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Luís Pedro Valente Gonçalves

SUBSTITUTOS ÓSSEOS INJECTÁVEIS PRONTOS-A-USAR

READY-TO-USE INJECTABLE BONE SUBSTITUTES

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em materiais e Dispositivos Biomédicos, realizada sob a orientação científica do Doutor José Maria da Fonte Ferreira, Professor Associado com Agregação do Departamento de Engenharia de Materiais e Cerâmica da Universidade de Aveiro e do Doutor Hugo Alexandre Gonçalves da Rocha Fernandes, Pós-Doc do Departamento de Engenharia de Materiais e Cerâmica da Universidade de Aveiro e Doutora Ana Filipa Marques de Brito, responsável pelos Assuntos Regulamentares da Reg4life.

À memória dos meus avôs

Aos meus pais, pela oportunidade que me proporcionaram e incansável apoio

o júri

presidente

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palavras-chave

Cimentos de fosfatos de cálcio, biovidro, substitutos ósseos, cimentos prontos-a-usar, *putty*, injectabilidade, tempo de presa, regeneração óssea, monetite, bioactivo, osteoconductividade

resumo

Nos últimos anos, o desenvolvimento e a inovação de novos substitutos ósseos tem revolucionado a vida de milhões de doentes. O objetivo deste trabalho é o desenvolvimento e caracterização de um sistema bioativo, injectável e pronto-a-usar (*putty*) para regeneração óssea. A fase sólida é constituída por pós de fosfato tricálcico beta (β-TCP), biovidro FastOs (FastOs®BG) e fosfato monocálcico monohidratado (MCPM), enquanto a fase líquida é o glicerol (G). A síntese dos pós de β-TCP foi obtida por reações de precipitação seguida de tratamento térmico; os pós de FastOs®BG foram obtidos por fusão e arrefecimento em água fria (fritagem) (*melt-quenching*). A caracterização dos pós foi feita por difracção de raios-X (XRD) e medição dos tamanhos de partícula.

O sistema injectável pronto-a-usar foi preparado através da mistura das fases sólida e líquida e colocado em seringas seladas com tampa roscada. Do ponto de vista de aplicação clínica, o sistema foi caracterizado tendo em conta a sua injectabilidade, tempo de presa (*setting time*, ST) e resistência mecânica. A análise estrutural do sistema também foi realizada, através de XRD, espectroscopia de infravermelho com transformada de Fourier (FTIR) e microscopia eletrónica de varrimento (SEM).

O sistema injectável pronto-a-usar tem uma razão em peso sólido/líquido (S/L) de 3,3, um ST médio de ~25 min, ~96% de injectabilidade, e 6 MPa de resistência máxima à compressão. Deste modo, o sistema injetável demonstrou excelentes resultados de injectabilidade, tendo-se verificado ainda a ausência do efeito de *filter pressing* e propriedades mecânicas aceitáveis. A análise estrutural dos cimentos endurecidos revelou a formação de cristais de monetite recobertos por uma camada apatítica amorfa após imersão em PBS e em água.

Os resultados obtidos são promissores e permitem concluir que o sistema injetável pronto-a-usar possui excelentes propriedades de manipulação do ponto de vista clínico.

De acordo com a Directiva 93/42/CEE o sistema injetável é considerado um dispositivo médico de classe III. Com o objectivo de contribuir para o seu processo de lançamento comercial e seguindo os requisitos essenciais estabelecidos no anexo I da Directiva 93/42/CEE foi elaborado um relatório tendo em conta a avaliação clínica do sistema injectável.

keywords

Calcium phosphate cements, bioglass, bone substitutes, premixed cement, putty, injectability, self-setting, bone regeneration, monetite, bioactive, osteoconductivity

abstract

In recent years, the development and innovation of new bone substitutes has revolutionized the lives of millions of patients. The aim of this work is the development and characterization of a bioactive, injectable and ready-to-use system (also called putty or premixed cement) for bone regeneration. The solid phase is constituted by beta-tricalcium phosphate (β-TCP), FastOs® bioglass (FastOs® BG) and monocalcium phosphate monohydrate (MCPM) powders, while the liquid phase comprises glycerol (G). The synthesis of β-TCP powder was obtained by precipitation reactions followed by heat-treatment; FastOs® BG was obtained by melt-quenching. The characterization of the obtained powders was made through X-ray diffraction (XRD) and measurement of the mean particle sizes and particle size distribution.

The putty was prepared by mixing the solid and liquid phases and placed in syringes with a screw cap. Regarding clinical application, injectability, setting time (ST) and mechanical strength were investigated to characterize the putty. Structural analyses of the putty were also performed by XRD, Fourier Tranform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM).

The putty has a solid/liquid weight ratio (S/L) of 3.3, mean ST of ~25 min, ~96% of injectability and a maximum compressive strength of 6 MPa. Therefore, the putty exhibited excellent injectability results, absence of filter pressing effect and acceptable mechanical properties. The structural analysis of the hardened cements revealed the formation of monetite crystals covered by an amorphous apatitic layer after immersion in PBS and water.

The results are encouraging and support the conclusion that ready-to-use injectable bone substitutes have excellent handling properties to be used clinically.

In accordance with the Directive 93/42/EEC the putty is considered a class III medical device. In order to pave the way towards its commercial release and in order to meet the essential requirements set out in Annex I of the Directive 93/42/EEC, a clinical evaluation has been carried out.

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Abbreviations

CaP – Calcium Phosphate

TCP – Tricalcium Phosphate

β-TCP – beta-Tricalcium Phosphate

BG – Bioglass

HA – Hydroxyapatite

CPC – Calcium Phosphate Cements

MCPM – Monocalcium Phosphate Monohydrate

MCPA – Monocalcium Phosphate Anhydrous

G – Glycerol

MD – Medical Device

PSD – Particle Size Distribution

ST – Setting time

FPE – Filter Pressing Effect

CS – Compressive Strength

PBS – Phosphate Buffered Saline

SBF – Simulated Body Fluid

XRD – X-Ray Diffraction

TG – Thermogravimetric Analysis

FTIR – Fourier Transform Infrared Spectroscopy

SEM – Scanning Electron Microscopy

CHAPTER I

INTRODUCTION AND AIM OF THESIS

1. Introduction

Over the last century, the emergence of new materials and surgical techniques has dramatically changed the lives of millions of patients. As defined by Chester in 1991, biomaterials are "materials intended for contact with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body". Biomaterials play a key role in modern healthcare, essentially in remediating musculo-skeletal diseases, such as osteoporosis or fractures associated with the aging of the population.[1]

The development of biomaterials used in orthopaedic surgery, traumatology and maxillofacial, particularly of bone substitutes has been remarkable. Following blood, bone is the second most transplanted tissue.[1]

According to Mainard[2], "all materials of human, vegetable or synthetic origin intended for human implantation with prospect of a reconstitution of bone capital, either for the strengthening of a bone structure or the filling of loss bone substance of traumatic or orthopaedic origin, can be considered bone substitutes".

The bone taken from the patient himself (autograft) is preferred for the treatment of bone defects, as it provides good results such as the reduced risk of rejection. The autografts are considered *gold standard*, but they also have disadvantages, as they expose the patient to a second surgery to retract bone tissue and can lead to weakening of the bone structure. The allografts, which consist in the implantation of bone tissue taken from one individual from the same species as the patient, may be an alternative but they have disadvantages, as causing the change of mechanical properties and the possibility to trigger a mechanism of immune rejection (that requires use of immunosuppressants). There are also xenografts, which originate from a non-human donor and have an increased risk of rejection and disease transmission. To solve all limitations associated to these grafts, synthetic bone substitutes (grafts) are now commonly used and represent a viable option for bone regeneration.[1,3]

Ceramic biomaterials based on calcium phosphates such as hydroxyapatites (HA), tricalcium phosphates (TCP) and calcium phosphate cements (CPC) have been extensively

used as bone substitutes for clinical applications due to excellent biocompatibility and osteoconductive properties.[4]

Bioactive glasses (BG) are also very popular and used among bone substitutes, due to their good biocompatibility in both bone and soft tissues. BG binds strongly to bone, promotes bone growth through the formation of an apatite layer and releases Ca, P and Si ions which stimulate osteogenesis.[5]

The current major disadvantage of all orthopaedic implants is their existence in hardened form, which requires the surgeon to adjust the site of surgery to the implant or has yet to change the morphology to obtain a specific form applicable to the injury site. These complications can lead to an increase of bone loss, trauma in the surrounding tissue and prolongation of surgery. By contrast, CPC can be adjusted to the bone cavity without machining and have self-setting ability.[6]

CPC can be mouldable or injectable and may be the solution for complex bone defects. However, CPC mixtures are prepared in the surgery room from components in powder and liquid that are supplied in fixed doses for each application. Even if it is not fully used, the surplus part sets and is discarded. Injectable systems based on bioactive materials such as bioactive glasses (BG) and calcium phosphates (CP) and a non-reactive liquid, supplied in syringes with screw cap, help overcome these difficulties. This type of ready-to-use medical device allows an administration of the required dose and keep the surplus part to other subsequent applications until all the contents have been used. These are profiled as a very recent application as an injectable bone substitute (also called premixed calcium phosphate cement or putty) and it is expected that they can overcome the current limitations of CPC and implants and promote fast bone regeneration.

1.1. Aim of thesis

The main aim of this work is the development and characterization of a ready-to-use injectable bioactive system for bone regeneration. It is intended to obtain a solid, bioactive phase, consisting of FastOs®BG and β-TCP powders and a liquid phase, non-reactive, consisting of glycerol (G). Afterwards, a certain amount of monocalcium phosphate monohydrate (MCPM) will be added to confer self-setting capacity of the putty when it is in contact with physiological fluid. The setting time will be evaluated by immersing a mould with putty in PBS, so the components of the non-reactive liquid will diffuse and be replaced by water molecules. The compositions, morphology/microstructure, injectability, phase separation/*filter pressing effect*, mechanical properties and the bioactivity and biodegradability *in vitro* will also be investigated. The classification and clinical evaluation of the developed bone substitute will be made in accordance with the Directive 93/42/EEC.

CHAPTER II

STATE OF THE ART

2. State of the Art

2.1 Bone anatomy and physiology

The skeletal system is multifunctional. Its functionality is assured by bone growth, formation and remodelling, throughout life, by bone cells and also by mineralization which is regulated by extracellular bone matrix. The bone strength depends on several factors: the weight, the geometry and composition, the material properties and the microstructure.[7]

The bone is a complex conjunctive tissue, highly organized and specialized, that performs different functions. It is physically characterized as a hard, rigid and strong tissue. Microscopic analysis reveals the presence of very few cells and intercellular substance formed by collagen fibres and hardened substance. The bone consists of bone cells (osteoblasts, osteocytes and osteoclasts) and extracellular matrix, differing from the other conjunctive tissues because its matrix is mineralized. In its composition is included 33% organic matrix and 67% of inorganic compounds. The most abundant ion in the tissue are calcium and phosphorus, which form the most important mineral constituent hydroxyapatite (HA), which has the following stoichiometric chemical formula: $Ca_{10}(PO_4)_6(OH)_2$.[8]

2.1.1 Bone Macrostructure

An adult human individual has a total of 213 bones, excluding the sesamoid. Each bone suffers constant changes throughout life. It grows to adapt to biomechanical forces, and remodels to remove old bone microscopically damaged, replacing it with new bone tissue, mechanically stronger to help preserve bone strength.[7]

The bone is composed by two types of bone tissue: cortical/compact bone (80%) and cancellous/trabecular/spongy bone (20%). The cortical bone forms the bone cortex and

has a dense structure. It is the main component of the long bones of the arm, leg and other bones on which we require higher strength and stiffness. Trabecular bone normally occupies the inner region of the bone and is composed by thin plates, or trabeculae, on a loose mesh structure. Each trabecula contains collagen fibres arranged in parallel lamellae. It is highly vascular and frequently contains red bone marrow where the hematopoiesis (process by which blood cells are formed) takes place. It has a larger surface area, but is less dense, softer, weaker and less hard than the cortical bone.[9]

The bones can also be classified as: long (e.g.: femur, tibia, humerus, radius), small (e.g.: carpal, tarsal), flat (e.g.: ribs, external, skull and shoulder blade), irregular (e.g.: vertebra) and sesamoid (patella and small bones of the foot). Each long bone can be divided into three regions, called epiphysis, metaphysis and diaphysis.[10]

The bone surface is covered by periosteal and endosteal as connective tissue. The periosteum coats the outer surface of the bone, except the links that are protected by articular cartilage and consist on a fibrous outer layer composed by collagen fibers, fibroblasts, and an inner layer comprising mesenchymal stem cells - osteoprogenitor cells with the capacity to divide and differentiate into osteoblasts. The endosteal is connected to the inner surface of the bone, particularly on the surface of the medullary cavity of long bones. It is composed by osteoprogenitor cells and a small amount of connective tissue. Both surfaces, periosteal and endosteal, provide a continuous reinforcement of osteoprogenitor cells or new osteoblasts for repair or new bone growth.[8] Figure 1 shows the structure of a long bone.

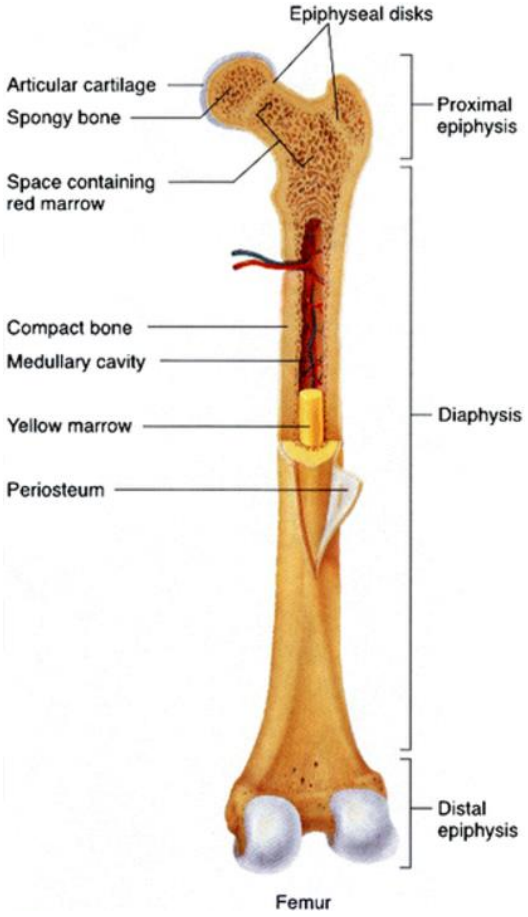

Figure 1 – Structure of a long bone (femur) (Adapted from Dorozhkin SV. [10]).

