGF-β; genetic determinant of maximum bone mass
	Hyaluronan (multigenic complex)	Can work together with versican to define the space designated to became bone
Glycoproteins	Alkaline phosphatase (1p36.1-p34)	hydrolyze mineral deposition inhibitors
	Osteonectin (5q31.3-32)	controls the collagen fibers diameter
SIBLINGS proteins	Osteopontin (4q21)	Inhibits bone mineralization and remodeling
	Bone sialoprotein (4q21)	Mineralization initiator
MEPE (4q21.1)		Phosphate metabolism regulator
glycoproteins containing RGD	Thrombospondin (15q15, 6q27, 1q21, 5q13, 19p13,1)	Cell adhesion
	Fibronectin (2q34)	Cell union

	Vitronectin (17q11)	Cell adhesion
	Fibrillin 1 and 2 (15q21.1, 5q23-31)	Regulates the formation of elastic fibers
Proteins which contain γ-carboxyglutamic acid	Matrix Gla proteins (12q13.1-p21.3	Mineralization inhibitor
	Osteocalcin (1q25-q31)	Osteoclast regulator; mineralization inhibitor
	S Protein (3p11.2)	Liver product, can be synthesized by osteoblasts

Table II-1 Main proteins constituents of the bone extracellular matrix and functions. Adapted from Clarke, 2008 [10]

- Bone cells

Cells implicated in the bone metabolism are: mesenchymal stem cells which are multipotent stromal cells that can be differentiated into a variety of cell types. Osteoblasts are cells responsible for the formation of the bone tissue. Osteocytes and Osteoclast (from the monocytic line) are responsible for the bone resorption.

- Mesenchymal stem Cells

Constitute a kind of stems cells namely as well progenitor cells. Each of these cells are defined to their functional features, which are: undifferentiated, self-maintenance, undifferentiated progeny production and tissue regeneration [13].

Mesenchymal stem cells (MSC) can differentiate into a variety of cell types [14-16] , including: osteoblasts (bone cells), chondrocytes (cartilage cells), and adipocytes (fat cells) cardio myocytes (Figure I.2).

The transcription factor Cbfa1/Runx-2 is specific to osteoblast differentiation [17], which increase the expression of alkaline phosphatase enzyme (ALP), collagen type I (COL1), osteopontin (OPN), osteocalcin (OCN), bone sialoprotein (BSP) and extracellular matrix calcification [16].

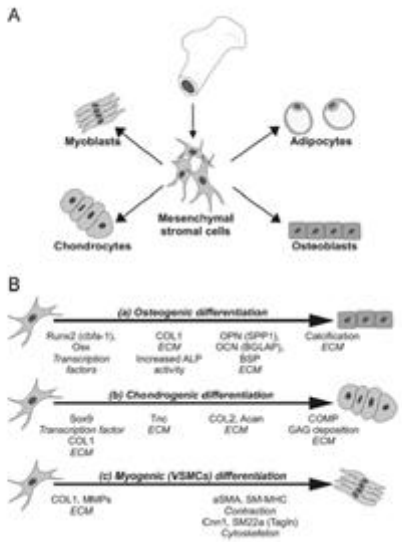


Figure II.2 Cell differentiation scheme

- Osteoblast

Osteoblasts are cells located on the surface of bone tissue. Their function is to produce bone tissue, forming collagen type I and calcifying it [18]. Osteoblasts are produced by MSCs differentiations, they have a diameter average size of 25µm and a polyhedral shape. Osteoblasts have a highly basophilic cytoplasm due to high ARN production, an extensive endoplasmic reticulum and a highly developed Golgi apparatus, as evidence of its high synthetic activity [1, 19].

Moreover, osteoblasts show great alkaline phosphatase enzyme activity in the cytoplasmic membrane. This enzyme performs a key role in the bone mineralization [18].

- Osteocytes

After bone formation, between 10% and 20% of osteoblast are housed in the bosom of bone and gradually are differentiated in osteocytes [1, 20].

Osteocytes are the most abundant cells in bone tissue. Its population may reach 90% of total cellular constituents [21, 22]. Located in the osteocytic gaps, osteocytes are more elongated than osteoblast, with a bigger nucleus and a less developed

endoplasmic reticulum. Its cytoplasm is basophilic and has cytoplasmic prolongations, which are inserted into bone canaliculi and kept in touch with others osteocites and osteoblast neighbors. This disposition enables to form an extracellular network and lets cellular communication among bone bosom cells and bone cells surface [21]. The nutrition of these cells depends on the connections through the canaliculis.

Osteocytes can detect mechanical changes and translate them into biochemical signals to act directly on the bone. Thus, the osteocyte network can address the bone remodeling and fix micro fractures [21, 23].

- Osteoclast

Osteoclasts are the only known cells able to break down the bone. Osteoclasts are formed by the fusion of monocytes which should have abandoned the circulating blood; hence, osteoclasts are derived from hematopoietic stem cells instead of mesenchymal cells. Osteoclasts are cells which have several cytological features such as:

- Large quantity of mitochondria and lysosomes
- Are multinuclear
- Are highly polarized

Their mission is to dissolve HAP crystals and digest the organic matrix by secreting diverse enzymes, highlighting proteases as the most important whose task is focused on bone breakdown and bone reabsorption [1].

1.1.2. Bone organization

Bone organization can be classified in different ways. Regarding its ripeness, it can be distinguish between immature bundle bone (also called woven bone) and mature lamellar bone.

Immature bundle bone is present in fetal development, newborns, fractured healing and growing metaphysis. This kind of bone is characterized by exhibiting great number of cells, its bulkiness and having thick non oriented collagen fibers type I, which provides mechanical isotropy to the tissue.

However, mature lamellar bone is formed in immature bundle bone. Primary bone contains interlaced collagen fibers. The immature primary bone is present in the developing skeletal system and bone regeneration, being subsequently replaced by lamellar bone tissue. Mature lamellar bone can be found in the entire mature skeleton, including both cancellous bone (spongy bone) and cortical bone. All of its collagen fibers are organized and oriented with the bone major axis. This fact confers mechanical anisotropy to the tissue which shows deformation resistance when the strength is parallel to the bone major axis.

Another classification could be granted regarding its structure, distinguishing between spongy bone (trabecular bone) and compact bone (cortical bone) (Figure I.3).

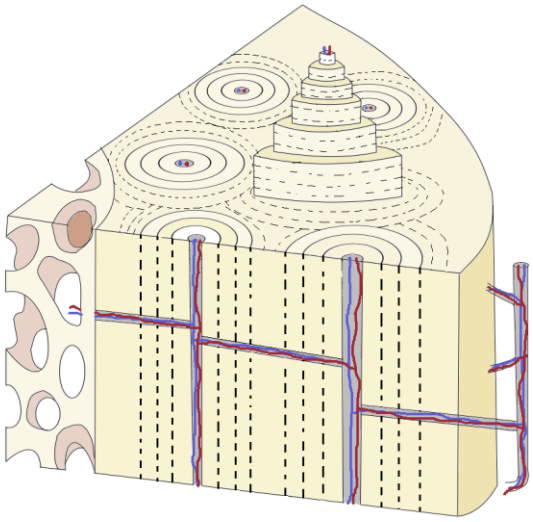


Figure II.3 Bone structure scheme

The ratio (trabecular:cortical) bone is stablishes as 1:4, although depending on the location it could exhibit different ratio [10].

- Trabecular bone (spongy bone) has a highly porous variable structure between 50 and 90% with interconnected pores. It forms series of trabeculae by plates and rods where is located the bone marrow.

- Cortical bone (compact bone) is denser than trabecular bone, with a 5% porous structure. Cortical bone is set out on bone surface, varying its thickness depending on its location. It is distributed in parallel disposition following the bone major axis forming cylindrical structures denominated osteons or Haversian system, which are wrapped

forming concentric layers (Figure I.3). Cortical bone external surface is limited by the periosteum and the internal surface by the endosteum. The cellular activity of periosteum surface is important regarding to appositional growth and fracture repair. Endosteum surface exhibits higher remodeling action than periosteum surface, likely, because of a high exposition of cytokines, due to bone marrow proximity [10].

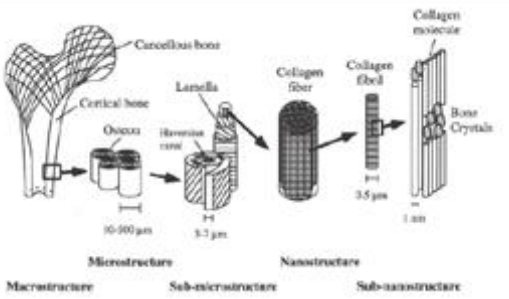


Figure II.4 hierarchical levels of bone structure

Figure I.4 shows a schematic representation of the bone tissue constituents, encompassing from nanoscopic to macroscopic scale. It could be observed the hierarchical levels and the bone structure organization where each level depends on its lower level to contribute to final bone architecture.

1.1.3. Ossification process

Intramembranous and endochondral ossification are the two ossification process [20, 24].

- Intramembranous ossification

Intramembranous ossification occurs in flat bones such as skull, bone jaw and collarbone. This ossification process is originated in mesenchymal membranes [20, 24].

Mesenchymal cells are differentiated to osteoblast, which form the extracellular matrix, and is subsequently mineralized (Figure I.4). Immature (woven) bone grows by a phenomenon known as appositional growth, which relies on the deposition of new bone layers on the outer surface through osteoblast, while osteoclast absorbs the inner layers [25, 26].

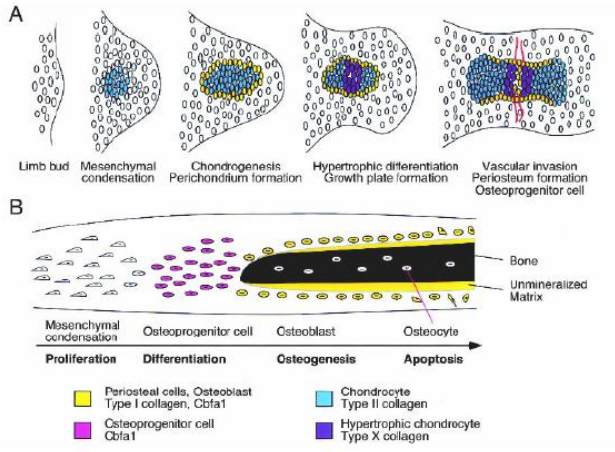


Figure II.5 Endochondral bone development scheme

- **Endochondral ossification**

Endochondral ossification leads to most of the body's bones (short and long bones, spinal column and the skull base bones) [27]. The formation process of this kind of bones begins with the mesenchymal membrane, which goes to an intermediate stage where is formed the hyaline cartilage from the mesenchymal cells differentiation to chondrocytes [24, 27-31]. The hyaline cartilage is used as a pattern for bone construction. In the center of the hyaline cartilage, chondrocytes begin to hypertrophy, the matrix calcified in vascular endothelial growth factor (VEGF), which induces to the blood vessels formation in the perichondrium. A primary ossification center forms as blood vessels and osteoblast, osteoclast and hematopoietic cells invade the calcified cartilage. Chondrocytes go into the apophyses and osteoblasts replace bone cartilage matrix, forming cancellous bone and leaving space to form the bone marrow. At the same time, bone collar formation occurs in perichondrium by osteoblasts, through the cartilage, where is located the primary ossification center inside a bone tube in the center of the diaphysis.

At the cartilage end is formed the secondary ossification center between the epiphysis and the diaphysis, constituted by a growth plate formed by cartilage. In the growth plate occurs a coordinate proliferation of chondrocytes, hypertrophy and apoptosis, which comes from longitudinal bone growth (Figure II.6). These processes are coordinate with the epiphysis growth and the diaphysis widening [24, 26-32].

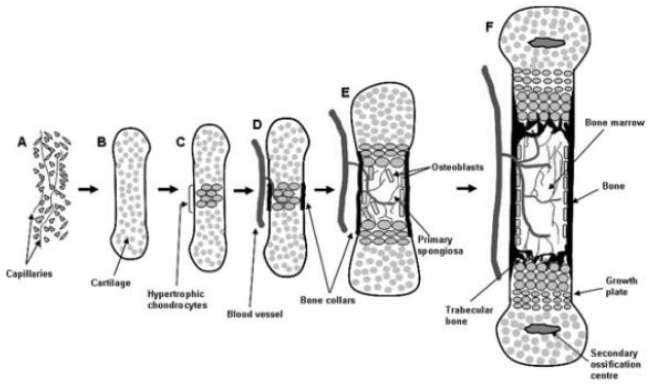


Figure II.6 Endochondral bone development scheme showing secondary ossification

1.1.4. Bone remodeling

Bone remodeling is a basic process which determinates bone quality, due to the architecture of the skeleton, mineralization process and bone geometric is conditioned by it [32]. Bone remodeling is a cyclic process which entails growth, modeling, remodeling and reparation of the bone. This process takes place in every bone of the body during the entire life, evidencing that bone tissue is an active tissue. The principles of this process are maintenance of the stronghold and mineral homeostasis. During bone remodeling, little portions of bone is absorbed (growth) due to osteoclastic activity, subsequently it is mineralized by the osteoblast activity (modeling) (Figure II.7). In the final process, during remodeling, reabsorption of the old bone occurs and new bone is formed. This process can repair micro fractures, avoiding their accumulation [33].

Osteopathy, in general, is produce by remodeling process alterations. Bone remodeling is regulated by hormonal and mechanic factors allowing bone adaptation to the body metabolic and mechanical necessities [34].

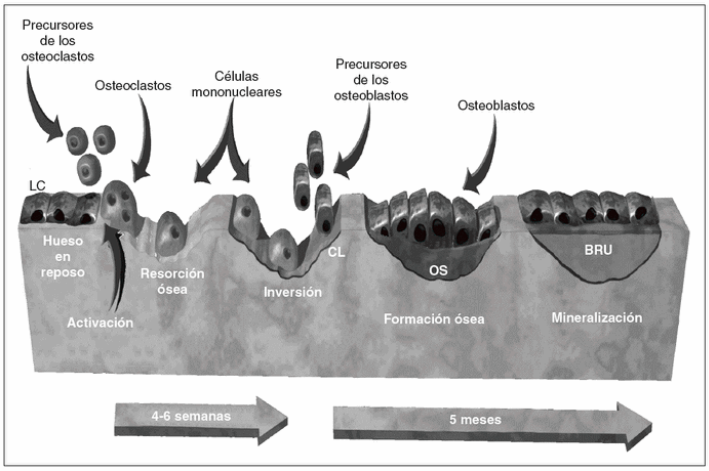


Figure II.7 Bone remodeling scheme

Each of remodeling process is carried out by osteoclastic/osteoblastic activation sequential cycles giving rise to a basic multicellular unit (BMU) [10, 35, 36].

The initial phase consists of recruitment and activation of a mononuclear precursor (Figure II.8). The osteoclast arrived to bone surface, linking trough integrin receptors to matrix proteins which contain the RGD sequence (arginine, glycine, aspartic acid), forming a sealed area between the bone and the osteoclast. This phase takes around three weeks in each remodeling cycle [37, 38]. As it can be observed in Figure II.8 and Figure II.9, several factors regulate the osteoclast activity, taking a lead role in the receptor activator for nuclear factor κ B ligand (RANKL) and the Osteoprotegerin (OPG), both produced by the osteoblast [10, 33, 35, 39-41].

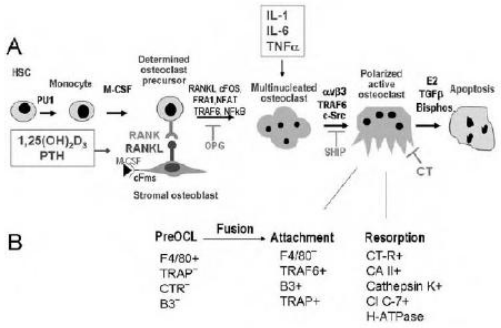


Figure II.8 Osteoclastogenesis Regulation

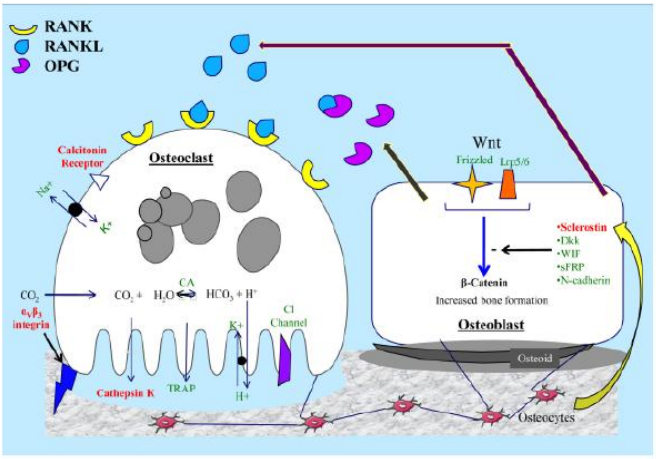


Figure II.9 Interaction among the osteocytes, osteoblasts and osteoclasts in bone remodeling

Once the osteoclast is fixed in the bone, tissue erosion begins, to perform it osteoclasts release H^+ through ATP-dependent proton pumps (H^+ ATPasa) with the propose of getting a constant PH of 4.5 inside the resorption chamber to make easier the HAP removal. Moreover, degradation of extracellular matrix by osteoclasts release enzyme, such as acid phosphatase, tartrate resistant, cathepsin K, metalloproteinase matrix and cytoplasmic gelatin lysosomes; results in resorption of the Howship´s lacuna with approximately 50µm in length in trabecular bone and 100µm in cortical bone [18, 38] (Figure II.9). This process takes around 2-3 weeks. Once resorption is finished, osteoclasts are eliminated by the apoptosis.

After resorption phase, remodeling entails a formation process, in which osteoblasts differ from their precursors (Figure II.10)[42]. It is presume that final resorption process and beginning of formation process involve different factors such as transforming growth factor beta (TGF-β), insulin-like growth factor 1 and 2 (IGF-1 and IGF-2), bone morphogenetic proteins (BMPs) and fibliblast growth factor (PDGF). The coupling of these two processes takes 1-2 weeks [18, 33, 39, 41, 42].

Bone formation takes between 4 or 5 months[10, 38]. During this time, osteoblastic cells secrete extracellular matrix and vesicles containing high amount of Ca^{2+} and phosphates concentrations, in order to get collage mineralization. Formation process has two phases: matrix synthesis and mineralization. Once osteoblasts are in contact with the surface; osteoid (formed by collagen I fibers, which are alienated before

integration to the mineralized bone) and other specific proteins are deposited forming and layer of 10µm. New matrix initiates its mineralization in 5-10 days of maturation, where hydroxyapatite crystals (HA) are formed. During extracellular matrix growth and mineralization progress, several osteoblasts get trapped inside the bone. These osteoblasts will be differentiate to osteocytes, forming an extensive network of tubules where the osteocytes are connected each other, also with the outer part of the bone [20, 43].

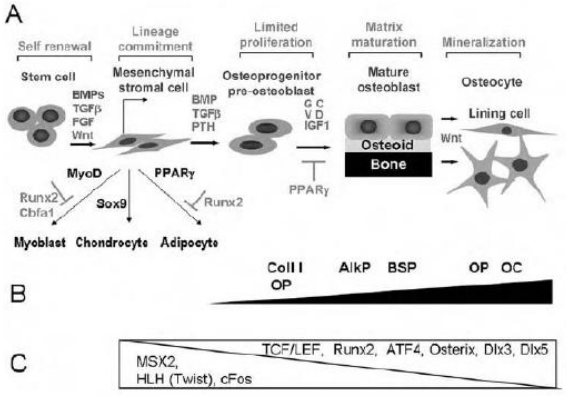


Figure II.10 Osteoclastogenesis regulation

When the process is finished, about 50-60% of the osteoblasts go into apoptosis, the rest of the osteoblasts become in osteocytes or bone-lining cells which surround the bone. Bone-lining cells work as a bone-blood wall but have the capacity to re-differentiate to osteoblast through mechanical or hormonal stimulation.

Remodeling process is quite similar in both cortical and trabecular bone [10, 44-46] (Figure II.11). In cortical bone remodeling, osteoblasts burrow a circular section tunnels from Haversian canals or Volkmann canals. Hence, osteons are formed with cylindrical shape. In trabecular bone remodeling, osteoclasts erode the trabecular surface leading shallow excavations and wide base. Therefore, trabecular places of remolding have plane lens convex shape [10]. Exist high probability of remodeling where a micro fracture is placed. Although, remodeling is a random process [38, 47].

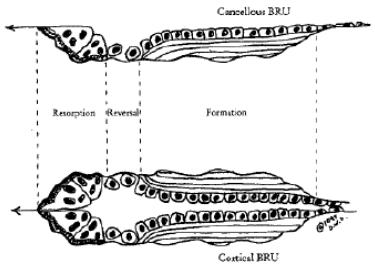


Figure II.11 Remodeling sequence of trabecular and cortical bone

1.1.5. Bone Modeling

Bone modeling process occurs frequently during bone growth. Unlike remodeling process, modeling does not involve bone resorption-formation coupling cycling process. But rather, resorption and formation are a separate process[48]. Bone modeling process has the goal of adapts structure to loading by changing bone size and shape and removes damage and so maintains bone strength [10, 35, 39, 42, 48, 49].

1.1.6. Bone Repair

The bone tissue is the only tissue capable to repair itself without any scar from the moment at which damage occurs [50, 51]. The damage repair process could be divided in sequential phases involving several kinds of cells and growth factors.

First stage involves an inflammatory response with a temporal delivery, especially during the first week, of different kinds of proinflammatory cytokines such as IL-1, IL-6 and TNF-α [52]. The localized painful inflamed swelling filled with clotted blood results from a break in a blood vessel (within the bone, the marrow space, the periosteum, or the surrounding tissue).

In the second stage osteoprogenitor cells, placed in the injury place, deliver bone morphogenetic proteins (BMPs), which recruit more mesenchymal cells with the action of proinflammatory cytokines. Connective tissue stem cells and capillary blood vessels penetrate the inflamed fracture hematoma clearing the debris from the injury (acting as phagocytes). If the injury is mechanical stable, the MSCs will be differentiate into osteoblast in order to regenerate the bone; but if the injury is mechanical unstable, the

MSCs will be differentiate into chondrocytes to form a collagen layer acting as a bridge between both fracture ends; resulting in fibrocartilaginous callus (soft callus) (Figure II.12). Procallus material usually extends beyond the volume previously occupied by the uninjured bone.

In the final stage (several weeks to months in duration) the soft callus is consequently calcified and forms bony callus (hard callus), which will be remodeling to form spongy bone. In the healing of a bone fracture; osteoclasts continue to dissolve away the fibrous and cartilaginous components of the injury site, while osteoblasts continue to replace the material with new bone matrix. Bone remodeling will continue until normal dimensions and composition of the bone are recreated; it represents the third and final stage in repair of a bone fracture, though some additional bone remodelling will often follow over time [50, 51, 53, 54].

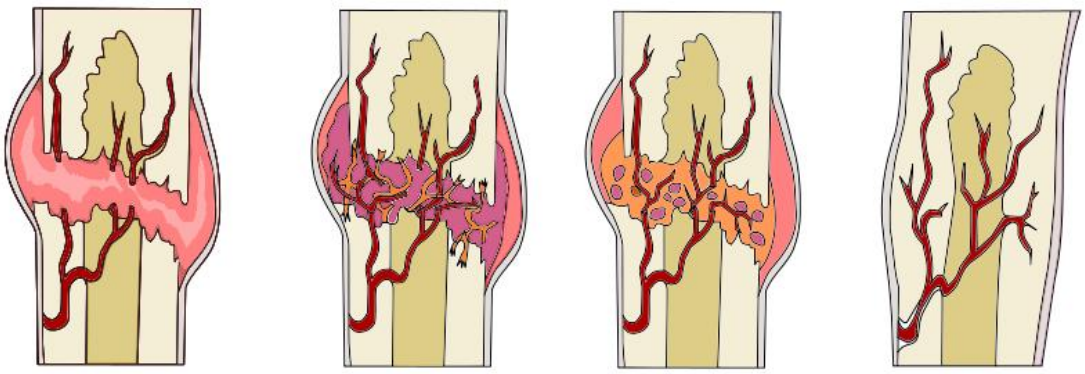


Figure II.12 Scheme of a bone callus during regeneration

1.1.7. Craniofacial injuries

Skull injures are the most frequent trauma seen in urban trauma centers [55, 56], leading causes of death and disability worldwide [57]. Accordingly big efforts are focused on the research of new materials capable to fulfill the requirements for bone fracture remodeling.

Elderly people is the most affected collective of craniofacial injuries, due to with normal aging, loss of estrogen increases and incurs bone loss and osteoporosis, which is especially prominent in women after menopause [58]. According to National Institute on Aging (US Department of Health and Human Services), in 2014, an estimated 8.1% percent of the world's population was over 61 years old. By 2050, this number is expected to triplicate.

Considering that compositions of cranial bone change with the age, physical properties are affected by this differences. A thin and non-homogeneous cortical bone layer compose fetal cranial bones, whereas mature adult cranial bones consist in a stiff inner and outer strata of cortical bone and a lightweight trabecular layer between them [59]. Care should be taken when both mechanical properties are compared. According the literature present for now, the most studies found related to cranial bone mechanical properties examine fetal or infant cranial bone [60-62]. Nevertheless, few studies have reported adult cranial bone [59, 63, 64]. Table II-1 shown below summarized a review of the literature comparing the reported average elastic moduli and maximum energy for failure for human cranial bone. It can be noticed a high degree of inter-individual variation between values. It must be considered that some factors influenced stiffer and stronger values of each skull, for example:

- Impact speed influences elastic modulus and maximum energy for failure, higher average speed increases those values.
- Morphological differences between individual cranial vaults play an important role, implying porosity, thickness and radius curvature.
- Bone site entails different composition and morphology of the bone between parietal and frontal bond. Parietal bone tends to be less thick, more porous and also has a lower percentage of bone volume compared to frontal bone [59]. All these facts entail that parietal bone resist less forces at fracture and also absorbs less energy before fracture.

	Gestation	*Ref*	*Child*	*Ref*	*Adult*	*Ref*
Elastic modulus	25 weeks 0.071 GPa	[60]	6 months 3.58 GPa	[60]	9.56 GPa	[65]
	40weeks 3.88 GPa	[66]	1 year 0.461 GPa	[61]		
			6 years 7.38 GPa	[66]		
Maximum energy for failure	30 weeks 0.0031 GPa	[60]	6 months 0.0446 GPa	[60]	0.12784 GPa	[59]
			1 year 0.03023 GPa	[61]		

Table II-2 a review of the literature comparing the reported average elastic moduli and maximum energy for failure for human cranial bone

Focusing our attention in adult cranial bone, different mechanical properties can be noticed depending on the specimen. As it was aforementioned, several factors influence the morphology and consequently the mechanical properties. Motherway et al. [59] state the maximum force of failure significantly affect depending of the position of the bond regarding to parietal and frontal bond. Also, the energy absorbed until failure is higher for frontal bound. In that study, it was noticed that elastic modulus and maximum bending stress increase with the increase in the percentage bone volume. Moreover, the author states that parietal bone tends to be less thick, more porous and also has a lower percentage of bone volume compared to frontal bone. All these facts entail that parietal bone resist less forces at fracture and also absorbs less energy before fracture.

From the statistical analysis, it can be noticed that mechanical properties show a dependence with the loading rate. It was found that higher speed entails noticeably higher maximum forces, 7.46±5.39 GPa (0.5 m/s), 10.77±9.38 GPa (1.0 m/s) and 15.54±10.29 GPa (2.5 m/s). Similar results were obtained to maximum bending stress, which was significantly larger at the highest speed. Nevertheless, the correlation between strain rate and elastic modulus is not significant.

2. Tissue Engineering

Tissue engineering relies on development of biomaterials combining scaffolds, cells, and biologically active molecules into functional tissues. The goal of tissue engineering is to assemble functional structures that restore, maintain, or improve damaged tissues or whole organs.

2.1. Osseointegration

Osseointegration is defined as “formation of a direct interface between an implant and the bone, without intervening soft tissue” [67]. This term refers to direct structural adaptation and functional connection between living bone and the surface of a load-bearing artificial implant. Process success depends on both previous processes function: osteoinduction and osteoconduction [68].

- Osteoinduction is the process where the stem cells (undifferentiated and pluripotent cells) are differentiated into osteogenic cells such as osteoblasts (in the inner layer of the periosteum) and will form the bone tissue (Figure II.13). New bone formation by these cells is known as osteogenesis (ossification).

- Osteogenesis is the bone formation process. Exist two kinds of osteogenesis [69]:

    - Contact osteogenesis: bone tissue is produced from the surrounding bone surface.

    - Distance osteogenesis: bone tissue formation is produced from the implant surface.

  Hence, bone tissue formation is given in both ways, from the surrounding bone surface to the implant and from the implant surface to the surrounding bone [70]. Nevertheless, contact osteogenesis forms bone tissue in an average of 30% faster than distance osteogenesis. Bone formation from the implant surface entails that the implant surface allows his colonization by the mesenchymal cells, called osteoconduction.