2.1.2 Bone Microstructure

Bone tissue is constituted by four kinds of characteristic cells: osteoblasts, osteocytes, osteoclasts and bone mesenchymal stem cells. Osteoblasts are mononucleated cells that synthesize collagen or non-collagenous proteins and are responsible for mineralization of the osteoid tissue process. Its main function is to produce bone matrix during the development or repair of bone tissue, which means it is responsible for bone formation.[8]

The osteocytes are flat and spindle-shaped cells that support the bone and are located inside the lamellas of cancellous and compact bone. These cells are completely surrounded by extracellular matrix, forming a space called gaps. The amount of bone

osteocytes existent per bone volume unit is directly dependent on the speed of the tissue forming process, i.e. the higher the speed of the formation of bone osteoblasts the greater will be the amount of osteoblasts that will later become osteocytes. Thus it can be said that their function includes bone formation (by osteoblasts), maintenance of the matrix and calcium homeostasis.[8]

Osteoclasts are cells that are different from others because they have higher volume and multiple cores. These cells play a crucial role in bone resorption and removal process (remodel the bone to reduce its volume) through chemical or enzymatic action. Thus, the bone is destroyed, releasing calcium or in conjunction with the process of bone deposition, leading to the remodelling of the tissue as a result of functional requirements. In addition to osteolytic functions, osteoclasts play a major role in the bone development and growth, through the release of polypeptide-like growth factors from the mineralized extracellular matrix.[8]

Finally, the undifferentiated mesenchymal cells are located in the conjunctive tissue between the trabeculae, throughout the vascular channels, and in the periosteum. Its main function is to differentiate into osteoblasts.[8]

At the molecular level, the bone comprises a bone matrix of lamellar or non-lamellar bone. The bone matrix is where the cells are located. The bone matrix is composed of organic and inorganic matter. The inorganic or mineral portion (approximately 70%) is mainly composed of small HA crystals. The organic phase is composed of Type I collagen fibres embedded in a homogeneous substance containing glycoproteins and proteoglycans.[8]

2.1.3. Bone Formation and Osteoconduction

Osteoconduction is critical to osteogenesis during the remodelling process in normal bone and consists of three main processes:

- Migration of progenitor cells of the bone via a transient matrix;

- Bone progenitor cell differentiation;

- Functional differentiated cells recruitment to initiate new bone formation.[11]

Osteogenesis is the result of osteoconduction and bone formation, occurring when the bone's first layer is directly secreted into the surface of the implant. During osteoconduction, pre-osteogenic cells are stimulated to migrate through a provisional matrix which can be represented by bone grafts, implant, or a blood clot. The migrating cells then begin a process of differentiation which results in the secretion of the new bone matrix. [12]

During bone formation, differentiated osteogenic cell secrete globular increases of a matrix devoid of collagen, called sealer. These afibrillar layers are located in the secondary osteons interface with surrounding tissue, and can also be seen in the bone-implant interface. This first layer provides nucleation sites for calcium phosphate nano-crystals which go through nucleation and growth inside the organic matrix. After deposition of the cement line matrix, the osteogenic cells differentiate into osteoblasts that produce extracellular collagen matrix as fibres. Finally, collagen fibres go through calcification and are separated from the underlying substrate by a calcified matrix without collagen.[12]

2.1.4. Bone Growth and Modelling

The bone undergoes longitudinal and radial growth, modelling and remodelling throughout life. The longitudinal and radial bone growth occurs during childhood and adolescence. The longitudinal growth occurs in the growth plates, where the cartilage proliferates in epiphyseal and metaphyseal areas of long bones, before subsequently being subjected to mineralization to form new primary bone.[13]

Bone modelling is the process by which bones change its overall shape in response to physiological influences or mechanical forces, leading to a gradual adjustment of the

skeleton in response to forces that are exerted. Bones may extend or modify the shaft by removing or adding bone to the appropriate surfaces, by the independent action of osteoblasts and osteoclasts in response to biomechanical forces. Bones typically extend with aging in response to periosteal apposition of new bone and endosteal resorption of old bone (native). Wolff's law describes the observation that long bones change shape in order to accommodate the stresses placed upon them. In adults, bone modelling is less frequent than remodelling.[13]

2.1.5. Bone Remodelling

Bone remodelling is the process by which bone is renewed to maintain bone strength and mineral homeostasis. Remodelling involves the continuous removal of discrete portions of old bone, its replacement with newly synthesized protein matrix and subsequent mineralization of matrix to form new bone.[7]

The remodelling process resorbs old bone and forms new bone to prevent the accumulation of bone micro injuries. Remodelling begins before birth and continues until death. The bone remodelling unit is composed of a tightly coupled group of osteoclasts and osteoblasts that sequentially perform the resorption of old bone and formation of new bone. Bone remodelling increases with age, especially along the perimenopause and post menopause. The remodelling cycle consists of four sequential steps: activation, resorption, formation and reversion, as represented in Figure 2. The remodelling sites may be developed randomly, but are also targeted to areas that require repair.[7]

The activation involves the recruitment and activation of monocyte-macrophage mononuclear osteoclasts precursors, of the circulation, endosteal elevation containing the lining cells of the bone surface and fusion of various mononuclear cells to form multinucleated pre-osteoclasts. These bind to bone matrix through interactions between the integrin receptor on their cell membranes and peptides containing RGD (arginine, glycine and asparagine) in matrix proteins.[7]

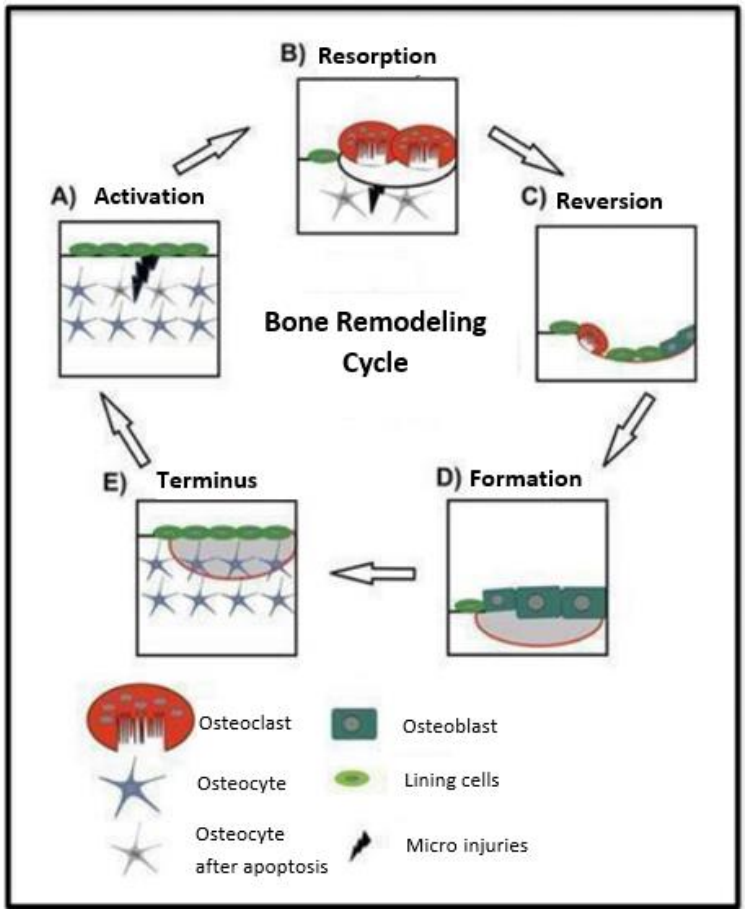

Figure 2 – *Scheme of normal bone remodelling cycle (Adapted from Clark B. [7]).*

During the reversion phase, bone resorption transits to bone formation. At the end of bone resorption, the resorption cavities contain a variety of mononuclear cells, including monocytes, osteocytes released from the bone matrix, and pre-osteoblasts recruited to start the formation of new bone, on which also participate various growth factors.[7]

Bone formation lasts from 4 to 6 months. Osteoblasts synthesize new collagenous organic matrix and regulate the mineralization of the matrix, releasing small membrane-bound matrix vesicles that concentrate calcium and phosphate and enzymatically destroy mineralization inhibitors, such as proteoglycans or pyrophosphate.[7]

Osteoblasts, located within the matrix become osteocytes with an extensive canaliculi network that connects it to the lining cells on the bone surface, to the osteoblasts and other osteocytes, held by gap junctions. The end result of each bone remodelling cycle is

the production of a new osteon. The main functions of bone remodelling include the preservation of the bone mechanical strength, replacing old bone with micro injuries by new bone, healthier and with calcium and phosphate homeostasis.[7]

2.2. Biomaterials for bone regeneration

Knowing the response of tissues in contact with a biomaterial is fundamental at the time of proceeding to the choice of a material to use in an implant.[14] Successful implementation of a biomaterial in the body depends mainly on two factors:
Biofunctionality, which is directly related to the biomaterial's ability to perform a particular function (or part of it) of the body.[14]

Biocompatibility, which is based on the analysis of the reactions occurred on the surface of the implant, not only at the time of implementation, but also over time when it undergoes a process of degradation and wear. Thus, in terms of biological response, after implanting a biomaterial a formation of a hematoma can occur, with an inflammatory-like response and the call of water and glycoproteins, which overlays and adhere to the implant.[14]

In general, biomaterials can be classified in two ways: its chemical composition and its biological behaviour.[15] Regarding chemical composition, biomaterials can be divided into 4 classes:

- Metals and metal alloys;
- Ceramic;
- Polymers;
- Composites.

The classification of biomaterials, considering their biological behaviour, is based on the host tissue response. Thus, biomaterials can be classified as:

- Bioinert - Do not cause the body's reaction to a foreign body, lying in direct connection to the host tissue (e.g. titanium, zirconia and alumina);

- Biotolerant - are moderately accepted by the host tissue, usually surrounded by a fibrous capsule (for example, stainless steel, cobalt-chromium alloys and poly(methyl methacrylate) (PMMA)).[15]

2.2.1. Bioglasses (BG)

In 1969 Larry Hench and co-workers successfully produced the first BG, being therefore considered the pioneers in the use of bioactive glasses for biomedical purposes.[5] The main feature of the BG is the ability to promote a rapid and durable chemical bond through an apatitic interface with bone tissue.[15] The traditional method of production of a glass by thermal fusion followed by cooling to solidify without crystallization, has proved to be limiting for some chemical compositions BGs, so the preparation by the sol-gel has been most commonly used for these cases.[15] The composition of BGs is essentially based on silica (silicate glasses) or phosphorus (phosphorus glasses), depending on the glass former used. These last are more easily melted and chemically more unstable than the silicate. The presence of phosphate groups (PO_4^{3-}) associated with calcium (Ca^{2+}) in the bone tissue is of great importance. The ability of a BG to bind to bone, undergo biodegradation and form an apatite surface layer varies depending on the composition and ratio of its components.[15]

Despite the successful use of BGs as bone graft substitutes, recent studies found high basicity of BG 45S5® when placed in contact with a mean, PBS or biological tissue. Thus, the surface of BG 45S5® becomes extremely reactive, leading to fast degradation rates *in vitro* and *in vivo* and poorer mechanical strength, which may not coincide with the growth rate of new bone. This limitation can compromise bone regeneration in defects, therefore new BG compositions have emerged, like the FastOs®, which is biocompatible, osteoconductive and promotes osseointegration, and has the particularity of being resorbed more slowly than BG 45S5, making it a potential candidate for bone regeneration. This BG is characterized by having excellent in vitro properties when cultured with mesenchymal stem cells, like high biomineralization rate, a more hydrophilic character and a higher modulus of elasticity.[16] FastOs®BG also has an

excellent densification capability, being possible to obtain very dense and sintered materials but amorphous, with flexural resistance of 85 MPa after sintering for 1 h at 800 °C.[16] All these features make FastOs®BG a strong candidate for incorporation into bioactive systems.

2.2.1.1. BG synthesis by melt-quenching

A glass can be produced by two processing methods: the traditional melt-quenching method and sol-gel. BG 45S5® and other commercial bioactive glasses are produced by the traditional method, in which the oxides are melted together at elevated temperatures (above 1300 °C) in a platinum crucible and cooled immediately (quenching) in a graphite mould or water to obtain a frit. These two methods of synthesis provide different physical properties to the BGs. The BGs prepared by sol-gel tend to have an inherent nano-porosity, while the BGs prepared by melt-quenching are dense.[17] FastOs®BG can be prepared by melt-quenching in platinum crucibles to 1570 °C. Then, the BG is poured into cold water to obtain a frit. The frit is firstly dried and then milled in a high energy mill to obtain the suitable particle size distribution.[16]

2.2.2. Calcium Phosphates (CaP)

The mineral part of normal bone and teeth consists essentially of carbonated hydroxyapatite deficient in calcium (Ca/P <1.67). This explains why a huge variety of bone substitutes is made from biphasic calcium phosphates consisting of hydroxyapatite (Ca/P = 1.67) and β-TCP (Ca/P = 1.5). Being bioactive and osteoconductive materials, they are distinguished by a strong organic bond that allows the growth and proliferation of the bone to the implant. β-TCP is biodegradable, and as it is resorbed, allows bone ingrowth up to full substitution of the implant. The most important property of CaP is probably its solubility in water due to the fact that the *in vivo* behaviour of the CaPs can be predicted by its solubility. Thus, according to solubility, CaPs can be ranked by decreasing order of

degradation rate *in situ* (pH 7.0)[18]: α-TCP> DCPD> OCP> β-TCP> HA[19]. The main calcium phosphates used for tissue regeneration are shown in Table 1.