- Osteoinduction is the process in which the bone growths into the structure of a bioactive implant or graft. This phenomenon mainly depends on the material biocompatibility and its surface features [71, 72]. As a result of implant surface colonization by osteoprogenitor cells, it is formed a contact interface between the implant and the surrounding tissues. There are two kinds of contact: bone or hard tissue contact and fibrous or soft tissue contact. Direct contact between live bone and the implant surface forms a strong extracellular matrix of union between both structural and functional, which increases over time. It promotes reparative osteogenesis in the interface and leads implant fixation due to the mineralization. In the other hand, in the interface between bone and soft tissue, epithelial cells form a strong collar around the implant without inflammatory response, forming a thin layer of connective tissue poorly vascularized in the surrounding implant surface, in which begins the tissues regeneration.

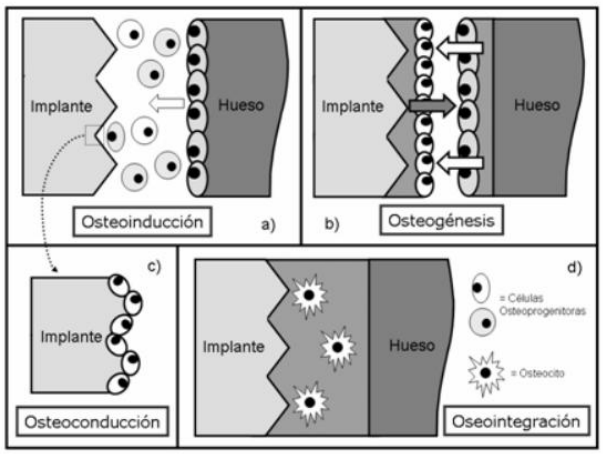


Figure II.13 Osteoinduction

2.2. Biomaterials

Biomaterial is defined as “ideated material to interact with biological systems to evaluate or substitute any tissue, organ or body function” according to Second Consensus Conference on Definitions in Biomaterials, celebrated in United Kingdom in 1992 [73]. Development of new biomaterials is an important field continuously in progress, focusing its efforts in bone regeneration due to bone pathologies entail an important average of physical disability.

Originally, bone implants approach only focused on achieving development of materials with the ability of supporting mechanic efforts in the fractured bone. Furthermore, materials should be biocompatible. This category includes metallic materials such as stainless steel and titanium alloys. Nevertheless, these systems need a second chirurgical intervention to remove the devise; entailing higher costs and promoting infections. Moreover, all material introduced in the body produce a response, albeit minimal.

In recent years, several authors such as Ikada [74], Meyer [75] and Barrere [76] describe bone implant materials with the following properties:

- Biocompatibility: integration in the organism without immune response, cytotoxic or genotoxic effects. This is a fundamental biomaterial property.

- Biodegradability: decomposition process (by hydrolysis) at rates that are as close as possible to the rates of new bone formation. This entails a challenge to the biocompatibility, due to degradation products should not be toxic.

- Strength and mechanical compatibility: Resisting mechanical stress as the position of the bone tissue it replaces. Mechanical properties, for example elasticity modulus, should be the most closer possible to the replaced tissue to prevent bone loss associated with the use of bone grafts or stress shielding.

- Osteoinductivity: promoting fixation and specific cells formation of the bone tissue. This is achieved with the recruit mesenchymal stem cells and osteoprogenitor with subsequent proliferation and differentiation into osteogenic line.

- Osteoconductivity: acting as a structural support in the formation and growth of new bone. This property is combined with biodegradability because the implant material should be absorbed to provide space to the new tissue.

- Radiolucency: radiographically distinguishable with regard to the tissue where it is implanted.

Development in tissue engineering regarding biomaterials require materials which fulfills these properties in different extents, with an appropriate response of the host.

There are material families with features that confer to the materials specific clinical applications. The biomaterial selection depends on the application.

- Organic materials

Natural materials are those which use bone tissue from the same individual (autograft), from different individual of the same species (allograft) or from different species (xenograft). Autografts usually fulfill the three desirable properties of an implant; osteoinductivity, osteoconductivity and osteogenicity. The disadvantages of this are the high cost and complexity of the chirurgical process. Furthermore, invasive surgeries present postoperative complications. Allografts require treatments such as freeze drying, irradiation, acid wash, etc. to prevent rejection by the recipient and eliminate possible infection in the implantable tissue. Xerografts, such as bovine bone also present some disadvantages from contagious diseases.

- Ceramic materials

Ceramics are inorganic not metallic materials with a crystalline structure, obtained from high temperature and pressure process [77]. In orthopedic applications are generally used two types of ceramic materials: metallic oxides and calcium phosphates.

Metallic oxides such as Alumina (Al_2O_3) or Zirconia (ZrO_2) are known as inert ceramic materials, which are highly resistant to corrosion and are broadly used as prosthetic joins surfaces [77].

Calcium Phosphates are known as bioactive ceramics due to their chemical fixation to the bone, involving bone regeneration. The two most important are Hydroxyapatite (HA) and Calcium Triphosphate ($Ca_3(PO_4)_2$); those are characterized in general for showing similar chemical composition with the bone mineral composition and exhibit similar mechanic properties compared with the bone.

In this category, the most widely used is hydroxyapatite (HA) which is a crystalline calcium phosphate (Ca_{10}) ($PO_4)_6$ ($OH)_2$). This material, is the principal bone compounding of mammals, around a 60-70% of dry residue from the bone. Depending on the source, hydroxyapatite structure could show similitudes with the bone tissue. Regarding osteoconductive properties, Hidroxyapatite enables the penetration of the surrounding connective bone tissue and performs a ossification process [78, 79].

According to Osrborn [80], Hidroxyapatite is the only material capable to set a primary union with the bone.

In order to understand how the osteoconductivity works, several researches were carried out. Okumura [81] research showed how osteogenesis begins on the porous surface of the implant and grows among the available space of the porous, process which was deeply studied by Trecant [82] to establish the proper density to optimize the process. Implant mechanical properties in vivo were also studied by Trecant et al.[82], who in 1994 designed a study to attest how the calcium phosphate implants improve their properties due to bone tissue growing and the formation of collagen and biologic apatite.

Even though, different types of calcium phosphate and variations of HA could show different biodegradability grades, Kitsugi et al. [83] demonstrated that there are not significant changes in material osteoconductivity properties. In 1996 Zongjian et al. [84] studied implants in diverse animals and demonstrated that hydroxyapatite and calcium phosphate could generate ontogenesis process; even though the implant was placed intramuscularly or subcutaneously instead of in the tissue bone. Depending on the animal, the tissue formation rate varies. So, in dogs was demonstrate an increase in vascular connective tissue in 15 days, mesenchymal cell accommodation in 30 days, matrix bone generation in 45 days and bone tissue remodeling in 60 days.

Superficial implant reactivity was studied by Ducheyne and Qiu [85], fact which affects fixation, proliferation, differentiation and mineralization of bone tissue cells. Cerroni et al. [86] carried out studies based on synthetic hydroxyapatite and exposed how bone tissue was generated inside the synthetic ceramic porous.

- Polymers

Concerning restorable polymers, the vast majority of researches are focused on polyglycolic acid (PGA) and polylactic acid (PLA). The first use of these polymers was reported in 1960 with the development of biodegradables sutures. The most widely studied material is PLA, a thermoplastic, amorphous and semi crystalline polymer, broadly used as a suture, also as a control drug deliver and bone implants. Hasegawa et al. [87] states that the implant degradation ratio is related with the place where is

implanted, hence the mechanical loads which should hold the tissue, influence the formation rate of it.

- Compounds

Currently, development of scaffolds based on polymer /ceramic compounds shows a great trending of study. This is due to, ceramic materials such as calcium phosphates have excellent osteoinductive properties despite of showing low biodegradability, mechanical strength and difficult process of conformation due to its geometry and physics properties. On the other hand, several polymers such as polylactic acid shows low osteoconductivity but better mechanical and degradable properties. Moreover, PLA has an easy conformation properties by diverse process which enables better control of its geometrical properties. Development of biodegradable polymer/ceramic compounds allows to obtain materials which fulfill properties as great mechanical strength, osteoconductive, osteoinductive and easy conformability. The table showed below (**¡Error! No se encuentra el origen de la referencia.**) grades the applications and properties of biomaterials.

In 1999 Shikinami [88] published his studies based on HA and polylactic acid compounds formed by pressure. These compounds showed closely mechanical properties to cortical bone; also it showed resorbability, bioactivity and osteoconductivity properties. In 2004 Ignjatovic y Uskokovic [89] developed HA and polylactic acid compound by pressure with heat. It was determinate that the variation in pressure entails different porosity. These materials were implanted in rats showing good compatibility and tissue adhesion of the implant. Weir et al [90] states that crystallinity changes and molecular weight are generated by sterilization process altering significantly mechanical properties.

2.3. Biodegradability

Biodegradability refers to polymer transformation and degradation due to enzymes activity or microorganism actuation such as bacteria and fungi. Biodegradation could be partial or total. Partial degradation consists in chemical structure alteration of the material entailing loose of specific properties.

Physiological environment satisfies proper conditions to carry out the hydrolytic process. For this purpose, the polymer must have hydrolytically unstable linkages and has to be hydrophilic to make the biodegradable process happen in a reasonable time. Hydrolysis process should be performed under PH physiological conditions (PH between 7 and 7.4).

In general terms, biodegradation occurs in the organism through via hydrolytic and is complete usually with an enzyme process. Accordingly, in new materials development should be studied different environment resistance. The most common biodegradability agents in the body organism are: water, inorganic salts (anions and cations), physiologic environment PH and enzymatic agents.

Biodegradable materials in medical applications should maintain their mechanic properties until they have complete their function. From that moment, they can be absorbed and excreted from the body without leaving traces. Chemical hydrolysis in weak links occurs in two steps. In the first step, water molecules penetrate the material, attacking the chemical links of the amorphous phase breaking the chains in smaller soluble water fragments. Therefore, this process occurs in the amorphous phase resulting in molecular weight loss without losing physical properties since the material matrix is supported by crystal phase. Once the amorphous phase is consumed, begins crystalline phase degradation. This selectivity can be attributed to a less ordered arrangement of amorphous phase, enabling the enzymes penetration into the polymer. The crosslinking factor of polycaprolactone also decreases degradation velocity. Due to limited chains mobility, enzymes have a difficult accessibility.

During the second step of the biodegradation process, named erosion bulk, the enzymatic attack produces fragments, leading to a faster polymer mass loss.

Degradation velocity is influenced by different factors:

- Environmental conditions: temperature, moisture, PH…
- Polymer features: presence of hydrolytically unstable linkages, stereochemistry, chain mobility, molecular weight, specific surface area, glass transition temperature and melting point, presence of residual monomer or additives, sequence distribution, presence of phenolic, hydroxyl or carboxylic

lateral groups (because several proteolytic enzymes specifically catalyze the hydrolysis of peptide bonds adjacent substituent groups), ...

- Microorganism features: quantity, variety, source, activity…

2.4. Fixing systems: Plates and Screws

Currently, the most popular method for rigid internal fixation of fracture bone is plates and screw, also called osteosynthesis plates. Conventionally, these rigid fixations were made of stainless steel, Cr-Co and Ti alloys and are designed to provide high axial pressures (dynamic compression) in the bone fracture, in order to facilities the bone heating and avoiding the formation of external callus. After one or two years from the operation, the plate and the screw are removed when a complete bone healing has been accomplished. However, it was reported that rigid fixation results in bone atrophy beneath the plate, and could cause refracture of the bone after plate removal [91-93]. This is attributed to stress shielding effect. Such effect occurs because the applied stress is lower than the normal physiological load, due to load is supported largely by the plate instead of the bone, giving rise to bone mass decrease and bone weakening. Hence, the bone placed underneath the plates adapts to the low stress and becomes less dense and weal. Several researches [94-96] have shown that degree of stiffness is proportional to the degree of stress protection mismatch. The stress-shielding affects bone remodeling and healing process leading to increase bone porosity (bone atrophy) [97-99]. It has been reported that a similar stiffness values between the implant and the host tissue, produces desired tissue remodeling. Taking this into account, the use of low-modulus polymers for these applications appears interesting.

According to that, it may be noted that the modulus of stainless steel is much higher than bone modulus (210-230 GPa vs 1-18 GPa). Analyzing this values, it can be understood that the plate transmit the majority of the stress [100]. The most similar stiffness enhance fracture healing mechanism. Due to the higher strain at the fracture sites, the primary healing is no longer possible and is replaced by physiological bone healing process, leading to the formation of an external callus and bridging the fracture. Hence, the cross-section of the newly formed bone is increased by the callus, prevents refracture.

3. Materials

Skull injures are the most frequent trauma seen in urban trauma centers [55, 56], leading causes of death and disability worldwide [57]. Accordingly big efforts are focused on the research of new materials capable to fulfill the requirements for bone fracture remodeling. Metal prostheses currently used are biocompatible and highly resistant but are heavy, expensive, do not promote the bone regeneration and neither are degraded over time. Consequently, a second intervention should be practiced in order to remove the metal prosthesis once the fractured bone is healed, entailing a slower recuperation process and possible infections. For the purpose of preventing the aforementioned stress shielding effect, several studies [101-104] focus their efforts in development of low-modulus biodegradable polymers in order to get similar stiffness values between the implant and the host tissue, resulting in a desirable tissue remodeling, avoiding the stress-shield effect.

Controlled delivery of osteogenic biomaterials is an alternative to traditional bone repair techniques, leading also a faster recuperation of the involved joint. Materials used for this kind of purposes must be able to overcome important medical issues e.g. implant rejection, chronic inflammatory reaction, infections, corrosion, metal toxicity, commonly present in typical metallic prostheses [103, 105]. Natural and synthetic polymers have been broadly studied as possible use in fixations, being bio absorbable synthetic polymers the most used, for instance Polylactic acid (PLA), polyglicol acid (PGA) or polycaprolactone (PCL) [101, 102, 104].

3.1. Polycaprolactone

Polycaprolactone (PCL) is an aliphatic polyester and degradable biomaterial. A great number of studies regarding in vitro and in vivo biocompatibility have been performed, accomplishing PCL as a suitable material for medical and drug delivery devices by US Food and Drug Administration approval [106-109]. The glass transition temperature of the PCL is around -60ºC, thus, it can be degraded under physiologic conditions as the same way as if it were a poly(α hydroxi acids) [110]. However, its applications are limited due to its low mechanical properties. For this reason it is necessary in many

cases to modify the PCL by adding fillers or blends [111-115]. The use of bio absorbable polycaprolactone for medical implants entail two significant challenge. First of all, it should be controlled the resorption degradation process. Secondly, mechanical properties will change with the rate of resorption, so the material should fulfill sufficiently high modulus until the regeneration of the broken bone. Comparing resorption kinetics degradation of others aliphatic polyesters (PLA, PGA), PCL shows considerably slower rate of resorption due to its hydrophobic character and high crystallinity [116], fact that encourages us to study PCL efficiency as a bioabsorbable plates. Craniofacial fractures might require implants with structural properties maintained for between 6 and 52 weeks [117]. Polycaprolactone is known to persist in vivo for more than 1 year [118, 119]. However, Polyglicol acid (PGA) loses practically all its strength in 6 weeks, being an hydrophilic polymer with a degradation rate within 3-12 months [120], notwithstanding its elevate degree of crystallinity, around 45-55%, thus resulting in insolubility in water [121]. Polylactic acid (PLA) remains intact longer than PGA, around 6 weeks due to its hydrophobicity and its isomers tend to form crystals during degradation process [122], with a complete resorption time for pure PLA between 1-6 years [120].

Marra et al. [123] reported that PCL is a suitable substrate for supporting cell growth promoting the regeneration of bone tissue.

Pego et al. [124] studied the degradation and the tissue response of polycaprolactone copolymers by immersing the explants in PBS containing 1 vol % of penicillin/streptomycin and kept at 4°C until further evaluation. Weight and number average molecular weight ($\bar{M}w$ and $\bar{M}n$, respectively), polydispersity index (PDI), and intrinsic viscosity ([η]) were determined by gel permeation chromatography (GPC) using chloroform as eluent at a flow rate of 1.5 mL/min. Up to 12 weeks the $\bar{M}n$ shown that degradation was slow. Despite the decrease in molecular weight observed in the 1-year implantation period, $\bar{M}n$ was still >90,000. One can expect that after this time the implants still possess suitable mechanical properties. Pego states that copolymers of polycaprolactone (90 mol %) had a constant strength during the first 50 weeks of degradation. Nevertheless, it was observed a linear decrease in σ_{max} during the second year, agreeing with the point in time that $\bar{M}n$ reached values below 75000. After 111 weeks, when the samples had values of $\bar{M}n$ of 25000, it presented fragility when were

tested. At this point, the samples seem to have a negligible tensile strength when it reaches those values of M̄n.

The degradation mechanisms of polycaprolactone have been studied for several groups, although it is still unclear. The products generated are either metabolized via the tricarboxylic acid (TCA) cycle or eliminated by direct renal secretion. It is known that the increase in polymer crystallinity reduce the accessibility of the polymer bulk, entailing limitation in both the rate of chain scission and weigh loss. Pitt and co-workers [109] proposed a first-order kinetic model which relates the rate of chain scission of an aliphatic polyester auto catalyzed by the generated carboxylic acid end groups to the decrease in M̄n with time.

$$\ln(\overline{Mn}) = ln(\overline{Mn^0}) - kt$$

Figure II.14

According to Pitt [109] and Pego [124], the first state of degradation process involve random hydrolytic ester and carbonate groups cleavage, although preferentially at the ester bounds. This involves the generation of carboxylic acid end groups by ester hydrolysis (Figure 1). Nevertheless, the second state stage begins with deviation from the initial linear relationship between ln(**η**) or ln(M̄n) and time in vivo.

3.2. Hidroxyapatite

Apatite term was used for the first time to reference to ores in 1788 by the German geologist William Alexander Deer [125], but the first attempt to establish the

hydroxyapatite´s composition by chemical analysis began in the second half of the 18th century by the Swedish chemist Jöns Jacob Berzelius (1779-1848) [126]. Currently this term is referred to a crystal family of materials related to the formula $M_{10}(RO_4)_6X_2$ where M refers to calcium, R to Phosphorus and X to an hydroxide or another halogen compound such as fluorine. The inorganic phase of the bone was determinate by X ray diffraction technique exposing that it was apatite in 1926 [127].

Hydroxyapatite is an apatite composed essentially with phosphorous and calcium with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. The atomic ratio CA/P is 1.67 with 39% by weight of Ca, 18.5% P and 3.38% of OH [128, 129].

Although several materials have been designed as hydroxyapatite by diverse researchers, actually, the Ca/P rate shown could be variable with a range of 1.3 to 2.0. These materials are seldom properly characterized, for this reason is though compare results from different sources of study. Nevertheless, the American Society for Testing and Materials (1987) determined the reference patterns both for Hydroxyapatite to tricalcium phosphates, in order to control and determinate the purity of these compounds[127] (Jaffe and Scott 1996). According to the explained before, the rate Ca/P should be 1.67, while the rate of tricalcium phosphates should be 1.5 [127].

- Contribution of hydroxyapatite in the bone

Bone composition varies according the position, age, food background and illness. Usually, the mineral phase represents between 60-70% of the bone tissue, water 5-8%, and organic matrix constitutes the rest (20%), which the 85-90% of this is collagen and 15-5% constitutes to non-collagenous proteins [130].

The mineral phase of the human bone includes different kinds of hydrated calcium phosphates; the most common is hydroxyapatite. This apatite is presented in bone as a flat crystal with a length of 20-80 nm and a thickness of 2-5 nm [130, 131]. It is calculated that an average of 65% of mineral phase in human bone is composed by hydroxyapatite [130].

Hydroxyapatites produced biologically does not contain only ions and radicals of the HAp but also traces of CO_3, Mg, Na, F and Cl because phosphate(PO_3) and hydroxide groups (OH) could be replaced by carbonate (CO_3) or chlorine(Cl) and fluorine(F) respectively, influencing physics properties of the ore. Impurity amounts vary according

to food background or the specific type of tissue, afecting properties and bioactivity of the bone [131].

The most highlight mechanic property of hydroxyapatite is the stiffness and toughness, which are combined with collagen elasticity. Collagen is the mainly component in bone organic phase, and provides particularity properties to the bone. Hydroxyapatite crystals improve bone matrix stiffness, in such a way that without them, bone could bend in an extremely easy way [132].

- Improvement of PCL mechanical properties depending of HA loading percentage.

Following the experimental data carried out by Biqiong Chen and Kang Sun [133] with the study of different amount of hydroxyapatite reinforced poly ε-caprolactone, they reported that the optimum percentage of hydroxyapatite is 20%. Is for that reason that we decided to introduce a load of 20% hydroxyapatite in our material. They studied a load of 0%, 20%, 40% and 60%, concluding that a load of 20% enhance the mechanical and thermal properties of the compound. Regarding the thermal properties, there are not significant, nevertheless the melting (Tm) and crystallization (Tc) temperature of the composite were increased by the presence of HA, suggesting slight interactions where HA facilitates the crystallization of PCL under cooling, acting as a nucleating agent.

On the other hand, it was shown an enhancement of mechanical properties. The strength reaches its greatest values when the concentration of HA was 20% and as the content of HA increased, the strength decreased significantly. It can be explain because when a matrix is loaded with certain particles, them limit the movement of the molecular chains in the matrix, when the amount is high the particles tend to agglomerate.

3.3. Halloysite Nanotubes

Halloysite Nanotubes (HNTs) is a clay material found as a raw mineral from natural deposits, fact that makes halloysite economically viable [134]. HNTs presents nanotubular structure where the outer surface can be compared to silica with oxygen

atoms, making the surface predominantly negative over most of the physiologically relevant range (PH 6-7) [135]. While the inner surface is relevant to alumina with hydroxyl groups giving a positive charge surface below 8.5 PH [136].

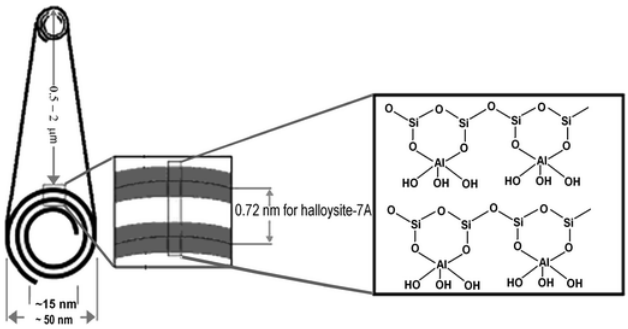


Figure II.15 Scheme of Halloysite Nanotube

The cylindrical structure is caused because alumina and silica layers and their water of hydration create a packing disorder resulting in the curvature of the sheets and rolling it selves giving a multilayer tubes [134]. The biocompatibility properties and tubular shape make HNTs a perfect material to be used for loading release applications with a small inner diameter of 15 nm and outer diameter of 50 nm [137-139].

The first study related to HNTs as a nanocontainer was carried out by Price et al. [138], demonstrating that HNTs is a viable and inexpensive nanocontainer for encapsulation of biologically active molecules. It has been also reported its physiochemical characterization as a novel drug delivery system [140-142]. Regarding the release rate, it can be compared the drug alone release with the entrapped drug in HNTs release, perceiving an improvement from 30 to 100 times longer with the encapsulation of the drug. Furthermore, the addition of polymer coating to the drug loaded HNTs can increase even more the release rate [143].

Halloysite entraps molecules in different ways, including adsorption to external and internal walls of the tubes [144, 145], intercalation [146-151] and loading of the substances into the lumen accompanied by crystallization of the loaded samples [140, 141, 152, 153].

Overloading of HNTs in a polymer matrix leads to nanotubes agglomeration, originating failure initiation sites in the material [154-156]. The agglomeration process occurs because of zig-zag structures generation by edge to edge and face to face interactions

between HNTs due to the chemical composition of HNTs [157]. The shape of nanotubes plus charge distribution (inner and outer face of tubule walls carry net negative charges and the edges are amphoteric with positive charge at low PH) favors face to end attachment [152]. Formation of aggregates can be possibly avoided by a surface modification of HNTs. Nevertheless, agglomeration effect can be minimized by a previous extrusion process to injection molding to improve the HNTs dispersion in the polymeric matrix, enhancing the mechanical properties. Moreover, it was observed by [158] that higher rotation speed of extruder, results in better nanotubes dispersion.

III. Objectives

1. Objectives

1.1. General objective

Improve mechanical and biologic properties of bio absorbable polymeric fixing systems to use in craniofacial injuries.

1.2. Specific objectives

1. Design blend formulations and manufacturing test specimens of bio absorbable materials based on Polycaprolactone additive with Hydroxyapatite and Halloysite Nanotubes.
2. Determine the optimum additive percentage.
3. Mechanical and thermal analysis of the different additived materials.

IV. Experimental

1. Materials

Polycaprolactone called commercially as CAPA 6500 was kindly provided by Solvay Interox UK. CAPA 6500 is a high molecular weight thermoplastic linear polyester derived from caprolactone monomer. The melting point is between 58-60ºC and the molecular weight is 50.000 Da.

In this study seven different samples of polycaprolactone (PCL) biomaterials were performed, consisting on different percentage of Hydroxyapatite (HA) and Halloysite nanoclay (HNTs) specified below in Table IV-1.

The chemical formula of the Hydroxyapatite is $HCa_5O_{13}P_3$ was supplied by Sigma Aldrich, Madrid, Spain. The mineral phase of the human bone includes different kinds of hydrated calcium phosphates; the most common is the hydroxyapatite. This apatite is present in the bone as a flat crystal with a large of 20-80 nm and 2-5 nm of thickness [131]; it is calculated that an average of 65% of the mineral phase of the human bone is composed by hydroxyapatite [130].

Halloysite Nanotubes, with molecular formula $Al_2Si_2O_5\ (OH)_4 \cdot 2\ H_2O$ was supplied by Sigma Aldrich, Madrid, Spain. HNTs is a clay formed by double layer of aluminum, silicon, hydrogen and oxygen. Its tubular shape results of a natural process which takes millions of years entailing the strain caused by lattice mismatch between adjacent atoms of the layers [159].

		Mass (%)		
	Sample ID	***PCL***	***HA***	***HNTs***
1	PCL HA 20	80	20	0
2	PCL HA 20 HNTs 2.5	77.5	20	2.5
3	PCL HA 20 HNTs 5.0	75	20	5.0
4	PCL HA 20 HNTs 7.5	72.5	20	7.5
5	PCL HNTs 2.5	97.5	0	2.5
6	PCL HNTs 5.0	95	0	5.0
7	PCL HNTs 7.5	92.5	0	7.5
8	PCL	100	0	0

Table IV-1 PCL/HA/HNTs mass percent composition for samples injected.

2. Procedures and methods

For the sample preparation, the first step was weighing the corresponding amount of PCL, HA and HNTs as it is specified in the Table IV-1. The different samples were mechanically mixed in a zip bag previously to be extruded in a twin screw corotating extruder at 40 rpm.

The profile for the four barrels of the extruder were set according the data summarized in Table II-2. After this process a period of cooling was needed before the pelletization of the samples. When the samples were pelletized with a ball mill, an injection molding process was made using a Meteor 270/75 injection (Mateu and Solé, Barcelona, Spain). The mold used was a steel mold with mirror finishing with the dimensions recommended by the corresponding standards.

Sample	***T1 (˚C)***	***T2 (˚C)***	***T3 (˚C)***	***V (rpm)***	***P (bar)***	***volumetric feeding (Kg/h)***	***Calculated flow (Kg/h)***
PCL HA 20	60	70	80	250	50	2.5	30
PCL HA 20 HNTs 2.5	60	70	80	250	50	2.5	30
PCL HA 20 HNTs 5.0	65	75	85	250	45	2.5	30
PCL HA 20 HNTs 7.5	65	75	85	250	45	2.5	30
PCL HNTs 2.5	65	80	90	250	60	2.5	30
PCL HNTs 5.0	70	80	90	250	65	2.5	30
PCL HNTs 7.5	70	80	90	250	65	2.5	30
PCL	90	85	80	75	60	2.5	30

Table IV-2 Extruder and injection parameters

In order to determine the possible applications of the prosthesis made with PCL/HA/HNTs composites, a complete study of the mechanical and thermal properties was required once they were injected. The aim of this assessment is to establish a relation between the properties and the internal structure of the materials with the subsequent selection of the mixture which best fulfilled the requirements.

2.1. Mechanical properties

The material mechanical properties describe how it respond to an applied load or force.

Firstly, the **hardness Shore D** was measure using a durometer Model 673-D (J. Bot Instruments, Barcelona, Spain) following the guidelines of the UNE-EN ISO 868. Each sample was evaluated at least 5 times obtaining an average value. Results are shown below (Table IV-3).

Sample	*1*	*2*	*3*	*4*	*5*	*x*	*s*
PCL HA 20	49	50	49	50	50	49.6	0.55
PCL HA 20 HNTs 2.5	50	50	51	51	51	50.6	0.55
PCL HA 20 HNTs 5.0	50	50	51	51	50	50.4	0.55
PCL HA 20 HNTs 7.5	52	52	52	53	52	52.2	0.45
PCL HNTs 2.5	48	48	48	47	47	47.6	0.55
PCL HNTs 5.0	47	47	46	47	48	47	0.71
PCL HNTs 7.5	46	46	47	47	47	46.6	0.55
PCL	48	48	47	48	48	47.8	0.45

Table IV-3 data hardness Shore D

With the purpose to evaluate dynamic fracture toughness, an **impact Charpy test** was carried out using a Charpy pendulum (Metrotec S. A., San Sebastian, Spain) with an energy of 1 J, according to ISO 179. Each value was obtained as a minimum of 5 notched samples and calculating the average value and not accepting a deviation upper than 5%. The data is shown below in Table 7.