Table 1 – *Calcium phosphates frequently used for tissue regeneration (Adapted from Dorozhkin SV. [10]).*

Ca/P Molar Ratio	Compound	Mineral	Formula
1.0	Dicalcium Phosphate anhydrous (DCPA)	Monetite	$CaHPO_4$
1.0	Dicalcium Phosphate dihydrate (DCPD)	Brushite	$CaHPO_4.2H_2O$
1.33	Octacalcium Phosphate (OCP)	-	$Ca_8(HPO_4)2(PO_4)_4.5H_2O$
1.43	-	Whitlockite	$Ca_{10}(HPO_4)(PO_4)_6$
1.5	Tricalcium Phosphate	-	α-$Ca_3(PO_4)_2$ β-$Ca_3(PO_4)_2$
1.67	-	Hydroxyapatite (HA)	$Ca_{10}(PO_4)_6(OH)_2$
2.0	Tetracalcium Phosphate	-	$Ca_4P_2O_9$

Commercial CaP biomaterials are available in different forms, e.g: beads, plates, coatings or cements.[20] The CaP granules are used to fill bone defects for the purpose of helping bone regeneration, without any structural support. CaP hard blocks can be used to fill bone defects with a defined geometric shape, supporting higher loads. For more complex bone defects, customised implants can be made by measuring the volume of the lesion, and producing the part by using additive manufacturing methods such as 3D printing or Robocasting. Given the advantageous biological properties of CaP coatings, these are applied in many metallic implants used in the bone to promote bone growth around implants.[20]

2.2.2.1. Tricalcium Phosphate (TCP)

Tricalcium phosphate can exist in two allotropic forms, β-TCP and α-TCP. Even in pure stoichiometric composition (Ca/P = 1.5), the β-TCP phase is only formed through heat treatment at ≥ 800 °C temperatures, being thermally stable until up to temperatures of ~ 1125 °C. Above this temperature, β-TCP is transformed gradually into the high-temperature phase, α-TCP, transformation that occurs with a volumetric expansion (increase of network parameters).[12] Both phases are resorbable, being the resorption rate of α-TCP faster than that of β-TCP. The resorption rate depends on the solubility, which determines the surface concentration of calcium and phosphorus available to stimulate phenotypic differentiation of osteogenic cells, initiating mineralization, leaving free space to promote osseointegration.[20] The degradation occurs through osteoclast activity. The main disadvantages of the TCP, when compared to HA, are related to the lack of structural support caused by its too fast resorption, because of its greater solubility, especially when associated with macroporosity.[20]

The use of biphasic calcium phosphate (BCP = β-TCP + HA) is also a common strategy to regulate the rate of resorption and adjust it to the growth rate of new bone.[10]

The biodegradability makes these bioceramics very attractive as bone substitutes, as it can present in different forms (granules, porous blocks, or mouldable / Injectable pastes). In the form of powder slurry, they are less effective due to its lack of macro porosity necessary for bone ingrowth. If it's combined with additives such as naphthalene or starch (porogens) it is possible to obtain macropores in a range of 100 to 300 microns, and consequent interconnectivity which provides faster osseointegration.[10] The granular shape is the most efficient of the three (granular, macroporous and gels) because the spaces between the beads increase the porosity of the matrix as well as its contact surface. Nevertheless, it presents an injectability problem, making it difficult to be formed into a 3D structure.

2.2.2.1.1. β-TCP powder synthesis

The synthesis of TCP high purity powders is not reported in the literature when compared to the HA. This can be accomplished using two methods: solid state reactions at high temperature or by precipitation at a low temperature, followed by calcination. The main problem of both methods relies on the variability of the composition of the powders.[21] The solid state reactions at high temperatures are hardly used for the synthesis of large quantities because of the difficulty in controlling the intimate mixture of reagents powders and the complete reaction between them.[21] Thus, the preparation of stoichiometric final products without second phases waste often requires successive grindings and/or corrections in the stoichiometry followed by sintering. Considering the wet methods, the β-TCP cannot be directly synthesized in aqueous solution.

The compound which is able to precipitate is apatitic tricalcium phosphate $Ca_9(HPO_4)(PO_4)_5(OH)$, which seems to be hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, where one ion an HPO_4^{2-} replaces PO_4^{3-}. The β-TCP anhydrous crystallization requires calcination of the apatitic compound at temperatures above 750 °C. The apatitic TCP synthesis is conceivable through the aqueous routes classically used for the synthesis of stoichiometric HA. The non-stoichiometric apatites $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ with $0 \leq x \leq 1$ can be precipitated, with the value of x dependent on the synthesis conditions.[21]

The most important parameters to control are the pH and temperature. Although the values reported in the literature are widely dispersed, a pH value maintained near neutral, slightly acidic, and low temperatures are generally used. The kinetics of precipitates formation is still little understood and the maturation times after total addition of reagents vary depending on the lack of maturity up to 12 h.[21]

Pure β-TCP is formed after calcining the powder with Ca/P = 1.500. For Ca/P values greater than 1.500, HA is formed as a second phase. A relative variation of 1% of the Ca/P molar ratio induces the formation of 10 wt % of HA. For Ca/P values smaller than 1.500 is formed another second phase: calcium pyrophosphate $Ca_2P_2O_7$. The biological and mechanical properties of calcium phosphate compounds strongly depend on its chemical

composition. Consequently, its preparation must be highly controlled to obtain reproducible properties and should be used accurate characterization techniques, such as the analysis of X-ray diffraction (XRD), infrared (FTIR) and scanning electron microscopy (SEM).[21]

2.2.3. Injectable bone substitutes

The discovery of calcium phosphate cements (CPC) in 1982 and 1983 marked a new era in which the handling properties of bone substitutes became extremely important.[9,22]

Continued research efforts are reflected in a rapid increase in the number of publications. For example, when performing a search on "Scopus" (www.scopus.com) using the two keywords "Injectable" and "Ceramic" is verified that almost 750 articles were published in 2015 (Figure 3).

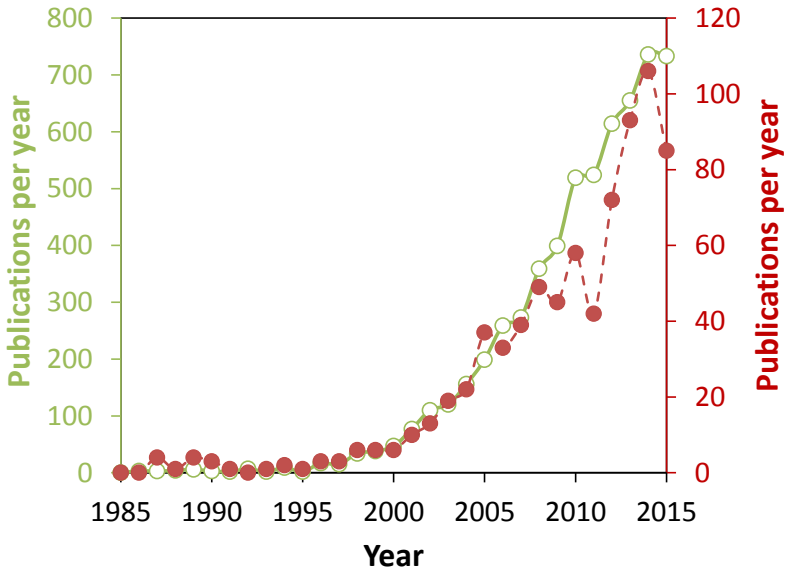

Figure 3 – *Number of articles cited per year in scopus (www.scopus.com) when selecting the following keywords (search in all fields): (green) "Injectable" and "Ceramic"; (red) "Putty" and "Ceramic".*

Over the last 15 years, a large variety of ceramic cements have been commercially introduced in the market as bone substitutes. Consequently, it is necessary to better understand the specific properties of these biomaterials, such as injectability, cohesion, setting time, and properties *in vitro* and *in vivo*.[23] There is a great diversity of new approaches proposed to improve them (Table 2).[23]

Table 2 – *List of commercially available ceramic cements. The main end product of the reaction can be either an apatite (calcium deficient, carbonate, among others), brushite (DCPD) or gypsum ($CaSO_4 \bullet 2\ H_2O$) (Adapted from Bohner M. [23]).*

Producer	Product name	Composition	Product
AG Digital Technology Corp	A-GRIX	Powder: calcium sulphate hemihydrate powder ($CaSO_4.^{1/2}\ H_2O$; CSH) & calcium sulphate granules; Solution: Aqueous solution	Gypsum
Berkeley Advanced Biomaterials (US)	Cem-Ostetic™	Powder: calcium phosphates (details unknown); Solution: Sterile water	Apatite
	Tri-Ostetic™	Powder: calcium phosphates (details unknown); Solution: Sterile water	Apatite
Biocomposites Ltd (GB)	Genex®	Composition: could not be found	Gypsum
Biomatlante (FR)	MCPC	Powder: mainly α-TCP, ACP, BCP = biphasic calcium phosphate (composite between HA and β-TCP); Solution: phosphate buffered solution (Khairoun *et al.*, 2005)	Apatite
Biomet (US) Interpore (US)	Calcibon®	Powder: α-TCP (61%), DCP (26%), CaCO3 (10%), PHA (3%); Solution: H2O, Na2HPO4 (Khairoun *et al.*, 1999)	Apatite
	Mimix™	Powder: TetCP, α-TCP, trisodium citrate (C6H5O7Na3·2H2O); Solution: H2O, citric acid (C6H8O7)	Apatite

Walter Lorenz Surgical (GER)	Quick Set Mimix™	Powder: Calcium phosphate powders, Na3C6H5O7·2H2O; Solution: Citric acid aqueous solution	Apatite
	Bone Plast® QS	Powder: CSH (CaSO4·½H2O); Solution: sterile aqueous solution	Gypsum
BoneSupport AB (SWE)	Cerament™	Powder: CaSO4·½H2O (60%), HA (40%); Solution: Aqueous solution of an iodine radiopacifier (http://www.bonesupport.com/)	Gypsum
Calcitec (US)	Osteofix	Powder: calcium phosphate and calcium oxide powders; Solution: phosphate buffer	Apatite
ETEX (US)	? -BSM; Embarc; Biobon	Powder: ACP (50%), DCPD (50%); Solution: Unbuffered aqueous saline solution (Lee *et al.*, 1999; Tofighi *et al.*, 2001)	Apatite
	β-BSM	Composition: could not be found (it has apparently a higher compressive strength and better injectability than α-BSM)	Apatite
	γ-BSM	Composition: could not be found (“putty” consistency)	Apatite
	OssiPro	Composition: could not be found; The cement is claimed to be macroporous after hardening	Apatite
	CarriGen	Composition: synthetic calcium phosphate, sodium carboxymethyl cellulose, sodium bicarbonate, and sodium carbonate	Apatite
Futura Biomedical (US)	OsteoCure	Powder: CaSO4·½H2O; Solution: sterile mixing solution	Gypsum
Graftys (FR)	Graftys® HBS	Powder: mainly ? -TCP, ACP, BCP = biphasic calcium phosphate (composite between HA and β-TCP); Solution: phosphate buffered solution (Khairoun et al., 2005)	Apatite
	Graftys® Quickset	Composition: calcium phosphate salts, hydroxypropylmethylcellulose (HPMC), and phosphate-based aqueous solution	Apatite

Kasios (FR)	Jectos Eurobone®	Powder: β-TCP (98%), $Na_4P_2O_7$ (2%); Solution: H_2O, H_3PO_4 (3.0M), H_2SO_4 (0.1M) (Frayssinet *et al.*, 2000)	Brushite
	Jectos+	Composition: could not be found (likely to be close to that of Jectos) (http://www.kasios.com/doc-pdf/JECTOS%2B699ed03-frgb.pdf)	Brushite
Kyphon (US)	KyphOs™	Powder: ? -TCP (77%), $Mg_3(PO_4)_2$ (14%), $MgHPO_4$ (4.8%), $SrCO_3$ (3.6%) ; Solution: H_2O, $(NH_4)_2HPO_4$ (3.5M) (Mulliez and Wenz, 2002)	Apatite
Lifecore (US)	CalMatrix	Powder: 90% $CaSO_4 \cdot ½H_2O$ and 10% carboxymethylcellulose; Solution: could not be found	Gypsum
Mitsubishi Materials (J)	Biopex®	Powder: α-TCP (75%), TetCP (20-18%), DCPD (5%), HA (0-2%) Solution: H_2O, Sodium succinate (12-13%), sodium chondroitin sulfate (5-5.4%) (when two values are indicated, the first value stems from reference (Kurashina *et al.*, 1997) and the second value from reference (Tanaka *et al.*, 2003))	Apatite
	Biopex®-R	Powder: α-TCP, TetCP, DCPD, HA, $Mg_3(PO_4)_2$, $NaHSO_3$ Solution: H_2O, Sodium succinate, sodium chondroitin sulfate (Tanaka et al., 2003)	Apatite
Orthogen Corporation	DentoGen	CSH powder and aqueous solution	Gypsum
Produits Dentaires SA (CH) CalciphOs (CH)	VitalOs4	Solution 1: β-TCP (1.34g), $Na_2H_2P_2O_7$ (0.025g), H_2O, salts (0.05M pH 7.4 PBS solution); Solution 2: MCPM (0.78g), $CaSO_4 \cdot 2H_2O$ (0.39g), H_2O, H_3PO_4 (0.05M) (Brendlen *et al.*, 2003)	Brushite
Shanghai Rebone Biomaterials Co (CN)	Rebone	Powder: TetCP, DCP; Solution: H_2O (Liu *et al.*, 1997)	Apatite

Skeletal Kinetics (US)	Callos™	Composition: α-TCP, CaCO3, MCPM; Solution: sodium silicate(Constantz, 2002)	Apatite
	Callos Inject™	Composition: α-tricalcium phosphate and unknown compounds (likely to be close to that of Callos™)	Apatite
	OsteoVation EX Inject	Probably similar to "Callos Inject™" (Product produced by S.K. but sold by OsteoMed)	Apatite
Stryker (US)	BoneSource	Powder: TetCP (73%), DCPD (27%); Solution: H2O, mixture of Na2HPO4 and NaH2PO4 (Brown and Chow, 1985; Brown and Chow, 1983; Chow, 1991)	Apatite
Leibinger (GER)	HydroSet™	Powder: TetCP, DCPD, trisodium citrate; Solution: H2O, polyvynilpyrrolidone, sodium phosphate (Hannink *et al.*, 2008)	Apatite
Synthes (US)	Norian® SRS Norian® CRS	Powder: α-TCP (85%), CaCO3 (12%) MCPM (3%); Solution: H2O, Na2HPO4 (Constantz *et al.*, 1995; Fernandez *et al.*, 1998)	Apatite
	Norian® SRS Fast Set Putty Norian® CRS Fast Set Putty	Composition: could not be found (likely to be close to that of Norian SRS/CRS)	Apatite
	Norian Drillable	Composition: calcium phosphate powder, bioresorbable fibers and sodium hyaluronate solution	Apatite
	chronOS™ Inject	Powder: β-TCP (73%), MCPM (21%), MgHPO4·3H2O (5%), MgSO4 (<1%), Na2H2P2O7 (<1%); Solution: H2O, sodium hyaluronate (0.5%) (Bohner *et al.*, 2003)	Brushite
Teknimed (FR)	Cementek®	Powder: α-TCP, TetCP, Na Glycerophosphate; Solution: H2O, Ca(OH)2, H3PO4 (S. Goncalves, Teknimed, private communication)	Apatite

	Cementek® LV	Powder: α-TCP, TetCP, Na Glycerophosphate, dimethylsiloxane; Solution: H2O, Ca(OH)2, H3PO4 (S. Goncalves, Teknimed, private communication)	Apatite
Wright Medical (US)	MIIG™ 115	Powder: CSH; Solution: Saline (Turner *et al.*, 2003)	Gypsum
	MIIG® X3	Composition: CSH; Solution: Sterile water (contains also traces of an accelerant)	Gypsum
	MIIG® X3 High-Visc	Composition: CSH; Solution: Sterile water (less than in MIIG® X3; contains also traces of an accelerant)	Gypsum
	Pro-Dense®	Composition: 75% CSH, 25% brushite and granular β-TCP	Gypsum

Combining the keyword "Putty" and "Ceramic" a smaller number of publications is obtained, but the development is remarkably similar.[23] Recently, attention shifted to hydrogels composites and bone substitutes, resulting in the discovery and commercialization of various products (Table 3).

Table 3 – *List of some non-setting non-allogenic pastes with indication of producer, product name, composition and form (pre-mixed or to be mixed). Denominations: BCP = biphasic calcium phosphate (composite between HA and β-TCP); CMC = carboxymethylcellulose; HPMC: hydroxypropylmethylcellulose (Adapted from Bohner M. [23]).*

Producer	Product name	Composition	Form
ApaTech (UK)	Actifuse™	HA, polymer and aqueous solution	Pre-mixed
	Actifuse™ Shape Actifuse™ ABX	Silicon-substituted calcium phosphate and polymer	Pre-mixed
Baxter (US)	TricOs T TricOs	BCP (60% HA, 40% β-TCP) granules and Tissucol (fibrin glue)	To be mixed
Berkeley Advanced Biomaterials	Bi-Ostetic Putty	Non-disclosed	Not disclosed
BioForm (US)	"Calcium hydroxylapatite implant"	HA powder embedded in a mixture of glycerine, water, and CMC1	Pre-mixed

Biomatlante (FR)	MBCP Gel®	BCP granules (60% HA, 40% β-TCP; 0.08-0.2mm) and 2% HPMC (Boix *et al.*, 2006; Gauthier *et al.*, 2005)	Pre-mixed
	Hydr'Os	BCP granules (60% HA, 40% β-TCP; micro and nanoparticles) and saline solution (Biomatlante, private communication)	Pre-mixed
Degradable solutions (CH)	easy graft™	β-TCP or BCP granules (0.45-1.00mm) coated with 10 µm PLGA, N-methyl-2-pyrrolydone (K. Ruffieux, private communication)	To be mixed
Dentsply (US)	Pepgen P-15® flow	Hydroxyapatite (0.25-0.42mm), P-15 peptide and aqueous sodium hyaluronate solution (product brochure)	To be mixed
DePuy Spine (US)	Healos® Fx	HA (20-30%) and collagen	To be mixed
Fluidinova (P)	nanoXIM TCP	β-TCP (5 or 15%) and water (company website)	Pre-mixed
	nanoXIM HA	HA (5, 15, 30, or 40%) and water (company website)	Pre-mixed
Integra LifeSciences (US)	Mozaik Osteoconductive Scaffold	β-TCP (80%) and type 1 collagen (20%)	To be mixed
Mathys Ltd (CH)	Ceros® Putty / cyclOS® Putty	β-TCP granules (0.125-0.71mm; 94%) and recombinant sodium hyaluronate powder (6%)	To be mixed
Medtronic (US)	Mastergraft®	BCP (85% HA, 15% β-TCP) and bovine collagen	To be mixed
NovaBone (US)	NovaBone® Putty	Bioglass and synthetic binder	Pre-mixed
Orthovita (US)	Vitoss Flow	Contains at least bioactive glass and saline solution (or blood marrow aspirate, or blood)	To be mixed
	Vitoss Pack	Contains at least bioactive glass and saline solution (or blood marrow aspirate, or blood)	To be mixed
Osartis / AAP (GER)	Ostim®	Nanocrystalline HA (35%) and water (65%) (Laschke *et al.*, 2007)	Pre-mixed
	JAX CS	CSD granules and an aqueous solution	To be mixed

Smith & Nephew (US)		(http://global.smithnephew.com/us/JAX_CS_OVERVIEW_7221.htm)	
	JAX TCP	β-TCP granules and an aqueous solution of 1.75% CMC and 10% glycerol (Clarke *et al.*, 2007)	To be mixed
Stryker (US)	Calstrux™	β-TCP granules and CMC	To be mixed
Teknimed (FR)	Nanogel	Nanocrystalline HA (100-200nm) (30%) and water (70%) (S. Goncalves, private communication)	Pre-mixed
Therics (US)	Therigraft™ Putty	β-TCP granules and polymer	Pre-mixed
Zimmer (US)	Collagraft	BCP granules (65% HA, 35% β-TCP; 0.5-1.0 mm), bovine collagen, and bone marrow aspirate (Bucholz, 2002)	To be mixed

Injectability describes how easy or difficult it is to inject a bioactive system from a syringe[24], being a very relevant property in minimally invasive surgical procedures in the area of orthopaedics and dentistry. Some authors define injectability of a paste as its ability to remain homogeneous during its injection.[24]

In other words, the paste must be able to be extruded through a needle without the occurrence of solid/liquid (S/L) phase separation. To this end, one of the crucial aspects that must be taken into account is the cohesion of the paste, because a CPC with ideal cohesion hardens even in contact with fluids without disintegrating and without phase separation during the injection of the product.[25] Another aspect, perhaps the most important one, is the study of the rheological behaviour, i.e., how the paste flows under the action of applied stresses. For a CPC paste to be injectable it should have relatively low viscosity in order to offer little resistance when it is leaving the syringe. However, the more viscous formulations are useful in situations where the surgeon chooses to manually place the paste in the place.[26]

There are several aspects that should be taken into account regarding the improvement of the rheological behaviour of bone cements. The first can be the injection apparatus

itself, since shorter cannulas and with a larger diameter favour the flow of the cement. The proportion between the solid and liquid phases is another relevant aspect to consider. The paste becomes less viscous with increasing the proportion of liquid and might therefore be easier to inject. This may have consequences related to mechanical properties, as well as lead to the annihilation of the paste's cohesion.[19] Another important aspect relates to the adjustment of size and size distribution of the particles of CPC components. Thinner particles pass better through narrower needles, but require a higher amount of liquid, which decreases the cohesion of the paste and leads to higher porosity and, also, worse mechanical properties.[19]

Another practical key aspect for all implantable materials is the expiration date after preparation. This describes how long an injectable cement can be stored with their properties intact. In a recent study[27] using dry powder mixtures of β-TCP, it was found that the powders tended to react with moisture in the surrounding atmosphere to form monetite. It was found that storage in dry atmosphere (argon), at low temperatures and the addition of retardants would help prolong the shelf life for the powder mixtures [28]. When the CPCs are stored in sealed syringes, the total amount of water present in the cement will be decisive for its validity.[24]

The size and distribution of sizes of the particles/agglomerates represent properties of extreme importance in all current bioactive systems. The flow behaviour is strongly dependent on these characteristics. The viscosity of the liquid is also of great importance. The flow behaviour is generally improved when the viscosity of the liquid increases, because a higher viscosity inhibits the segregation of the particles and the *filter pressing effect*.[23]

The final product characteristics can vary depending on the composition of the phases, the S/L ratio, the calcium/phosphorus (Ca/P) molar ratio and the presence of additives. Therefore, the properties of the cement such as the initial plasticity, time of cohesion/consolidation and of hardening must be taken into account, as well as the final mechanical resistance and injectability.[27]

2.2.3.1 Calcium Phosphate Cements (CPC)

The first cements based on calcium phosphates (CPC) could be shaped in any way, and it would make the utilization of CaP as bone substitutes easier.[29] The first publications reported on the use of CPC in dental cavity filling, where its superior biocompatibility and absorption reportedly enabled the best results. Today, the use of CPC in dentistry is limited since they do not have the mechanical properties required for repair of the cavity. Still, there are various applications of these biomaterials in orthopaedics, maxillofacial surgery and bone defects filling in general.[29]

Reactive CPCs pastes are malleable for a certain period of time, allowing the surgeon an easy accommodation in the bone defect with his own hands. Moreover, CPCs pastes can be placed in syringes and injected directly into bone defects in minimally invasive surgeries, preventing infections and reducing patient recovery time. These handling facilities confer to CPCs great advantages when compared to other non-malleable shapes.[29] These injectable systems need to be prepared (mixed) before application and meet a number of key aspects summarised in in Table 4.

Table 4 – *Important properties for injectable calcium phosphate cement and the questions to be investigated in this thesis (Adapted from Aberg J.* et al [30]*).*

Stage	Property	Question to answer
Handling (before injection)	**Working time**	- How much time does the surgeon dispose for injection?
	Injectability	- Is the cement easy or difficult to inject?
Hardening (0-24 h after Injection)	**Setting time**	- How long time before the patient can be moved?
	Final phase composition	- What chemistry will the biology encounter?

	Mechanical properties	- How strong is the cement? - For what applications can it be used?
Biology (0-12 weeks, post implantation)	***In vitro***	- What is the cellular response?
	In vivo	- Will the cement be resorbed? - Can bone grow onto the cement?
Shelf life	**–**	- For how long can the cement be stored?

The development of the fundamental properties of CPCs for biomedical applications can be divided into the three stages indicated in Table 4. The first stage refers to properties that are important before the cement is inside the body[24]. The second phase begins immediately after injection and continues until the complete hardening of the cement. Thereafter, the immediate and long term biological response is discussed in the third stage. Finally, the shelf life of cement is equally an important aspect, although it is not directly a part of the surgical procedure.[24]

In a clinical situation, the time that the surgeon has to prepare and inject the cement in a bone defect is designated working time (WT). Conventionally, the WT is measured using two methods: the Gilmore's needle method or the Vicat needle. The first Gilmore needle weighs 113.4 g and has a tip diameter of 2.12 mm. It is placed on the surface of the cement paste and the depth of its printing is registered. When the needle leaves no visible impression on the cement, it is considered that its WT was achieved, also called initial setting time. The same method is also used to measure the final setting time using the second Gilmore needle that weighs 453.6 and has a tip diameter of 1.06 mm [24]. The sequence of events along the cement setting is schematised in Figure 4. Additives are commonly used method to control the setting kinetics and the rheological properties of CPC [24].

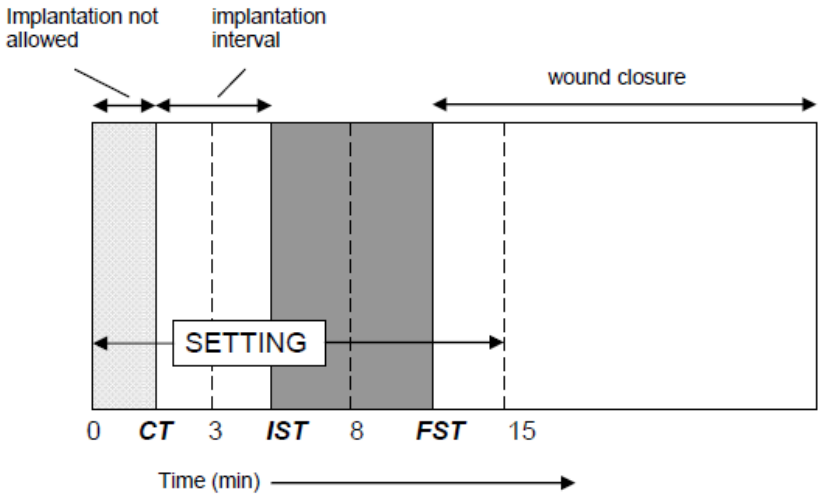

Figure 4 – *Setting time scheme (Adapted from Driessens FCM.* et al [31]*).*

After applying the Gilmore needle method, the initial setting time (IST) and final setting time (FST) values are obtained. However, there is still no consensus on what should be the ideal values of IST and FST, and its clinical meaning. Some researchers have suggested that cement should be injected before the IST, while the wound must be closed and after FST, making sure that the cement has not suffered deformation between these two periods.[31]

For Ginebra[32] the clinical meaning of these times is that the paste should be implemented before the IST and the wound can be closed after the FST. The cement should not be deformed in the solidification period between IST and FST because any deformation may induce cracks in it. That said, the period between IST and FST should be the time that the material takes to solidify until it becomes a material strong enough to withstand a certain pressure without deforming or breaking. The three requirements for the proper handling of the CPC, with numbers expressed in minutes (min), are presented below.

$3 \leq IST < 8$ (I)

$IST - CT \geq 1$ (II)

$FST \leq 15$ (III)

The requirement (II) means that the cohesion time (CT) should occur at least one minute before the IST, so the doctor has at least one minute to apply and shape the material. Since the mixing of the components takes about 1 min, the shorter CT that is permitted is only two min. Therefore the doctor has at least a minute to remove the paste from the spot where the mixing is carried out, putting it on the spatula or syringe in which the placement of CPC in the bone defect site will be effected. This should occur after CT and before IST. Alternatively, the doctor may also apply the CPC directly with his hands, shaping it with his fingers until the desired shape for use is achieved, as long as that implementation takes place also between CT and IST. For dental applications, the IST should be close to 3 min, while for orthopaedic applications it should be close to 8 min. Finally, the FST cannot exceed 15 min.[32]

The use of CPC as an injectable system can make the process longstanding, since the CPC is prepared in full operation by the clinician. After making the mixture it is necessary to transfer the paste to the syringe, which can take some time. In this case, it may be desirable that the initial setting time is the closest possible to 8 min in order to have time to prepare the syringe, while the deformation of cement without incurring structural damage is still possible. Until now the majority of cements are prepared manually, placing the solid and liquid phase in a kind of mortar, and mixing it with a pestle and a spatula.[33]

The mechanical behaviour of CPCs is also an important parameter, since in most of its clinical applications they are put in direct contact with the human cancellous bone, thus being expected that the mechanical strength required for the cement should be at least as strong as the trabecular bones. Unfortunately, CPCs are sufficiently strong only when submitted to compression. In practice, after setting, the mechanical strength of the cements is low when compared to that of bones, teeth or even calcium phosphate bioceramics. Thus, the products obtained after the hardening of all the CPCs are fragile, since they have a low impact resistance, while the resistance to compression ranges from 10-100 MPa.