Sample	*1*	*2*	*3*	*4*	*5*	*x*	*s*
PCL HA 20	0.56	0.52	0.54	0.56	0.56	0.55	0.02
PCL HA 20 HNTs 2,5	0.43	0.43	0.46	0.45	0.44	0.44	0.01
PCLA HA 20 HNTs 5,0	0.38	0.39	0.38	0.33	0.39	0.37	0.03
PCL HA 20 HNTs 7,5	0.3	0.33	0.34	0.32	0.32	0.32	0.01
PCL HNTs 2,5	0.47	0.53	0.49	0.48	0.51	0.50	0.02
PCL HANTs 5,0	1.18	1.17	1.06	1.14	1.09	1.13	0.05
PCL HNTs 7,5	0.78	0.85	0.8	0.8	0.81	0.81	0.03
PCL	0.53	0.53	0.53	0.52	0.5	0.52	0.01

Table IV-4 Data Charpy test

To complete the study of the mechanical properties, **flexural and tensile test** were performed using an electromechanical universal test machine Elib 30 (Ibertest S.A.E, Madrid, Spain). The flexural test was carried out according to ISO 178 with an extension rates of 5 $mm \cdot min^{-1}$. In the case of the tensile test, the extension rate was 10 $mm \cdot min^{-1}$ according the UNE-EN ISO 527. The flexural and tensile strength (MPa) were evaluated in each assay. All the samples shown an elongation higher than 1000%. Data is shown below (Table IV-5 and Table IV-6).

Sample		***1***	***2***	***3***	***4***	***5***	***x***	***s***
PCL HA 20	**Load (N)**	9480	7820	7720	7440	7540	8000	0.08
	Elastic modulus (Mpa)	179.49	160.90	147.76	148.14	152.36	157.73	13.2
PCL HA 20 HNTs 2,5	**Load (N)**	8960	7930	7820	7510	7980	8040	0.05
	Elastic modulus (Mpa)	188.05	228.46	239.04	239.38	244.38	227.86	23.0
PCLA HA 20 HNTs 5,0	**Load (N)**	-	7440	8080	8030	-	7850	0.04
	Elastic modulus (Mpa)	-	225.54	233.24	231.88	-	230.22	4.11
PCL HA 20 HNTs 7,5	**Load (N)**	-	7760	-	7580	7490	7610	0.01
	Elastic modulus (Mpa)	-	173.86	-	157.89	157.92	163.22	9.21
PCL HNTs 2,5	**Load (N)**	8580	6870	6740	6780	6720	7140	0.08
	Elastic modulus (Mpa)	140.91	144.15	143.21	140.32	149.90	143.69	3.81
PCL HANTs 5,0	**Load (N)**	9250	6540	6440	6120	6520	6970	0.13
	Elastic modulus (Mpa)	134.76	145.68	143.56	136.30	148.72	141.80	6.04

PCL HNTs 7,5	**Load (N)**	8920	6830	5740	6560	6630	6940	0.12
	Elastic modulus (Mpa)	142.59	126.59	158.58	150.31	151.73	145.96	12.2
PCL	**Load (N)**	9400	9410	9520	9690	-	9500	0.01
	Elastic modulus (Mpa)	174.20	145.11	140.12	155.80	-	153.80	15.0

Table IV-5 Data Tensile strength test

Sample		***1***	***2***	***3***	***4***	***5***	***x***	***s***
PCL HA 20	**Load (N)**	-	47.80	48.20	47.00	51.40	48.60	1.93
	Flexural modulus (Mpa)	-	667.64	636.58	607.93	594.82	626.74	32.36
	Tensile strength (Mpa)	-	28.80	29.10	28.40	31.00	29.33	1.15
PCL HA 20 HNTs 2,5	**Load (N)**	50.90	53.50	50.30	52.90	53.30	52.18	1.47
	Flexural modulus (Mpa)	785.25	733.03	807.77	705.15	823.33	770.91	50.18
	Tensile strength (Mpa)	30.80	32.30	30.30	31.90	32.20	31.50	0.90
PCLA HA 20 HNTs 5,0	**Load (N)**	55.60	54.60	52.30	51.20	55.30	53.80	1.95
	Flexural modulus (Mpa)	884.04	885.33	883.70	810.84	855.16	863.81	32.20
	Tensile strength (Mpa)	33.60	33.00	31.60	30.90	33.40	32.50	1.19
PCL HA 20 HNTs 7,5	**Load (N)**	53.10	51.70	54.20	54.00	56.00	53.80	1.58
	Flexural modulus	871.11	817.48	944.43	892.85	908.16	886.81	47.08

	(Mpa)							
	Tensile strength (Mpa)	32.10	31.20	32.70	38.22	33.80	33.60	2.75
PCL HNTs 2,5	**Load (N)**	40.20	42.40	41.50	40.60	40.20	40.98	0.95
	Flexural modulus (Mpa)	391.91	463.86	447.52	446.79	445.90	439.20	27.46
	Tensile strength (Mpa)	24.30	25.60	25.10	24.50	24.30	24.76	0.57
PCL HANTs 5,0	**Load (N)**	41.60	39.50	39.30	39.00	39.30	39.74	1.05
	Flexural modulus (Mpa)	471.70	437.59	444.76	463.21	401.73	443.80	27.22
	Tensile strength (Mpa)	25.10	23.80	23.70	23.50	23.73	23.97	0.64
PCL HNTs 7,5	**Load (N)**	41.00	42.70	41.80	40.00	43.80	41.86	1.47
	Flexural modulus (Mpa)	483.66	463.60	540.53	404.95	525.19	483.59	53.75
	Tensile strength (Mpa)	24.80	25.80	25.20	24.20	26.50	25.30	0.89
PCL	**Load (N)**	41.80	41.90	38.00	40.40	42.00	40.82	1.71
	Flexural modulus (Mpa)	430.15	430.28	387.98	387.64	452.24	417.66	28.69
	Tensile strength (Mpa)	25.20	23.60	21.40	22.70	23.60	23.30	1.39

Table IV-6 Data Flexural strength test

2.2. Thermal properties

Polymers are very sensitive to temperature changes and many factors contribute to affect their thermal behavior. The thermal properties of polymers are equally as important as the mechanical properties. Thermal analysis involve several techniques to assess the material behavior vs temperature changes. The aim of theses analysis is to set a relationship between the material physic properties and the temperature.

To study the thermal stability, different techniques were used. In order to study the main thermal transitions and the possibility of modification these in the composites, **calorimetric analysis (DSC)** was performed using a DSC Mettler-Toledo DSC 821e (Mettler-Toledo S.A.E., Barcelona, Spain). Every sample (between 5 and 10 mg) was studied with the following heating rate (30-350 at 10ºC·min^{-1}) in atmospheric air (Table IV-7).

Sample	*Mass (mg)*	*Integral (J/g)*	*Tm (˚C)*	*Td (˚C)*
PCL HA 20	8.8	-108.96	64.69	268.11
PCL HA 20 HNTs 2,5	7.0	-58.71	62.80	268.89
PCLA HA 20 HNTs 5,0	9.2	-50.11	62.40	274.69
PCL HA 20 HNTs 7,5	9.3	-60.30	63.08	271.86
PCL HNTs 2,5	7.1	-56.88	63.17	238.78
PCL HANTs 5,0	9.0	-79.32	61.12	262.60
PCL HNTs 7,5	6.6	-51.26	61.74	261.61
PCL	6.9	-72.46	63.76	250.26

Table IV-7 DSC data

Thermo gravimetric analysis (TGA) was performed to study possible modifications of physical properties as a consequence of the temperature. The equipment used was a (Mettler-Toledo Inc., Schwerzenbach, Switzerland) with a heating rate of 30-350 at 20ºC·min^{-1} (Table IV-8).

Sample	*Mass (mg)*	*Mass loss %*	*Td (˚C)*
PCL HA 20	8.8	-77.63	300-500
PCL HA 20 HNTs 2,5	5.9	-76.62	300-500
PCLA HA 20 HNTs 5,0	6.4	-74.22	300-500
PCL HA 20 HNTs 7,5	8.5	-70.74	300-500
PCL HNTs 2,5	8.1	-88.58	300-500
PCL HANTs 5,0	6.7	-89.64	300-500
PCL HNTs 7,5	6.8	-87.41	300-500
PCL	8.8	-91.75	300-500

Table IV-8 Thermo gravimetric analysis (TGA) data

VICAT test was performed to determine the softening point of the different mixtures. The test consists in the obtainment of the temperature at which the specimen is penetrated to a depth of 1mm by a flat-ended needle with a load of 5 Kg. The equipment used was a Vicat/HDT VHDT 20 (Metrotec SA, San Sebastian, Spain). The experiment conditions are contemplate in the standard ISO 306 and D1525. In Table IV-9 is summarized the different registered temperature.

Sample	*T1 (˚C)*	*T2 (˚C)*	*x*	*s*
PCL HA 20	56.8	56.6	56.7	0.14
PCL HA 20 HNTs 2,5	55.6	55.6	55.6	0.00
PCLA HA 20 HNTs 5,0	55.0	55.2	55.1	0.14
PCL HA 20 HNTs 7,5	54.8	55.0	54.9	0.14
PCL HNTs 2,5	55.4	55.6	55.5	0.14
PCL HANTs 5,0	54.6	54.6	54.6	0.00
PCL HNTs 7,5	54.2	54.6	54.4	0.28
PCL	54.8	53.8	54.3	0.71

Table IV-9 VICAT data for different samples of PCL

The **head deflection temperature test** (HDT) is the temperature at which a polymer deforms under a specific load (200g) as a consequence of a temperature program increase (50˚C/h). The equipment used was a Vicat/HDT VHDT 20 (Metrotec SA, San

Sebastian, Spain). The test was carried out following the standard ISO 75 and ASTM D648. The values obtained are summarized in Table 13.

Sample	*T1 (˚C)*
PCL HA 20	35.8
PCL HA 20 HNTs 2,5	37.6
PCLA HA 20 HNTs 5,0	37.4
PCL HA 20 HNTs 7,5	38.6
PCL HNTs 2,5	32.0
PCL HANTs 5,0	38.8
PCL HNTs 7,5	34.6
PCL	37.8

Table IV-10 HDT data for different samples of PCL

2.3. Dynamical Mechanical Analysis (DMA)

Finally, **Dynamic Mechanical Thermal Analysis (DMTA)** of all the composites were done with AR G2 from TA Instruments (TA Instruments, New Castle, USA). The analysis was made in torsion mode with samples of 40x10x4 mm^3. The heating rate used was from 25ºC to 80ºC at 2ºC·min^{-1} using a constant frequency of 1 Hz and a strain of 0.1% as a controlled variable. The storage modulus was obtained with the following expression showed below:

$$E' = \frac{\sigma_0}{\varepsilon_0}\cos\delta$$

Where E´ is considerate the storage modulus (MPa), σ and ε are the stress and strain respectively, and δ is the phase lag between stress and strain.

2.4. Scanning electron microscope (SEM)

A FEI model Phenom (FEI Company, Eindhoven, The Netherlands) **scanning electron microscope (SEM)** has been used to study the fractured surface from impact test. The

accelerating voltage was 5 kV. Previously, samples were metallized with a gold-palladium alloy in a sputter coater EMITECH mod. SC7620 (Quorum Technologies Ltd., East Sussex, United Kingdom).

V. Discussion

1. Mechanical properties

As a first approximation to determinate the mechanical properties of different composites of PCL/HA/HNTs, **Shore D Hardness** was measured, the average value is shown in Figure V.1 Shore D hardness. It can be observed the evidence that an introduction of HA and HNTs filler increases Shore D Hardness of the composites. On the other side, samples with only a filler of HNTs show a decrease in Shore D Hardness average values. However, the best results are obtained when two filler components have been added (Samples PCL HA 20 HNTs 2,5, PCLA HA 20 HNTs 5,0, PCL HA 20 HNTs 7,5). Analyzing the data, it seems evident that a synergic effect between the addition of HA and HNTs has been produced, increasing a 5% the Shore D Hardness value. Therefore, it is possible that the filler acts filling the empty spaces between the polymer chains as different authors have demonstrated [160-162].

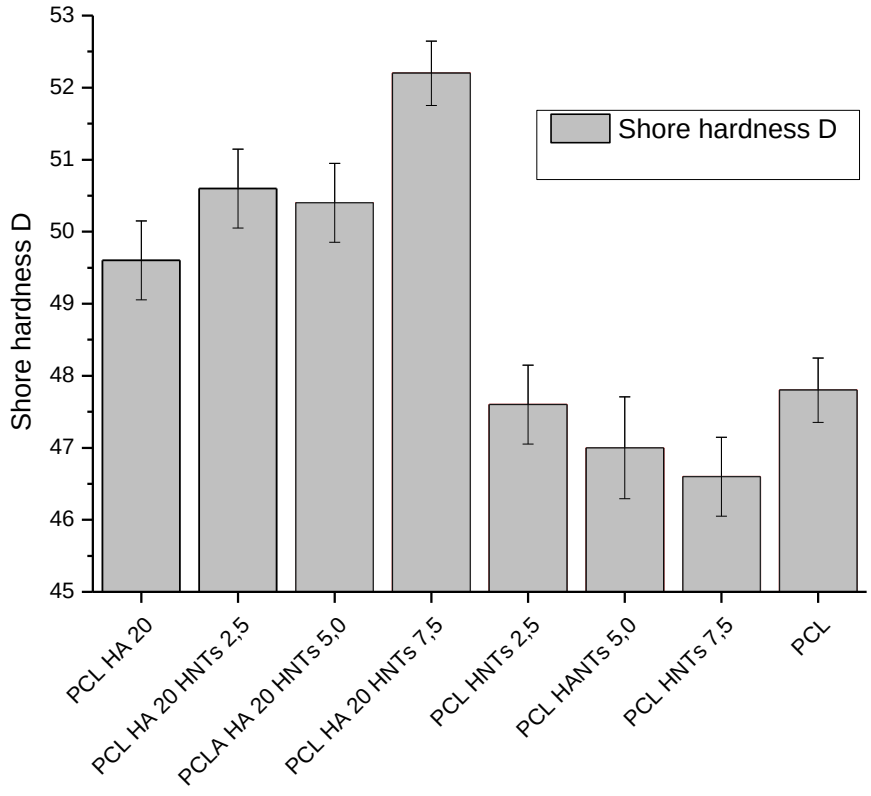


Figure V.1 Shore D hardness

It can be noticed that addition of HA and HNTs provides an enhancement of the mechanical properties. Moreover, it supplies intrinsic properties, for example

biocompatibility and tubular shape which can be used as drug delivery system [163, 164]. Notwithstanding, one of the main drawbacks of loading polymers is the decrease of absorbed energy by the polymer during the impact or deformation [165]. The data of Charpy Impact Test carried out is summarized in Figure V.2.