The poor mechanical properties reduce the clinical applicability of the CPC. There are some factors that can induce changes in the measured mechanical resistance, such as factors related to sample preparation and also in the measurement process.[33] Some of these factors are summarized in Table 5.

Table 5 – *Factors that affect CPC (Adapted from Driessens FCM.* et al [33]*).*

Factor	Consequence
S/L Ratio	Higher ratio means higher solid phase resulting in higher resistance to compression.
Ca/P ratio of the reagents	Influence the nature of the, however the maximum compressive strength may vary depending on the formula.
Particle size	Smaller particles may lead to higher resistance to compression.
Additives	Can lead to increased or decreased mechanical strength depending on the type and amount used.
Chemical stability	The metastable products such as DCPD or OCP can be transformed under physiological conditions, becoming more stable and with low solubility such as CDHA, maintaining its strength indefinitely during storage in almost neutral aqueous solutions at 37 °C.
Temperature	Can lead to dehydration of the cement causing more porosity and consequently lower mechanical strength.

The setting time of CPC is based on a dissolution reaction which occurs when powders are mixed with reactive liquid. However, their poor injectability, low strength and lack of macroporosity to bone growth have limited its use in clinical applications. The injectability of CPCs is extremely important in clinical applications involving defects with limited accessibility or narrow groove, when there is a need for a very precise placement of the paste to conform the bone defect area, or when using minimally invasive surgical techniques.[29]

2.2.3.2. Ready-to-use Injectable Bone Substitutes

Given the limitations of CPCs, new (pre-mixed) injectable bioactive systems that could be used immediately were created. These bone substitutes are easier to use because they do not require any mixing or any transfer to an appropriate delivery system. Furthermore, there is no time restriction to use the product when it is open. The only disadvantage is linked to the predefinition of the mixture's composition.[23]

Since none of bone replacements proposed so far have load bearing, the principal strategy currently used to repair bone defects is to use a bone substitute that is rapidly resorbed and replaced by new mature bone. To achieve this, not only the chemical composition but also the geometry of the bone substitute must be optimized [18]. The use of a bone substitute that can easily be crossed by blood vessels and cells is particularly important. For this purpose, it must have a completely interconnected porous structure with pore diameters and pore interconnections larger than about 50 μm.[18] An approach to obtain a pasty bone substitute and macroporous is to combine granules with a hydrogel, e.g: dextran[34] or sodium hyaluronate [35]. Since the hydrogel solids content is generally very low, the cells can easily pass through the macroporous hydrogel gaps present between the granules.

The size of the gaps is controlled by the percentage of hydrogel in the mix and by the size distribution of the granules.[28] Thus, a bone substitute with superior biological properties is obtained.

NovaBone Putty®, a calcium phosphosilicate and BG platform with additives, for application in bone regeneration is an example.[36] The solid phase, bioactive, is composed by BG 45S5® particles and calcium phosphosilicates. The liquid phase comprises glycerol and polyethylene glycol (PEG). The bioactive phase allows the initial release of CaP and improves the physical characteristics of the paste, facilitating its handling. PEG occupies the spaces between the particles of the bioactive phase and also facilitates the handling of the putty, giving it a soft surface. Glycerol serves as a binder, has high hydrophilicity, and allows the consistency of the paste, retaining together all phases present.

The product overcomes the limitations of CPC and similar existing materials, is easy to handle, is conformable to the site of injury, is hydrophilic (mixes with blood), it has excellent retention at the injury site (remains locally) and a very smooth surface. All these properties result in rapid and complete resorption, osteoconduction and osteostimulation, leading to bone regeneration.[36]

2.3. Legislation of Medical Devices

Injectable bone substitutes are considered medical devices, as described in Directive 93/42/EEC June 14th 1993 of the European Council and amended by M1 Directive 98/79/EC, M2 Directive 2000/70/EC, M3 Directive 2001/104/EC, M4 Regulation (EC) 1882/2003 and M5 Directive 2007/47/EC.[37] According to it, a medical device is meant by "any instrument, apparatus, appliance, software, material or other article, whether used alone or in combination, including the software intended by its manufacturer to be used specifically for diagnostic and/or therapeutic purposes and necessary for the proper functioning of the medical device intended by the manufacturer to be used for human beings for the purpose of:

- Diagnosis, prevention, monitoring, treatment or alleviation of disease,

- Diagnosis, monitoring, treatment, alleviation or compensation for an injury or handicap,

- Study, replacement or modification of the anatomy or of a physiological process,

- Conception inspection, on which the main intended action in the human body is that it's not achieved by pharmacological, immunological or metabolic means, but its function can be supported by such means; ".[37]

According to the directive, to market a MD in the European Union is necessary to have the CE marking. To obtain the CE marking, there are certain requirements that need to be fulfilled, as schematised in Figure 5. Based on Figure 5 and Directive 93/42/EEC, there are

two ways to proceed to clinical evaluation of a MD and later obtain the CE marking. The first way is a critical evaluation of the scientific literature in order to obtain equivalence of the MD that is intended to release in the market, relatively to an already marketed MD which is reported in the literature. The other way is characterized by conducting an independent clinical trial that after drawing up a research plan, it is required an approval by the competent Ethics Committee. After the clinical evaluation, whether by equivalence or clinical trial, a design dossier must be developed and later submitted to the assessment of a notified body. In case of approval the CE mark is obtained. After approval by the notified body, it is necessary to notify the Competent Authority of the respective country (e.g: Portugal – INFARMED) of the intention to place the MD on the market, which complies with European accreditation.

For the production of the MD, the entire procedure has to be done according to the a Quality Management System for example, according to EN ISO 13485:2003.[38]

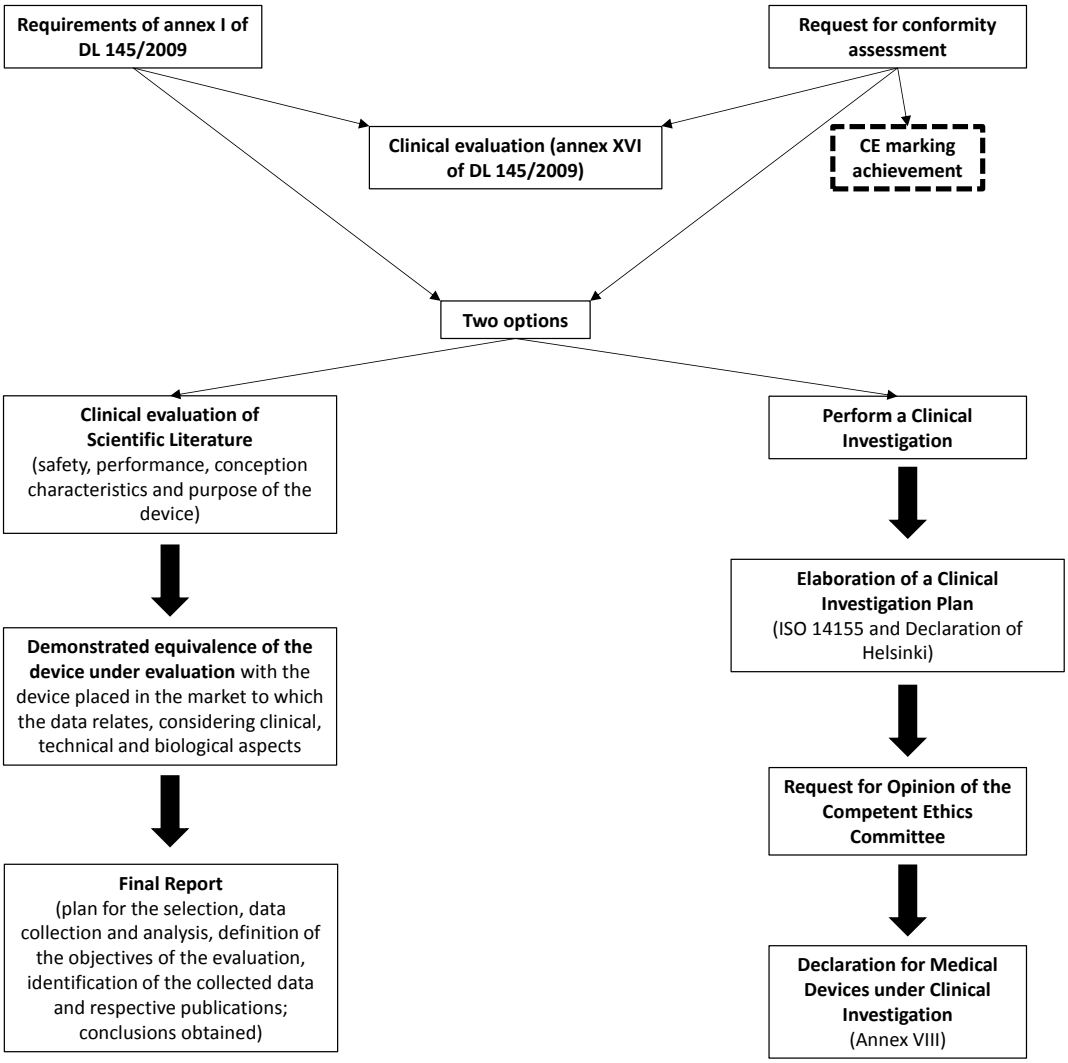

Figure 5 – *Representative scheme of the requirements to carry out a clinical evaluation and obtain the CE marking.*

CHAPTER III

MATERIALS AND METHODS

3. Preparation and Characterization of the Putty

3.1. Experimental procedure

3.1.1. β-TCP Synthesis

β-TCP powder was obtained by chemical precipitation from calcium nitrate tetrahydrate [$Ca(NO_3).4H_2O$] (Panreac) and di-ammonium hydrogenphosphate [$(NH_4)_2HPO_4$], (Panreac) as chemical precursors for calcium and phosphorus, respectively (Table 6).

Table 6 – *Precursors used in the synthesis of β-TCP powders.*

Reagent	Chemical Formula	Precursor	Concentration (g/L)
Di-ammonium hydrogenphosphate	$(NH_4)_2HPO_4$	P	79.236
Calcium nitrate tetrahydrate	$Ca\ (NO3)_2.4H_2O$	Ca	211.163

The calculations for the preparation of the solutions for use in the synthesis of β-TCP powder were based on a ratio Ca/P = 1.49. Assuming that the initial concentration of the P solution was fixed at 0.6 M, concentration of solution of the Ca precursor was calculated so that this ratio could be maintained. Therefore, the total concentration of the Ca precursor had to be 0.894 M. The di-ammonium hydrogenphosphate solution was slowly added via peristaltic pump with 88.6 mL/min flow rate to the calcium nitrate tetrahydrate solution previously placed in the reactor under stirring at a speed of 1000 rpm. After complete admission of the solutions of precursors to the reactor, the pH of the solution/suspension mixture was controlled to 7 and maintained at this value by the initial addition of 70 mL of ammonium hydroxide (NH_4OH; Sigma-Aldrich, Germany) solution. The pH value was measured and recorded, in an interval of 15 min, with a pH meter, within 3 h of synthesis, and maintained at pH 7 by adding ammonium hydroxide when necessary. The reaction was carried out at 30 °C under constant stirring speed of 1000 rpm for 3 hours (h). The experimental details are summarised in Figure 6.

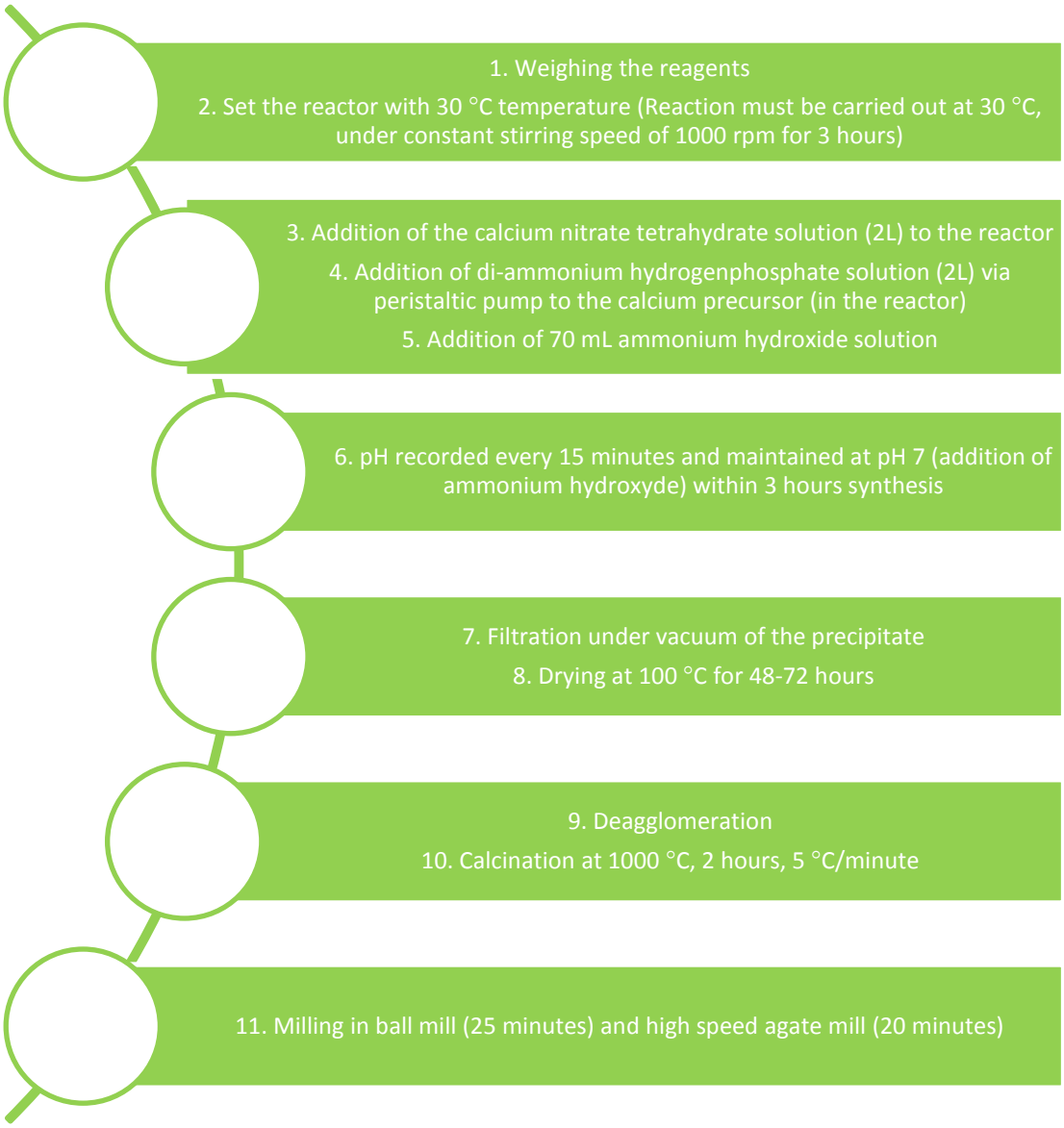

Figure 6 – *β-TCP powders synthesis.*

The suspension of the precipitate was withdrawn from the reactor after 3 h of reaction. The precipitate was separated by filtration under vacuum and was then dried at 100 °C for 48 h. Then, it was deagglomerated and calcined at 1000 °C for 2 h, 5 °C min^{-1}. Finally, the powders were initially milled in a ball mill for 25 min and in an high speed agate mill for 20 min to obtain particles with mean particle size of 1~3 µm (determined by light scattering technique). The crystalline phases of the calcium phosphate powder were then investigated by X-ray diffraction (XRD) analysis.