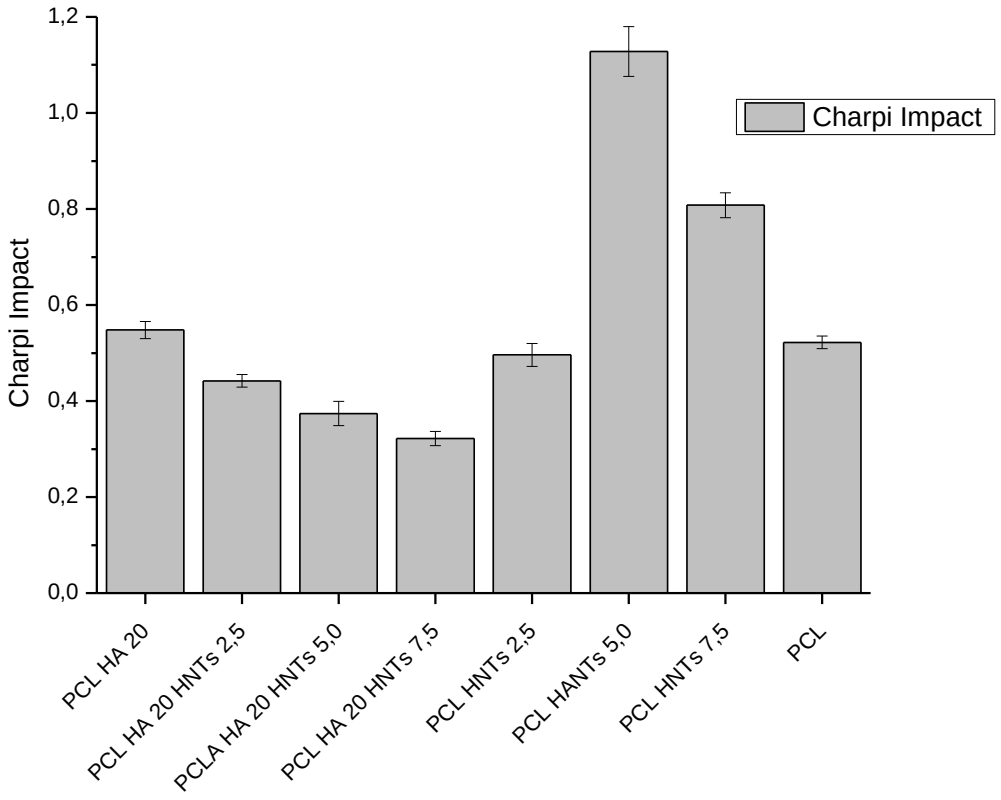
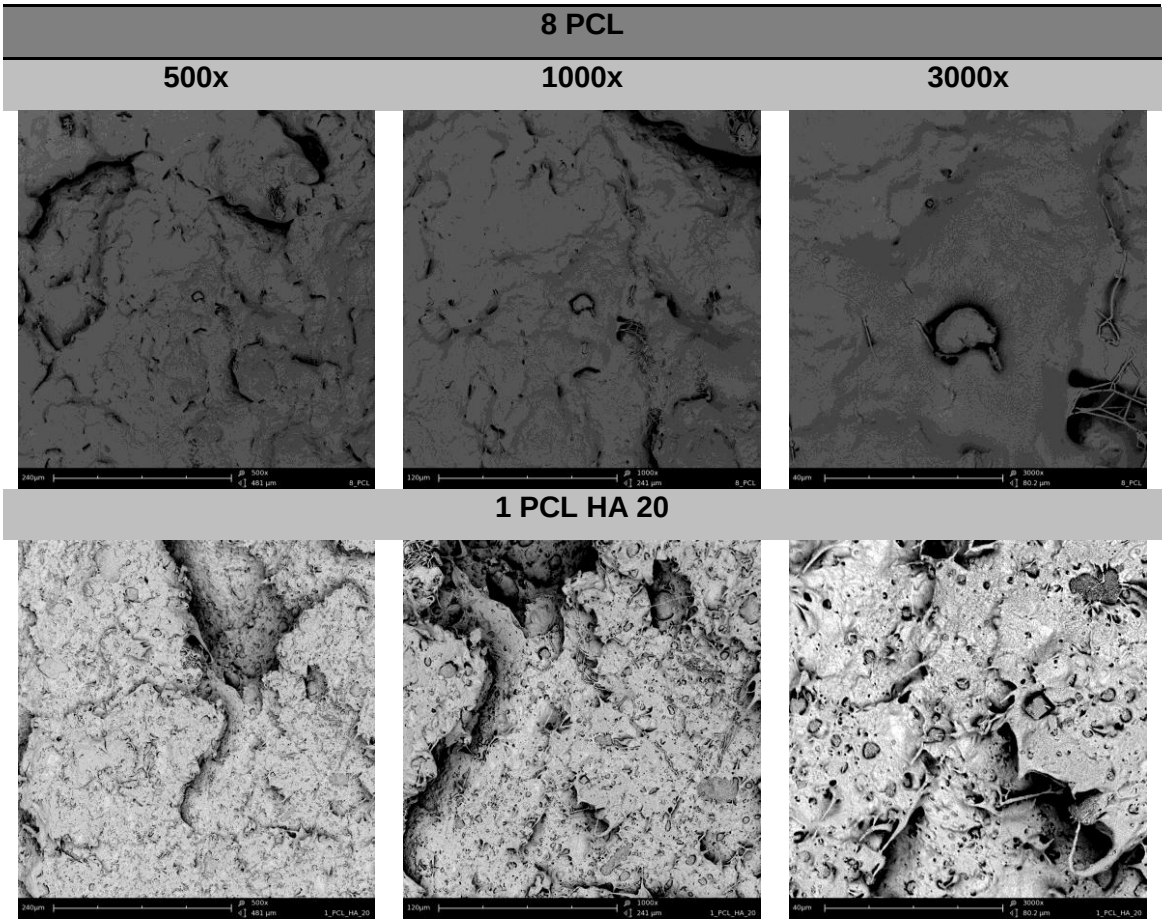


Figure V.2 Charpy Impact Test (kJ/m^2)

Charpy Impact test (kJ/m^2) shows a different behavior as a function of filler type. For example, HA provides a decrease in toughness with the increase of the amount of HNTs. But, without the presence of HA, the capacity to absorb impacts increase considerably with the presence of HNTs in the polymer. The enhancement is upper to 50% when a 7.5% of HNTs is added. These results suggest that strong interactions between PCL and HNTs are achieved, allowing more efficient load transfer [166].

Analyzing the Charpy impact test, a failure surface morphology was study for two reason: first of all, to give a logical explanation to the impact test results. Secondly, to observe the proper distribution of the HA and HNTs fillers in PCL matrix. The study of the different images obtained by SEM showed two types of morphologies (Figure V.3 and Figure V.4). As a representative sample of composites with low toughness, the

images of PCL HA 20 HNTs 7.5 sample (3,2 KJ/m^2) showed a homogeneous surface, without excessive cracks, feature which is typical present in brittle materials [167]. These samples are characterized for the lack of failure surface. Moreover, in this samples it is possible to observe a homogenous distribution of particles of HA and HNTs, so a correct compatibility between the fillers and the PCL matrix is attributed. This fact can be confirmed by the absence of large gaps between both components [168]. On the other hand, samples with high toughness, for example PCL HTNs 7.5 sample (8,1 KJ/m^2), have a high roughness surface. In this samples the heterogeneity of the samples is higher than in samples with low toughness. The surface is characterized by non-uniform appearance, with parallel lines of PCL product of the advance rate of the cracks during the fracture. This kind of morphology can be detected in ductile samples. In the same way, samples with low toughness showed distribution of dispersed particles, which could facilitate the correct delivery of drugs presents in the lumen of the HNTs. It should be taking into account that some samples have small amount of filler, is for this reason that the distribution is optimal.



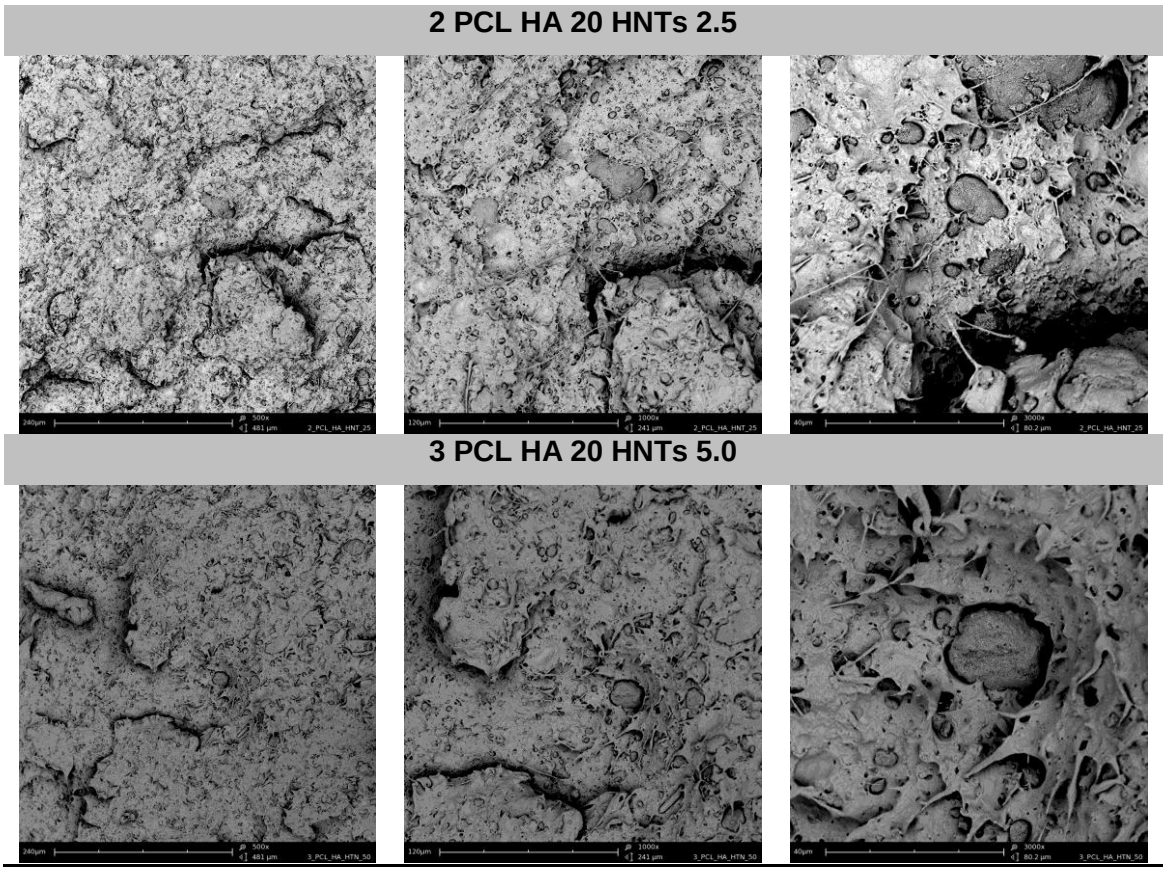
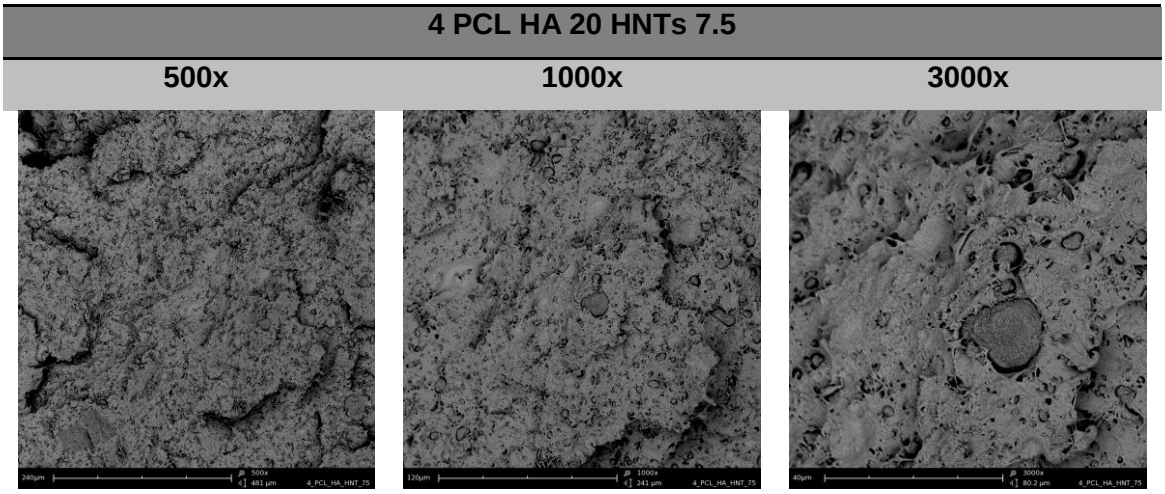