3.1.2. Bioglass Synthesis

FastOs®BG was prepared by the melt-quenching method, as diagrammed in figure 7. Homogeneous mixtures of batches (~100 g), obtained by ball milling (~5 minute mixture), were preheated at 900 °C for 1 h for decarbonization and melted in Pt crucibles at 1570 °C for 1 h. The glass was obtained in frit form by quenching the glass melt in cold water. The frit was dried, ball milled for 15 min and milled again in a high speed agate mill for about 20 min, resulting in fine glass particles with mean particle size of ~10–15 µm. The amorphous nature of glasses was confirmed by XRD analysis.

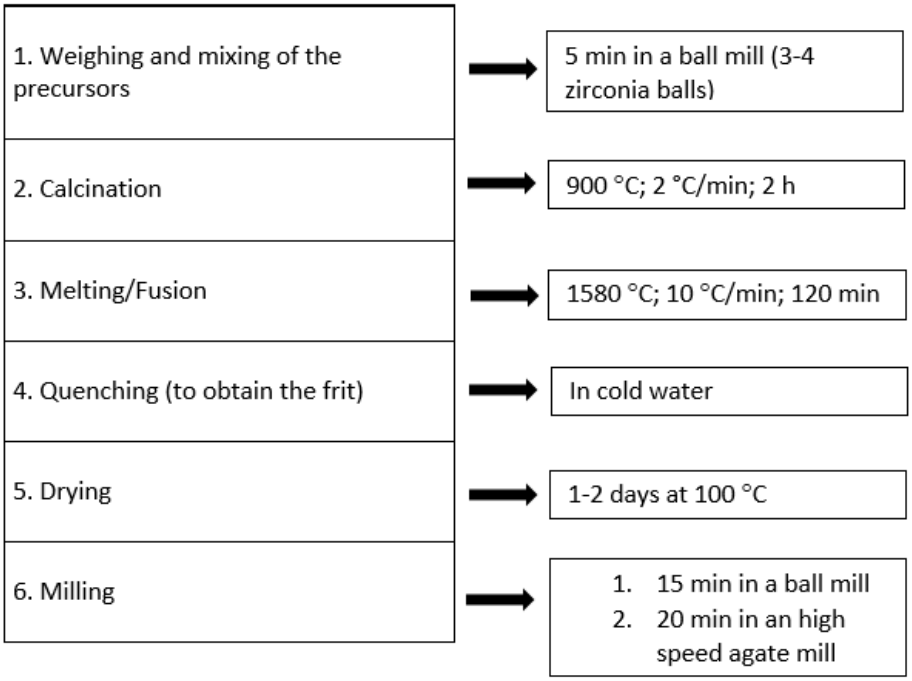

Figure 7 – *Steps followed for the synthesis of FastOs®BG.*

3.1.3. Dehydration and milling of monocalcium phosphate monohydrate (MCPM)

Monocalcium phosphate monohydrate, ($Ca(H_2PO_4)_2.H_2O$ - MCPM, Sigma-Aldrich) was selected as the acidic component to react with β-TCP to promote setting after implantation when in contact with physiological fluid. Besides the stoichiometric water, it is also susceptible of attracting and holding water molecules from the surrounding environment at room temperature. The presence of water in the powder mixture (MCPM + β-TCP + FastOs®BG) is not desired as it would promote its early partial setting of the putty, therefore, compromising its shell life. Therefore, MCPM was previously dehydrated at 80 °C in a laboratory oven, followed by ball milling, drying and sieving as schematized in Figure 8.

Figure 8 – *MCPM treatment steps.*

The process had the duration of 3 days (d) and after that the MCPM powder with D_{50} <100 µm was saved into a desiccator. A thermogravimetric (TG) analysis would give a more accurate description of the dehydration process of MCPM. Unfortunately the equipment was broken and the requested analysis is still pending.

3.1.4. Putty preparation

The calcium phosphate cement putty consisted of a solid phase (a mixture of FastOs®BG, MCPM and β-TCP powders in the following volume ratios of 40:30:30) premixed in a liquid phase (Glycerol ≥ 99.5 %, Sigma-Aldrich). The planned solid/liquid (S/L) weight ratio was 3.3. Glycerol was firstly added to the container and then the powders were added according to their mean particle sizes, starting from the finest one (β-TCP) followed by FastOs®BG, and finally MCPM. The mixture was stirred after each powder addition for 2–5 min at 800–1200 rpm in order to obtain a homogenous putty. Several syringes (1, 3 and 5 mL) were then filled with the putty using a vacuum syringe charger.

The overall putty preparation process is schematized in Figure 9. The syringe charger is not yet fully optimized for this kind of viscous pastes/putties and complete vacuum conditions could not be granted.

Figure 9 *– Putty preparation scheme.*

3.2. Characterization of starting powders

The starting powders were characterized regarding crystalline phase composition and particle size distribution (PSD). The crystalline phases were analysed in a XRD diffractometer. The particle size distributions (PSDs) of β-TCP and FastOs®BG powders were determined by light scattering technique using the Fraunhofer optical model. The PSD of MCPM powder cannot be assessed through a wet characterization technique using water as dispersing liquid due to its solubility in water. Therefore, the milled MCPM

powder was passed through a 100 μm mesh before being added to the putty formulation and characterized though Scanning Electron Microscopy (SEM).

3.3. Putty Characterization

The putty developed in this work is intended to be used clinically during surgery. To reduce the risk of errors the putty should be predictable, easy to use and it's behaviour when applied in the body well known.

3.3.1. Injectability

To evaluate the injectability, only 5 mL syringes were used, as the syringes with lower capacities are smaller and won't fit the wooden support of the injectability device available. Therefore, 5 mL syringes with putty were mounted in a universal testing machine, as shown in figure 10. A cross-head speed/force of 15 mm min^{-1} was used to extrude the putty from the syringe. The extrusion rate was kept constant, while the force was recorded in the software.

Figure 10 – *Injectability evaluation.*

The fraction of putty that could be extruded was determined by measuring the weight of the plastic syringe before and after extrusion. Therefore, the maximum fraction (injectability) could never reach 100 % since residual putty was always left. Injectability was calculated with the following formula:

$$I = \frac{m\ injected(g)}{m\ initial\ (g)} * 100\ \%$$ **(Equation 1)**.

In this case, I is the injectability, $m_{injected}$ and $m_{initial}$ are the weight of the paste injected through the syringe and the paste initially contained in the syringe. All values were obtained from the average of three tests.

3.3.2. Phase and size separation

In order to evaluate the phase and size separation of the putty, during injectability, in other words, to investigate the occurrence of filter pressing effect, putty samples were collected every 60 seconds counted from the beginning of the extrusion process. Samples were submitted to TG in order to investigate which thermal treatment was more suitable, since the liquid and organics need to be removed to investigate if S/L ratio remains the same before extrusion. After the TG result, the samples were weighed before and after calcination at 400 °C for 1 h using 2 °C min^{-1} to determine their S/L values.

3.3.3. Setting time

To evaluate the setting time (ST) of the putty, it was injected into three cylindrical moulds (13 mm Ø; 10 mm height). At t = 0 the filled moulds were immersed in 10 ml of freshly prepared phosphate buffered saline solution (PBS, pH 7.4, approximately) at 37 °C, to stimulate *in vivo* conditions. The putty was considered to have set when the sample could support the weight of a 453.6 g Gilmore needle with a tip diameter of 1.06 mm without leaving a mark on the sample (FST)[39].

The three samples were tested consecutively every 5 min. However, the setting of the putty is very dependent on the diffusion of water into the putty replacing the glycerol. Therefore, the surface of the samples hardened before the bulk. Considering this, the time at which the tip of the needle didn't leave any mark was when the bulk could support the weight without breaking. The putty was considered to have set when both sides supported weight of the needle.

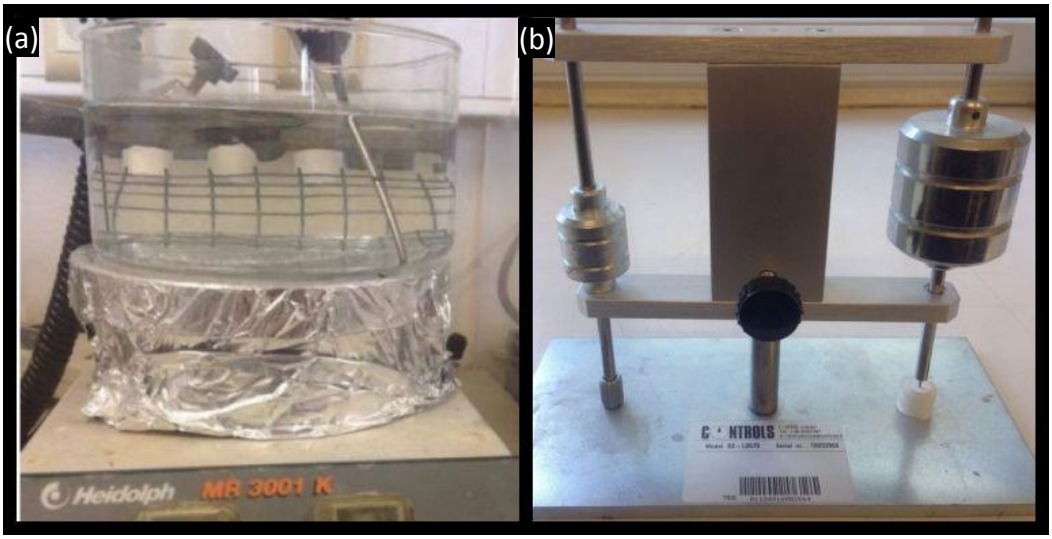

Figure 11 – *a) Method used to measure setting time in PBS at 37 °C; b) Application of the Gilmore needle in the putty (already set).*

3.3.4. Compressive Strength

To measure the compressive strength (CS), the putty was injected into cylindrical moulds (13 mm Ø; 10 mm height) and immersed in 10 ml PBS at 37 °C in a sealed plastic cup. After 24 h, 7 and 28 d, the samples were removed from their moulds and smoothly polished to obtain the correct height and parallel surfaces. The maximum compressive strength until failure was measured using the same universal testing machine. The cross-head speed was 11 mm min^{-1} and a thin aluminium film was placed between the sample and the crosshead to reduce the effect of possible defects deriving from the putty surface or mould (Figure 12). At least three measurements were made for each sample.

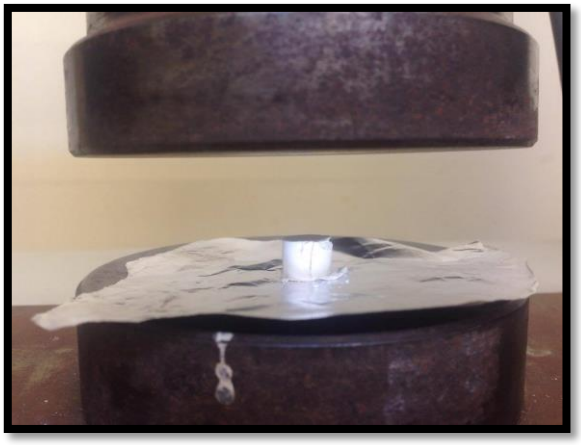

Figure 12 – *Compressive strength measurement of the putty (after failure).*

3.3.5. Bioactivity and Degradation in Simulated Body Fluid (SBF) and Phosphate Buffered Saline Solution (PBS)

SBF, which has ion concentrations and a pH value similar to those of human blood plasma, was prepared in accordance with the Cuneyt Tas method. Initially, 960 mL of high purity deionized water was added to a 1000 mL capacity glass beaker, which was then placed in a hot plate/magnetic stirrer (solution must be stirred step by step). The Cuneyt Tas–SBF recipe was then prepared as follows:

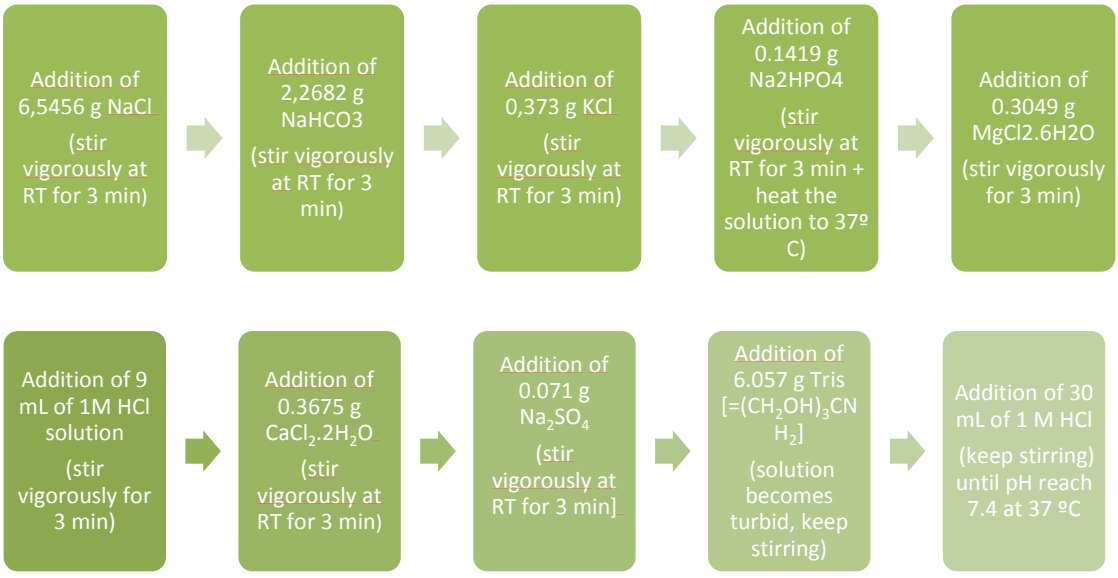

Figure 13 – *Steps to obtain the Cuneyt Tas-SBF recipe.*

27 mM HCO_3-Tris SBF, with pH 7.4 at 37 °C is obtained. SBF solution was kept in a clean glass media bottle (1000 mL capacity), tightly capped, in a refrigerator. SBF solutions older than 30 d were never used.