2 PCL HA 20 HNTs 2.5
3 PCL HA 20 HNTs 5.0

Figure V.3


4 PCL HA 20 HNTs 7.5
500x
1000x
3000x

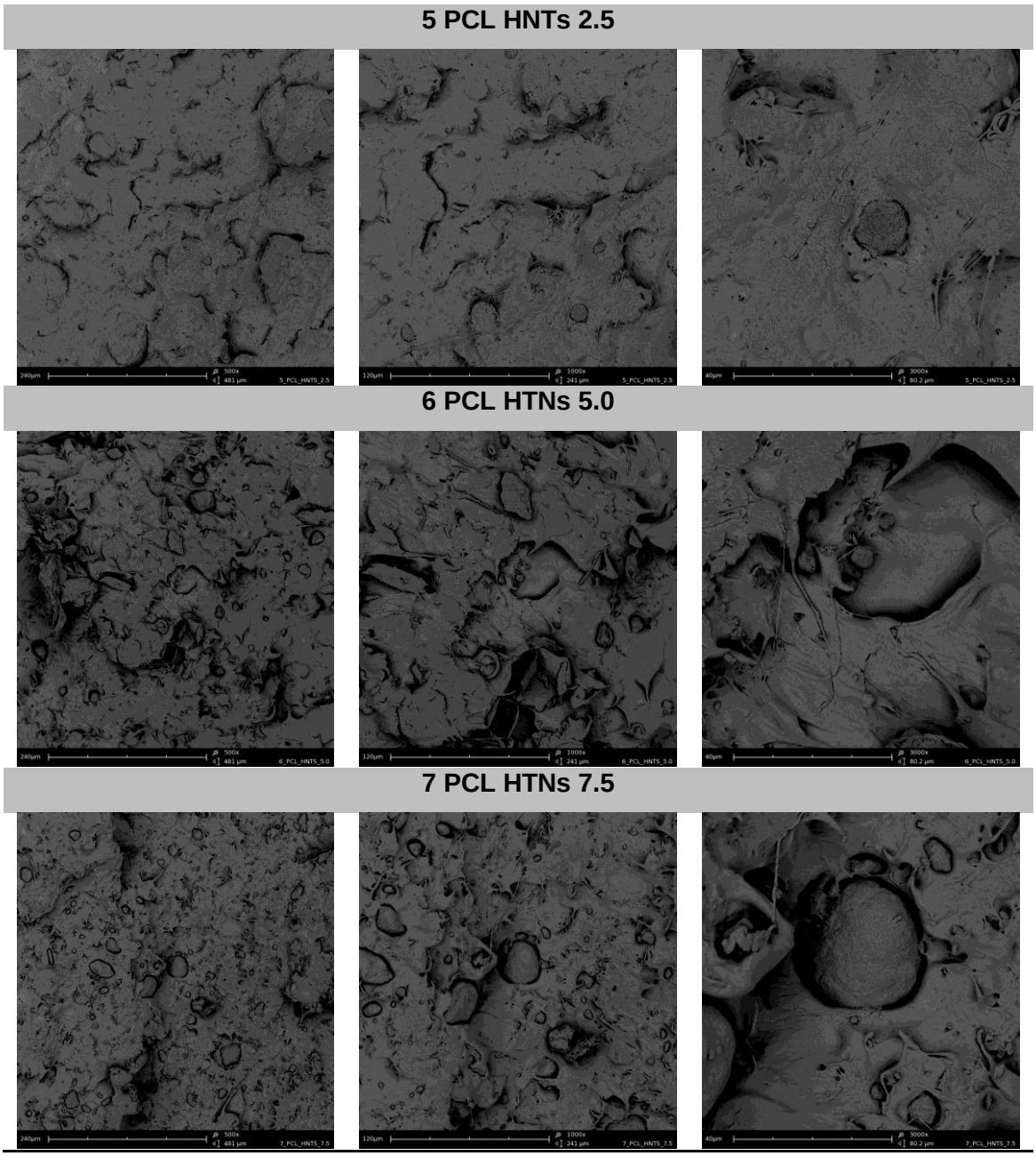


Figure V.4

Mechanical properties are completed with the determination of the **flexural behavior** of the different composites. As it can be seen, flexural strength and flexural modulus improved with the introduction of HA. Samples with 20% of HA reach values of up to 44% compared with the raw PCL value in flexural strength and 105% in flexural modulus. On the other hand, different behavior is registered with the lack of HA. This behavior agree with hardness results. Samples containing PCL and HNTs have practically identical flexural properties that PCL pristine sample. The material with the

best mechanical properties observed is the sample PCL HA 20 HNTs 7.5, which has a flexural modulus of 886.8 MPa. Decreasing of flexural properties exceeding 7.5 wt% of HNTs was tested by Liu et al. [154] and Prashantha et al. [155], correlating the overloading of HNTs with the generation of agglomerates acting as a weak points and failure initiation sites.

However, the values of flexural modulus obtained are lower compared to the majority of human bones, it has been related in diverse bibliography [169]. Nevertheless, the values obtained can be compared with human trabecular bone. According the literature present for now values of human bone modulus is typically between 7.000 and 25.000 MPa, these values depend on different variables such as type of bone, ages, location, and health factors [7, 170-172].

For this reason, the composites developed from natural sources as PCL, HA and HNTs are forced to be applied in low mechanical stress bones, for example mandibular bones and, especially, craniofacial bones [173]. Usually, craniofacial reconstructive surgery is performed on young children or infants, being necessary a reshaped with appropriated contour materials such as, mechanical properties, biocompatibility and positive esthetics aspects [103]. All these aspects could be covered with the development of bio-composites made with PCL, HA and HNTs, that moreover, allows the subsequent release of drugs.

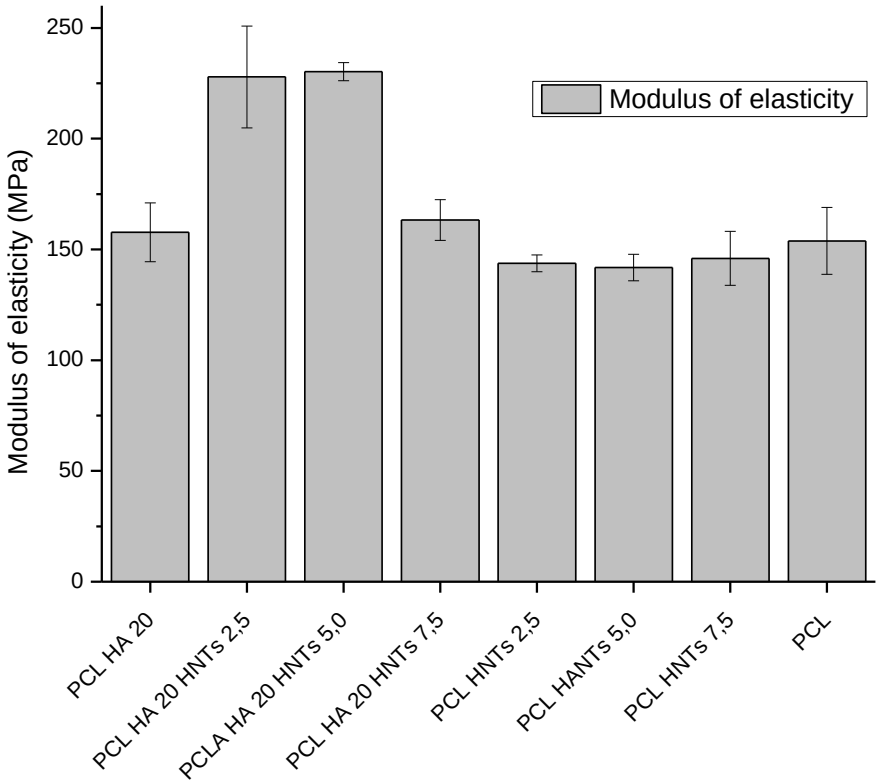


Figure V.5 Tensile strength results

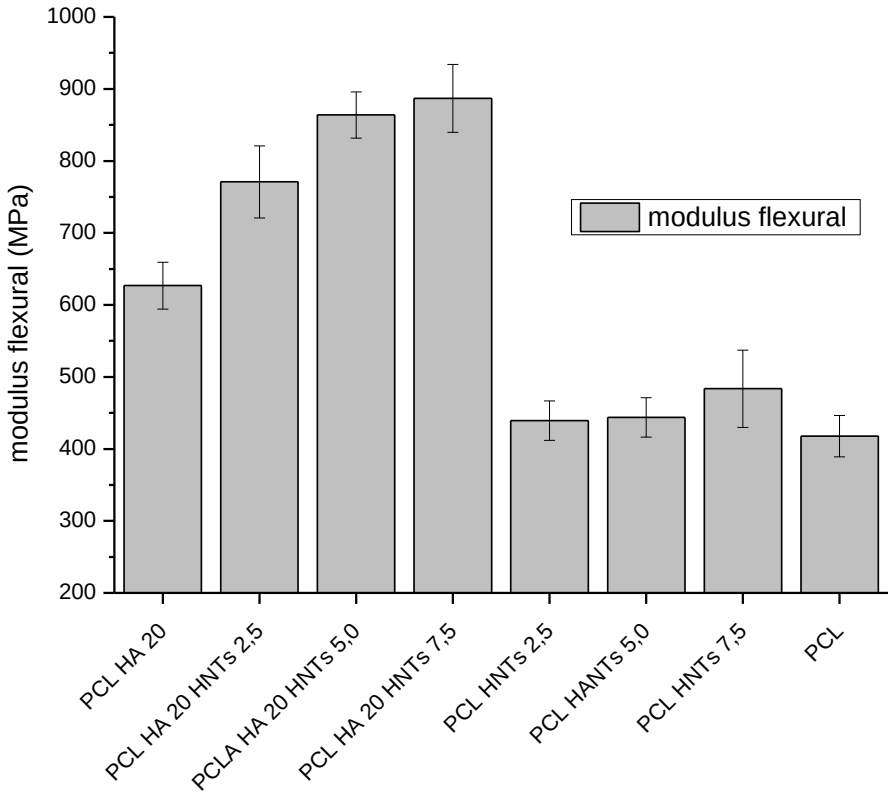


Figure V.6 Flexural results

2. Thermal properties

In a first stage, in order to determine the possible changes in thermal properties of the different composites developed, **Dynamic Scanning Calorimetry (DSC)** and **Thermo gravimetric Analysis (TGA)** were done. The data obtained is necessary to determine ease of processing and large-scale manufacturing. The results are summarized in Table V-1.

	DSC		TGA		
Sample	**Melting Temp Tm (ºC)**	**Degradation Temp Td (ºC)**	**Initial Temp (ºC)**	**End Temp (ºC)**	**Mass Residual Ratio (%)**
PCL HA 20	64,6	268,1	300,0	500,0	22,4
PCL HA 20 HNTs 2,5	62,8	268,8	300,0	500,0	23,4
PCLA HA 20 HNTs 5,0	62,4	271,4	300,0	500,0	25,8
PCL HA 20 HNTs 7,5	63,1	274,8	300,0	500,0	29,3
PCL HNTs 2,5	63,1	261,9	300,0	500,0	11,4
PCL HANTs 5,0	61,2	262,6	300,0	500,0	10,4
PCL HNTs 7,5	61,7	261,6	300,0	500,0	12,6
PCL	63,7	250,2	300,0	500,0	8,2

Table V-1 Summary of thermal values obtained by DSC and TGA

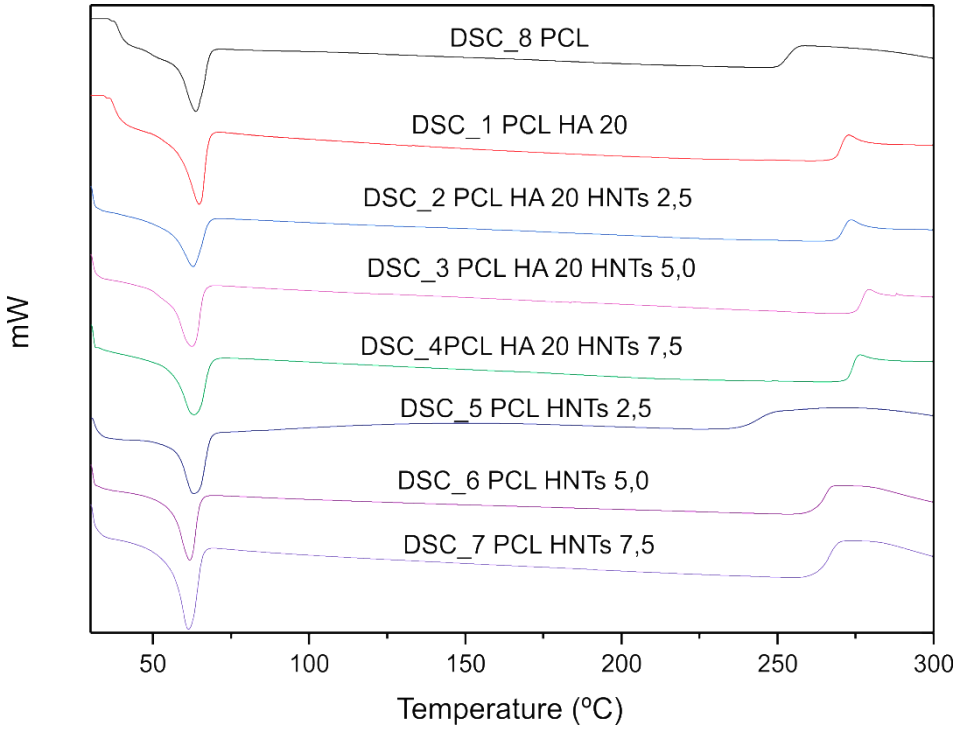


Figure V.7 DSC analysis

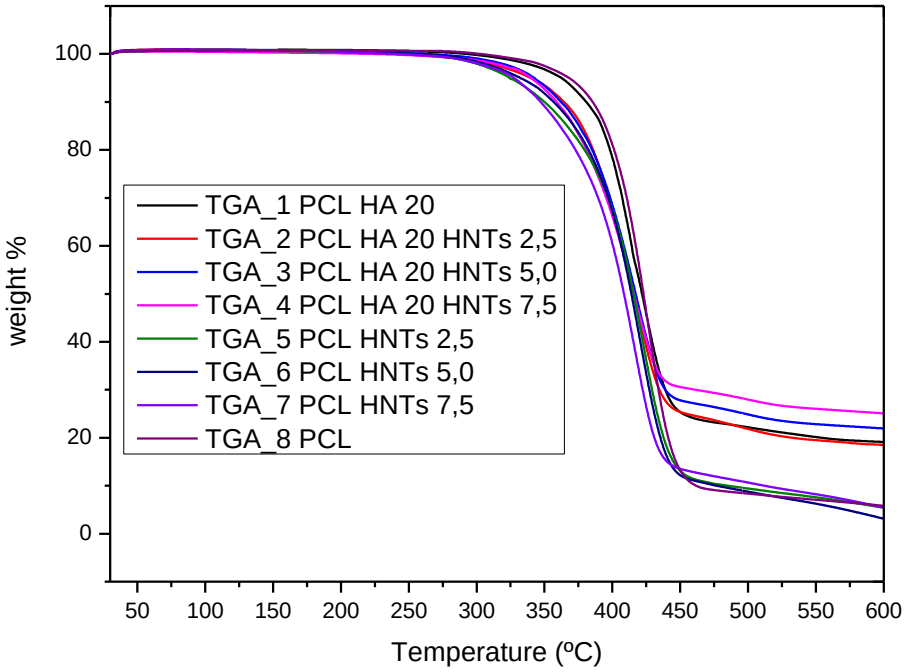


Figure V.8 TGA analysis

Similar values melting temperatures were obtained for all the composites, only a maximum difference of 2ºC respect raw PCL was detected. This slight difference is a positive aspect for processability by techniques as injection molding, extrusion, etc. because it does not affect an increase in operating temperature, with the consequent energy saving [174]. Moreover, the degradation temperature obtained in the seven composites developed has high value than the PCL pristine sample. The degradation temperature of PCL pristine sample is around 250 ºC, while in the different composites presented this temperature is higher than 274ºC. This difference, bigger than 20ºC, provides a greater range of thermal stability. This process could be justified by the introduction of high contents of filler that affects increasing the degradation mechanism by a complex two-step nucleation-drive reaction [175]. On the other hand, in PCL pristine sample this mechanism is only based on a chain scission process [176].