A litter of PBS, which is isotonic (osmolality and ion concentrations of the solution usually match those of the human body), was prepared as follows in a 1000 mL glass cup under constant stirring:

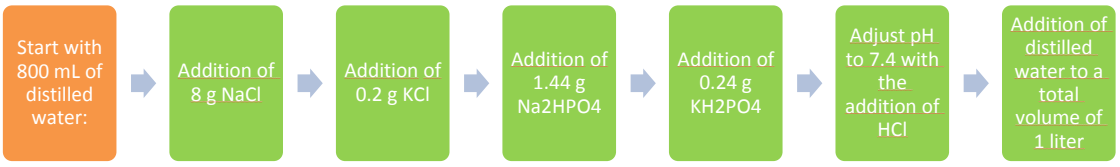

Figure 14 – *Steps to obtain PBS.*

PBS, with pH 7.4 at room temperature was obtained and stored in a plastic container, in a refrigerator. PBS solutions older than 7 d were never used.

After setting for 24 h (1 d), the putty samples (13 mm Ø; 10 mm height) were immersed in 10 mL of both solutions at 37 °C for 7 and 28 d. For the evaluation of *in vitro* bioactivity, the samples were removed after incubation for the specified time periods, rinsed in deionized water and dried at room temperature until reaching a constant weight. The samples were characterized with XRD, and the surface morphologies were observed through SEM.

Regarding *in vitro* degradation, the 24 h, 7 d and 28 d samples were immersed into PBS and SBF solutions at 37 °C. For this evaluation, the samples were removed after incubation for the specified time periods, rinsed in deionized water, dried at 80 °C for 24 h and weighed. *In vitro* degradation was calculated through the following formula:

$$D = [Wo - WT)/Wo] * 100\ \% \qquad \textbf{(Equation 2)}.$$

In which, D is the degradation rate and W_0 and W_T are the dry weight of the initial sample and the degraded sample, respectively. These measurements were made in triplicate.

3.3.6. Structural analysis

The existence of crystalline phases in the developed putty was investigated through XRD. The infrared spectra of the putty was obtained using Fourier transform infrared spectroscopy (FTIR), in order to identify the functional groups present in the putty and support the results of XRD. The putty microstructure was analysed through SEM. The samples were placed in an aluminium holder with carbon glue. After drying in a laboratory oven during night, samples were coated with a carbon film using a carbon evaporator.

3.3.7. Shelf life

The shelf life is an important feature for putties and all implantable premixed cements. It tells about how long the putty can be stored while maintaining intact the relevant properties. Gbureck et. al.[28] showed that dry powder mixtures of β-TCP and MCPM tend to react to form monetite in the presence atmospheric humidity. It was found that shelf life could be prolonged by storing the powder mixtures under dry (argon) atmosphere and low temperature, or by adding a retardant. In the present study, the syringes were filled with the putty, screw capped and stored in sealed plastic sleeves. Putty injectability, cohesion and hardening were checked week after week.

3.4. Equipment, software and devices used

This subchapter is intended to report on the equipment, software and devices used in the production and characterization of raw materials and putties. These are presented in Table 7.

Table 7 – *Equipment, software and devices used in the production and characterization of raw materials and putty.*

Type of equipment	Brand and model name
Production of starting materials of putty	
Ovens used in the calcination of β-TCP powders and fusion of FastOs®BG	Termolab
pH meter	Consort C1010
Ball mill	Ceramic Instruments, Sassuolo - Italy
High speed agate mill	Retsch PM14
Laboratory ovens	MMM Medcenter ECOCELL
Mixer	THINKY ARE-250
Vacuum syringe charger	THINKY ARC-40H
Syringes	SOFT-JECT Luer Lock
Characterization	
Setting time	Controls 63-L0075
XRD	Rigaku Geigerflex D/Max, Tokyo, Japan; C Series; (CuKa radiation; 2h angle range 10–80°; step 0.02°s^{-1})
Particle Analyser	Coulter LS 230
Injectability and compressive strenght	Universal testing machine: SHIMADZU AG-IS ; software: TRAPEZIUM 2
SEM	HITACHI S-4100 (constituted of an electron emission system with tungsten filament, acceleration 10 kV and 15 Å maximum resolution)
Carbon evaporator	EMITECH
FTIR	Mattson Galaxy S-7000, USA (powders were mixed with KBr in the proportion of 1.5/200 (by weight) and pressed into a pellet using a hand press). In total, 128 scans for background and 64 scans per sample were made with a signal gain of 1. The resolution was 4 cm^{-1}.

3.5. Legislation of Medical Devices

The clinical evaluation of the MD is a key step to pave the way towards its commercial release. To proceed to the clinical evaluation of the MD, a critical evaluation of the scientific literature was made, in order to obtain equivalence of the MD relatively to an already marketed MD, reported in the literature.

Therefore, in order to meet the essential requirements set out in Annex I of the Directive 93/42/EEC, a clinical evaluation report (CER) has been carried out. Its structure is supported on the document MEDDEV. 2.7.1 Rev.3.[40]

CHAPTER IV

RESULTS AND DISCUSSION

5. Results and Discussion

5.1. Powder Characterization

5.1.1. β-TCP

The XRD pattern of the precipitated calcium phosphate powder after being heat treated at 1000 °C with a heating rate of 5 °C/min is displayed in Figure 15. The diffractogram was analysed using PDF files (Powder Diffraction Files) of ICDD (International Centre for Diffraction Data) and the standard diffraction lines for β-TCP (PDF file 04-006-9376) and for Calcium Pyrophosphate (CPP) (PDF file 04-009-3876) are also plotted in Figure 15 for comparison. Figure 15 also shows the results of crystalline phase analysis determined by using the Reference Intensity Ratio (RIR), revealing that the β-TCP powder consists of 81% β-TCP and 19% CPP phases. This significant amount of CPP phase is likely due to an insufficient alkalinity of the media during precipitation.

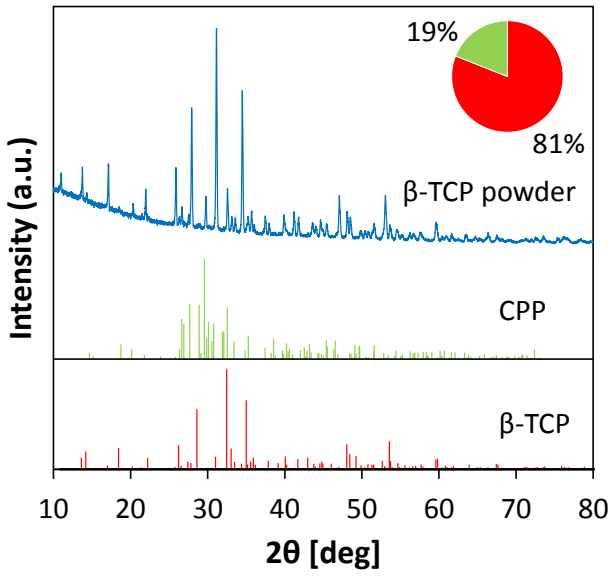

Figure 15 – *X-Ray Diffractogram of β-TCP powder. (CPP: Calcium Pyrophosphate, $Ca_2(P_2O_7)$, ICDD 04-009-3876; β-TCP: Beta-Tricalcium Phosphate $Ca_3(PO_4)_2$, ICDD 04-006-9376).*

The calcined β-TCP powder was dry milled in order to obtain an adequate particle size distribution. The particle size distribution curve of the β–TCP powder is presented in Figure 16. The particle diameters (D_{10}, D_{25}, D_{50}; D_{75}, and D_{90}) below which typical volume percent values (10%, 25%, 50% 75% and 90%) of the distribution lie below are reported in Table 8. The D_{50}, the median, is defined as the diameter where half of the population lies below this value. It can be seen that the β–TCP powder has a bimodal particle size distribution, with median particle size (D_{50}) of ~1.5 µm. The coarser particle population centred at about 8–9 µm in Figure 16 is likely due to the presence of some agglomerates that were not completely destroyed upon milling.

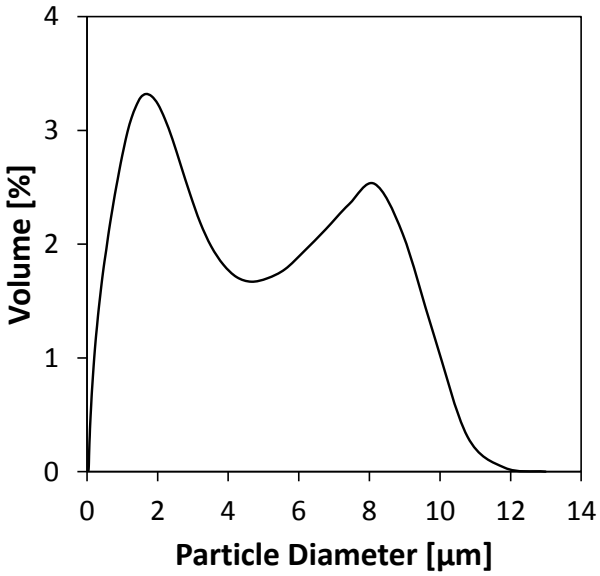

Figure 16 – *Particle size distribution of β–TCP powder.*

Table 8 – Particle size distribution intervals of β–TCP powders.

Volume [%]	< 10	< 25	< 50	< 75	< 90	Mean
Particle Diameter [µm]	0.263	0.632	1.509	3.270	6.963	2.491

5.1.2. FastOs®BG

The XRD pattern of FastOs®BG powder is presented in Figure 17. The absence of crystalline phases is obvious, which confirms the amorphous nature of the BG.

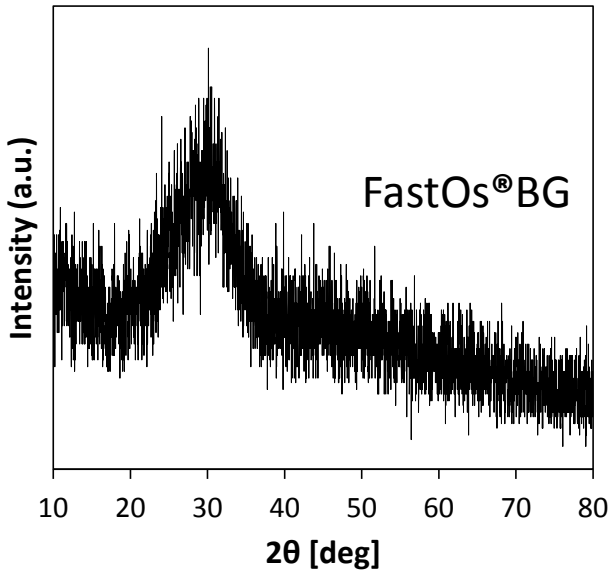

***Figure 17** – X-Ray Diffractogram of FastOs®BG frit.*

FastOs®BG was then submitted to milling, to obtain the required particle size distribution. The particle size distribution curve of the FastOs®BG frit dry milled for 35 min is presented in Figure 18. Like for the β–TCP powder, the particle diameters (D_{10}, D_{25}, D_{50}; D_{75}, and D_{90}) for the FastOs®BG frit are also reported in Table 9. The results indicate a relatively broad particle size distribution with D_{50} ~14 µm. According to Aberg[30], the injectability of the putties is enhanced when a broad particle size distribution of the materials is used.

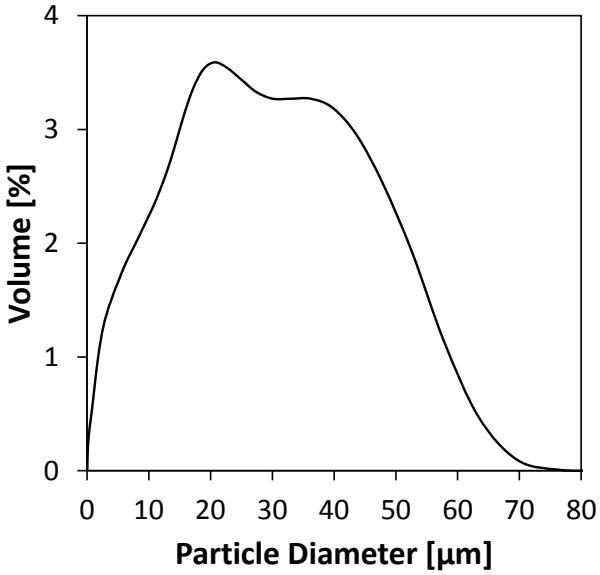

Figure 18 – *Particle size distribution of FastOs®BG.*

Table 9 – Particle size distribution intervals of *FastOs®BG.*

Volume [%]	< 10	< 25	< 50	< 75	< 90	Mean
Particle Diameter [µm]	1.203	4.213	13.72	27.73	42.57	17.92

5.1.3. Monocalcium Phosphate Monohydrate (MCPM)

MCPM is a highly soluble and hygroscopic compound. Therefore, the assessment of its particle size was made by SEM observations. Figure 19 shows the size/morphology of the MCPM particles as received (a) and after the dehydration and milling processes (b). It can be seen (Figure 19 (a)) that the as received powder consists of well faceted and rhombohedral particles, some of them with several hundreds of microns in size. After milling, the MCPM particles show clear evidenced of comminution (smaller and isometric particles, as seen in Figure 19 (b)). The sizes observed in Figure 19 (b) are in fair agreement with the expected ones for a powder passed through a 100 µm mesh.