Thermo gravimetric analysis (TGA) shows a higher residual mass ratio when the filler content increases, reaching almost 30% corresponding to the sum of the content of HA and HNTs. This behavior is due to the high thermal stability supplied by calcium apatite present in the HA. Only a 4% of weight loss of HA is attributed to the absorbed water and CO_2 registered from 600 to 1000ºC [177]. Therefore, the incorporation of HA and HNTs as fillers in PCL matrix has not drawbacks in usual manufacturing polymers process. If the viscosity of the mix is increased in any case, only some adjusts in pressure or time cycle would be necessary.

To complete the thermal analysis VICAT and HDT test were performed in order to determine heat resistance characteristics of the material and to define precisely the thermal behavior. These properties should be determine in order to define the processability. In injection moulding process, the removal of the piece from the mold has to be neart or below the HDT temperature, this means that part deformation will be held withing acceptable limits after removal.

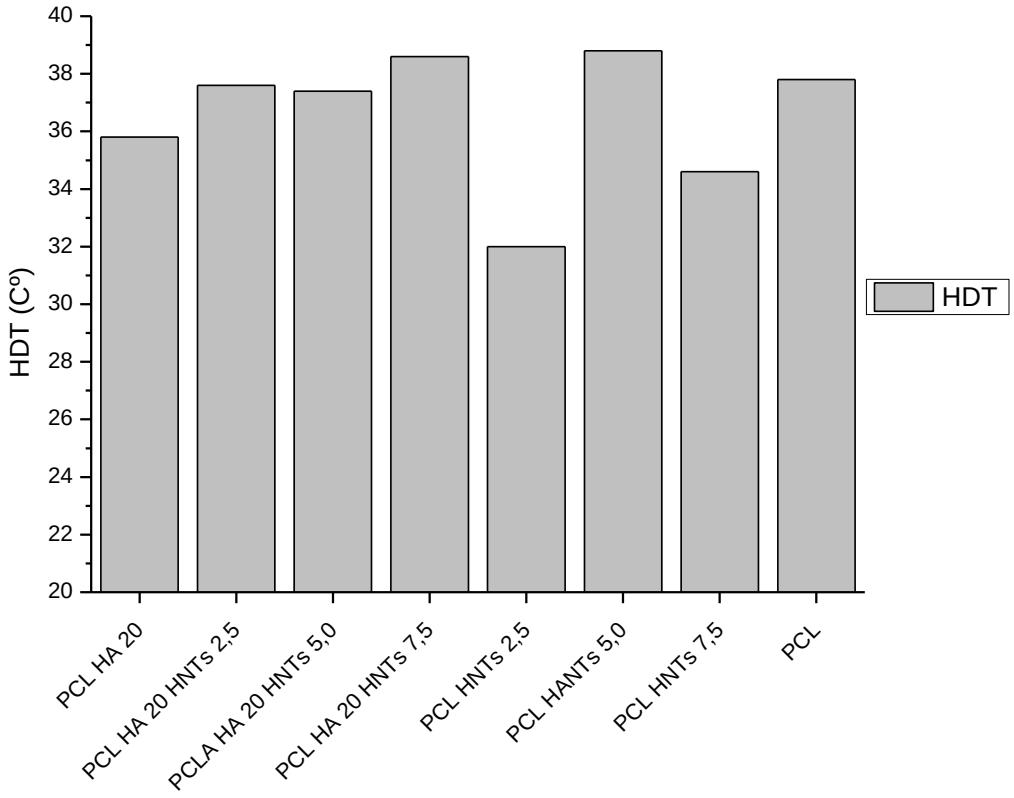


Figure V.9 HDT results

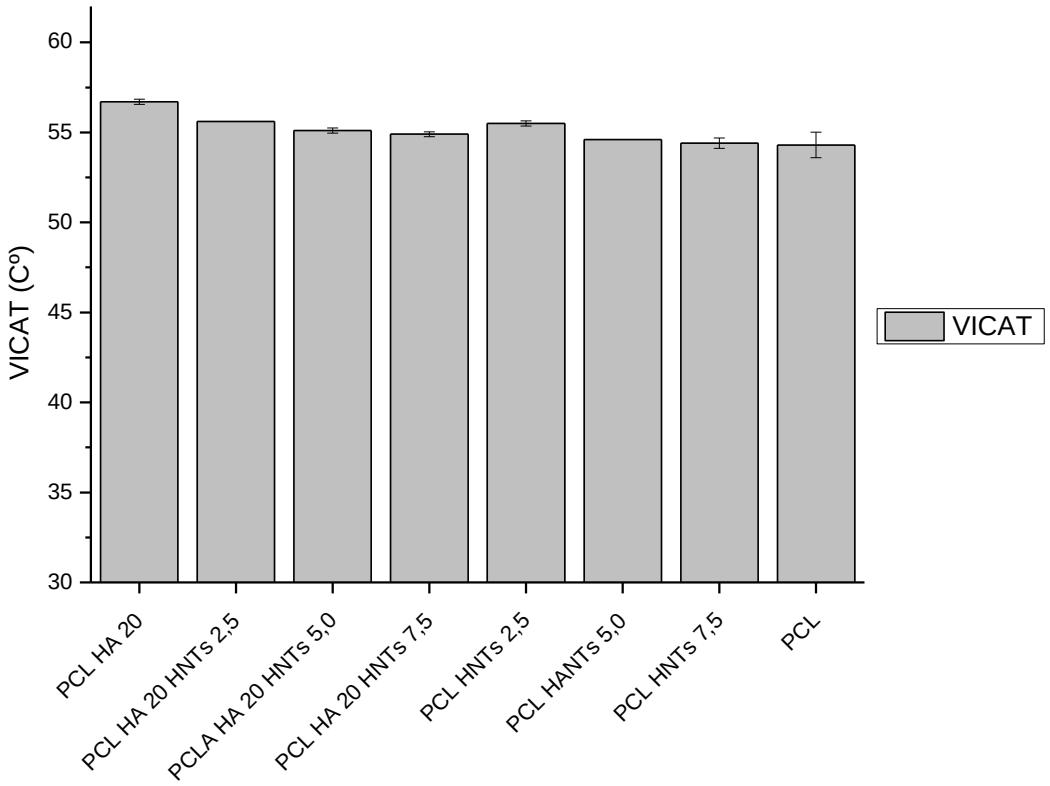


Figure V.10 VICAT results

If we assess the data from our test, show above in Table IV-9 and Table IV-10, it can be noticed that temperatures between samples have a variance not more than 2˚C in Vicat and 3˚C in HDT. The increase in temperature with the addition of HNTs should be related with the hydrogen bounding interactions between the hydroxyl group present in HNTs surface and the carboxyl present in ester linkage of PCL molecular chain [154].

Results obtained don't provide substantial information, however we obtained the required information to perform an optimum injection process for the material.

3. Dynamical Mechanical Analysis (DMA)

In order to complete the mechanical and thermal properties study of different composites destined to plates and screws, prosthesis, bioresorbable fixation, etc, Dynamical Mechanical Analysis (DMA) was carried out. Taking into account the glass transition of PCL (-60ºC), one of the most important thermal transitions, previous studies have demonstrated that the glass transition is not affected by the addition of different amounts of filler in polymeric matrix as PCL or polyethylene [165, 178]. For these reason, the temperature range studied in DMA analysis was from 25 to 80ºC, because this is the working temperature range where a good thermal stability of composites are desired. Figure IV.11 plots the evolution of the storage modulus as a function of the temperature.

First of all, the results indicate a decrease in the storage modulus around 50-55ºC as a consequence of the softening due to the melting of PCL matrix (increase in chain mobility). It can be concluded that the addition of HA as a filler entails an enhancement of the storage modulus. If attention is focused in the human body temperature, 37ºC, and the work temperature of this composites, samples with a 20% of HA reaches an enhancement around of 109% in the storage modulus. On the other hand, consistently with the previous mechanical test, the addition of HNTs just results in an increment of mechanical properties. Nevertheless, the main purpose to add HNTs beside the increment of mechanical properties is the future use as a delivery systems [163]. According to Lun et al. and Schmitt et al. which result agree with our own results, it was consider that the composite made with 7,5% of HNTs guarantees enough amount of tubular structures to be used as storage of specific drugs [179, 180].

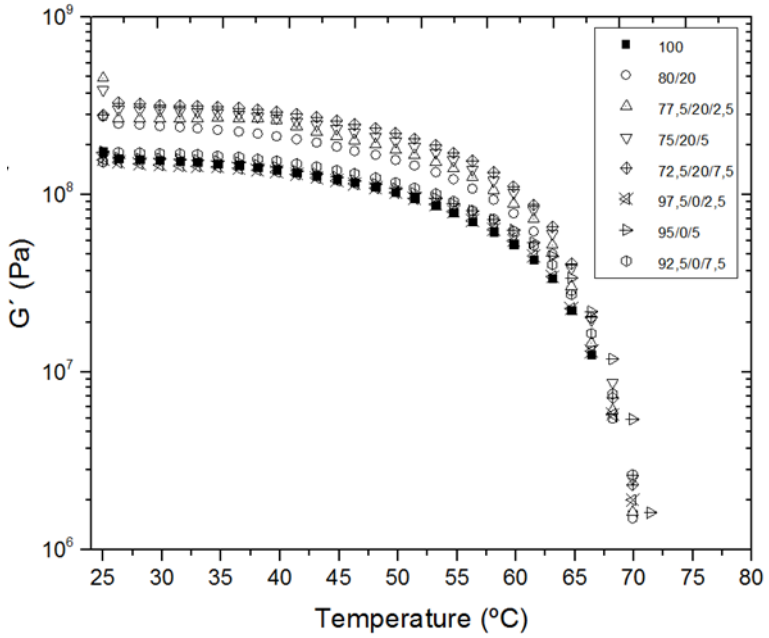


Figure V.11 Dynamical Mechanical Analysis results

VI. Conclusions

The main objective of this study is to improve mechanical and biologic properties of bio absorbable polymeric fixing systems to use in craniofacial injuries. Taking into account that facial trauma is one of the most common injuries seen in urban trauma centers, development of new bio absorbable materials capable to fulfill the requirements of bone fracture remodeling is a promising alternative to current metal prostheses. Commonly, metal prostheses are heavy, expensive, do not promote bone regeneration, neither are degraded over time and could lead to bone refracture after the required removal of the plates attributed to the stress shielding effect.

Controlled delivery of osteogenic biomaterials is an alternative to traditional bone repair techniques, leading also a faster recuperation of the involved joint. Polycaprolactone (PCL) is a degradable biomaterial under physiologic conditions, persisting in vivo for more than one year, fulfilling sufficient high modulus until the regeneration of the broken bone. However, its applications are limited due to its low mechanical properties. For this reason two fillers are used to modify the PCL strength. Hydroxyapatite (HA) provides osteoconductive properties, without inflammatory response, neither toxic. Halloysite nanotubes (HNTs) is biocompatible, with high mechanical strength and abundant in the nature.

Designing blend formulations and manufacturing test specimens is the first specific objective of this work. Supporting our design blend formulations after considering studies already done, a 20 wt% of HA is kept constant while the percentage of HNTs varies from 2.5 wt% to 7.5 wt%.

Once design blend formulations are established, determination of the optimum HNTs percentage is mechanically and thermally tested. After evaluating the results, the materials which show the best properties compared to the bone properties are “3 PLC HA 20 NTHs 5.0” and “4 PLC HA 20 NTHs 7.5” with a 5 wt% of HNTs and 7.5 wt% of HNTs respectively. Although, both materials show similar mechanical properties, a 7.5 wt% of HNTs is established as the optimum percentage due to the amount of Halloysite Nanotubes loaded to be functionalized in futures works is higher. These results agree with the studies carried out by Liu et al. [154] and Prashantha et al. [155], who tested the decrease of flexural properties exceeding 7.5 wt% of HNTs, correlating the overloading of HNTs with the generation of agglomerates acting as a weak points and failure initiation sites.

VII. Future works

The present study exhibits promising results capable to compete with current researches based on osteoconductive biocompatible polymers to be applied in complex bone fractures treatment as fixing and screws.

Professor Turng Lih-Sheng, Chair of BIONATES Laboratory at UW-Madison and a recognized authority in tissue engineering, proposed the possibility of a future collaboration to me, extending this study to my PhD Thesis. Fact that encouraged me to further explore this area of research with the promising study of seeding, growth and culture of cells on the developed bio material. This potential field of study supplies the desirable value to my research work thanks to the recognized experience on the discipline by the research group led by Professor Turng lih-Sheng. Two different assignments are proposed bellow in order to provide continuity to the study.

Considering the improvement of Polycaprolactone mechanical properties with the addition of 20 wt% of HA and 7.5 wt% of HNTs, a future work will focus on the implementation of this devices with curcumin in order to give them antioxidant, anti-inflammatory and potentially chemotherapeutic properties. To achieve this propose, Halloysite Nanotubes will be functionalized with the target compound and subsequently mixed with Polycaprolactone (PCL) and hydroxyapatite (HA). Different goals can be established:

- First of all loading procedure of Halloysite Nanotube with curcumin should be designed using different solvents to compeer the process efficiency (ethanol, acetone and water). Following the previous procedure carried out by Abdullayev et al. [134, 136].

- The introduction of curcumin in Halloysite Nanotubes lumen has to be proved (Scanning Electron Microscopy SEM, Transmission Electron Microscopy TEM, and Thermo Gravimetric Analysis TGA).

- Determination of the best solvent performance.

- Determination of amount of functionalized Halloysite Nanotubes to be loaded in Polycaprolactone.

To verify the new material as capable to be use in biomedical applications, Cytotoxicity and Cell Viability test should be carried on to examine the interaction of the new material with fibroblast cells (NIH 3T3), enabling the drug screening and prediction of potential toxic effects of chemicals. Proliferation and viability analysis is crucial for cell growth and differentiation studies. Different tasks can be proposed:

- Cell seeding and culture of Mesenchymal stem cell (MSCs cells), following the protocol approved by University of Wisconsin-Madison IRB [181]. In order to characterize cell attachment and cell morphology on the surface of the plates, Scanning Electron Microscopy (SEM) will be use.

- Cell proliferation will be carried out following the procedure MTS assay (CellTiter 96® AQ One Solution Cell Proliferation Assay, Pomega, Madison, WI) [182].

- In Vitro degradation studies using a phosphate buffer saline (PBS) will be performed according to the procedure proposed by Cui et al. [183].

VIII. Reference

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IX. Appendix

Tables list

Figures list