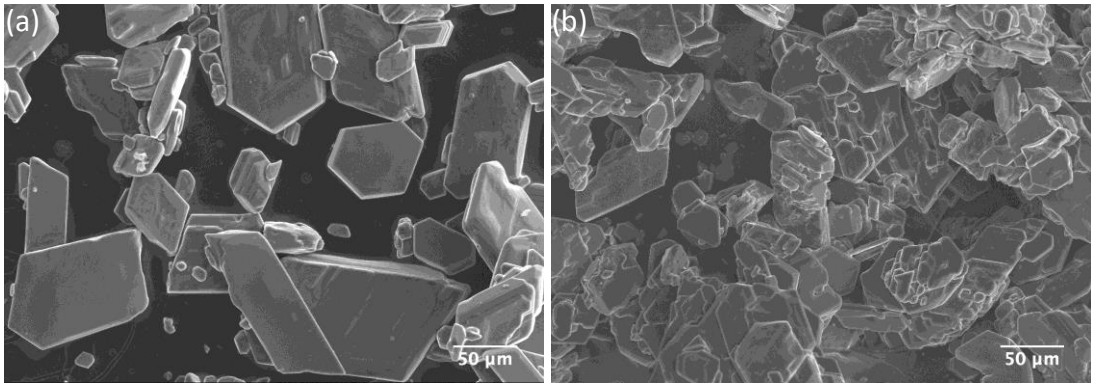

Figure 19 – *(a) SEM image of as received MCPM powders; (b) SEM image of dehydrated and milled MCPM powders (before mixing).*

5.2. Putty Characterization

5.2.1. Injectability

The predefined volume ratios of the powders used in the putty formulation were FastOs®BG : β-TCP : MCPM = 40:30:30 and the total S/L volume ratio was 60/40. Considering the density of each powder and of the Glycerol, the resulting S/L weight ratio is 3.3. These conditions ensured good injectability results as shown in Table 10. The three syringe samples showed that the putty was easily expelled and kept cohesion. The percentages of Injectability (%I) were always > 95%, with a mean of ~96%. These values are excellent considering the relatively high S/L ratio used.

Table 10 – *Injectability percentage Results. SN – Syringe Number; SaN - Sample Number; S – Syringe weight (g); S + P – Syringe + Putty weight (g); S + LO – Syringe + Leftover's weight (g); S + LO – Syringe + Leftover's weight (g); GC – Glass Container weight (g); GC + P – Glass Container + Putty weight (g); EP – Extruded Putty weight (g); TEP – Total Extruded Putty weight (g); P – Putty weight (g); % I - % Injectability; Mean – Mean % Injectability*

SN	SaN	S (g)	S+P (g)	S+LO (g)	GC (g)	GC+P (g)	EP (g)	TEP (g)	P	% I	Mean (%)
1	1	5.29	15.8	5.69	47.1	49.57	2.47	10.13	10.51	96.38	96.38
	2				47.87	52.01	4.14				
	3				29.53	33.05	3.52				
2	4		15.64	5.7	47.37	49.97	2.60	9.91	10.35	95.75	
	5				29.65	33.50	3.85				
	6				47.86	51.32	3.46				
3	7		16.01	5.60	44.62	47.92	3.30	10.41	10.72	97.11	
	8				50.39	53.85	3.46				
	9				43.72	47.37	3.65				

The injectability curves are displayed in Figure 20. They include a first initial increase of the extrusion force up to a kind of roughly constant plateau, and end by a steep increase of the extrusion force when almost all the putty has been expelled.

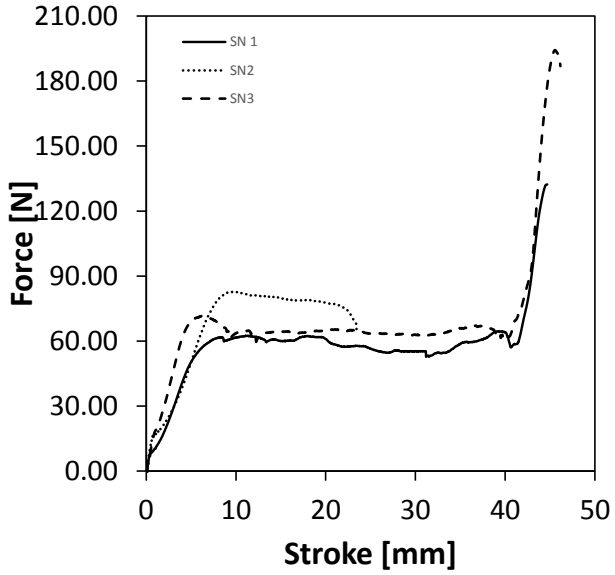

Figure 20 – Injectability curves for SN 1, 2 and 3.

The first initial increase is related to the yield stress of the putty and the gradual breaking down of its internal structure. The plateau means the force required for keeping a constant flow. And finally, the last steep increase is solely due to the exhaustion of the putty in the syringe.

The injectability profiles also show small irregularities, which are attributed to some deficiencies of the syringe filing process. Namely, the vacuum could not be completely granted due to the relatively high viscosity of the putties and some air bubbles became entrapped inside. This explains the shape of the injectability curve 2 (SN 2) in which ~96% of the putty was extruded under an applied maximum force of ~80 N. Therefore, the injectability curves 1 and 3 (SN 1 and 3, Figure 20) seem to be the most representative of the extrusion behaviour of the putties. The easiness of the extrusion process suggests that filter pressing effect is likely to be absent.

5.2.2. Phase and size separation

The injectability performance is evaluated based not only on the percentage of extruded paste but also in terms of its homogeneity along the entire process. Therefore, it is essential analysing the eventual occurrence of solid/liquid phase segregation during injection.[41] If the firstly extruded putty sample contains a higher proportion of liquid than exists in the initial mixture, this is an indication that the FPE has occurred. In order to investigate this, three putty samples from each syringe were collected at different time points along the extrusion, submitted to thermal treatment at 400 °C and the S/L ratio was determined.

Regarding TG results (Figure 21), the total weight loss of the putty was 14% up to 400 °C, followed by a constant plateau with further increasing the temperature up to 500 °C. Therefore, all the collected putty samples were heat treated at 400 °C to determine the S/L ratios. The data reported in Table 11 show that the experimental weight S/L ratio values determined after the heat treatment at 400 °C ranged from 2.76 to 2.94.

These values differ from the theoretically planned S/L weight ratio value of 3.3, assuming that the MCPM was a solid. However, as its name indicates, this compound contains one mole of water per mole of MCPM, which is lost upon heat treating at 400 °C. Moreover, MCPM is a hygroscopic compound and is likely to further hydrate in contact with the atmosphere. This explains why the differences between the planned and the experimental S/L ratio values are higher than expected.

Figure 22 shows the evolution of the S/L over the measurement numbers for each syringe. The results show that the calculated S/L in each measurement number (MN) for each syringe are very similar (and almost the same when rounded to one decimal place) and the S/L in the first MN for each syringe is always higher than the following MN. Having this in mind and observing the S/L curves for each syringe the filter pressing effect is clearly absent.

Table 11 – *Phase and size separation results. SN - Syringe Number; MN – Measurement Number; GC+EP – Glass Container + Extruded Putty weight (g); GC+CP – Glass Container + Calcined Putty weight (g); ΔP – Liquid Phase weight (g); EP – Extruded Putty weight (g); S – Solid Phase weight (g); S/L – Solid to Liquid Ratio; Mean – S/L mean; SD – Standard Deviation; MM – Mean of mean; S/L (T) – Theoretical S/L*

SN	MN	GC+EP (g)	GC+CP (g)	ΔP (g)	EP (g)	S (g)	S/L	Mean	SD	MM
1	1	49.57	48.93	0.64	2.47	1.83	2.86	2.85	0.02	2.86
	2	52.01	50.94	1.07	4.14	3.07	2.87			
	3	33.05	32.13	0.92	3.52	2.6	2.83			
2	1	49.97	49.31	0.66	2.6	1.94	2.94	2.91	0.03	
	2	33.5	32.51	0.99	3.85	2.86	2.89			
	3	51.32	50.43	0.89	3.46	2.57	2.89			
3	1	47.92	47.07	0.85	3.3	2.45	2.88	2.83	0.06	
	2	53.85	52.95	0.9	3.46	2.56	2.84			
	3	47.37	46.4	0.97	3.65	2.68	2.76			

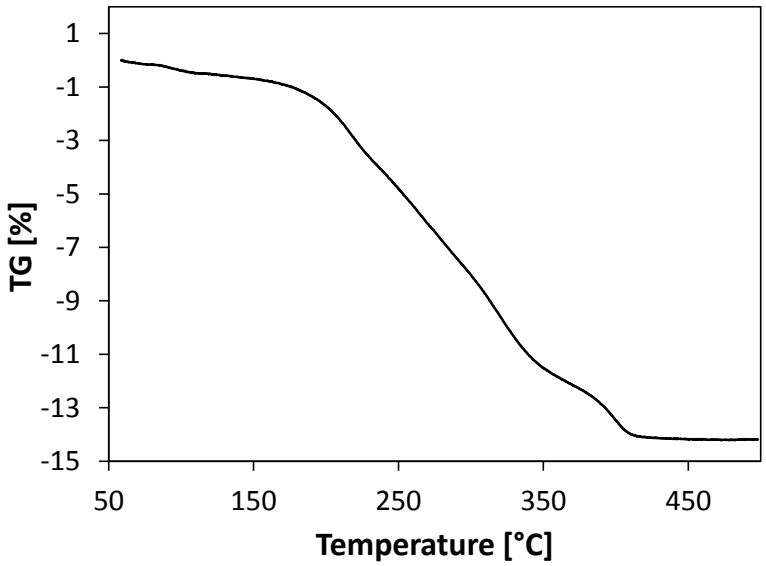

Figure 21 – *TG results of the putty when calcined at 500 °C for 1 h with a heating rate of 2 °C/min.*

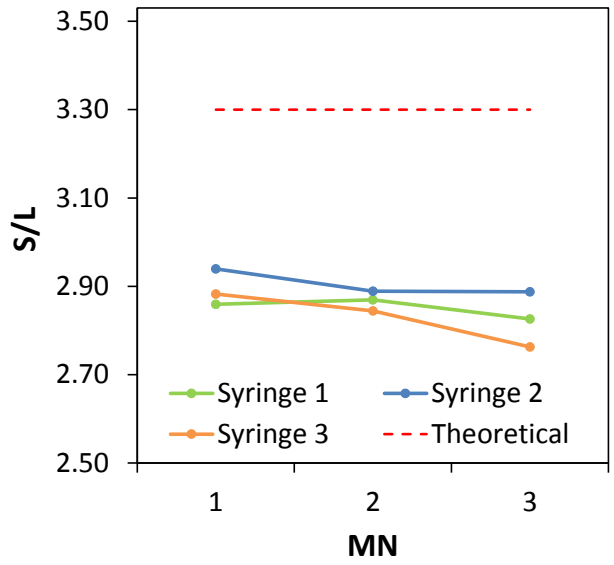

Figure 22 – S/L evolution over the measurement numbers (MN) for each syringe. Theoretical value is indicated by the red dashed line for comparison purposes.

5.2.3. Setting time

Regarding this premixed cement/putty, it is not only the chemistry determining the setting time but also the water-glycerol exchange rate, the dissolution rate of MCPM particles and the strength of the putty after hardening are important factors.[24] These will determine how fast the putty obtains sufficient strength to bear the load of the Gilmore test needle. The setting time results of the putty, when immersed in PBS (pH 7.4) at 37 °C are presented in Figure 23.

In this specific case S/L weight ratio was kept constant (3.3), since the putty formulation was predefined. Measuring the ST of this putty can be tricky since the surfaces of the putty sample set first than the bulk. This is determined by the diffusion rate of water molecules into the bulk of the putty sample.

ST results ranged from 24 to 27 min with a mean of 25.33 min, which are reasonable. However, a target ST would be below 20 min might require the addition of acidic components to the mixture that act as hardening accelerators.[30] Decreasing the particle sizes of the reacting solid components (β-TCP, MCPM) is another alternative approach towards shortening the ST, as reported elsewhere.[30] The resulting higher specific surface areas make reactions to go faster.

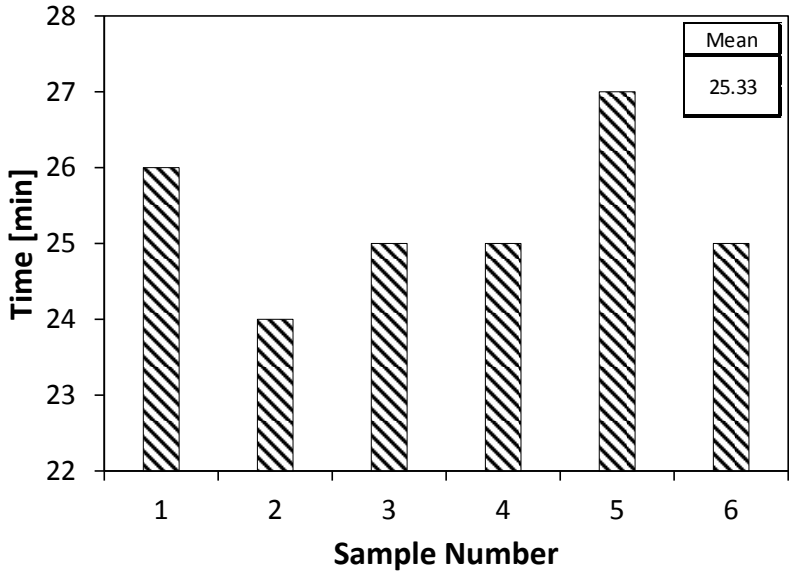

Figure 23 – *Setting time results of the putty when immersed in PBS (pH 7.4) at 37 °C.*

5.2.4. Mechanical Properties

The parameters used to control the mechanical properties of CPC that are also valid for putties are S/L ratio and particle size. Higher S/L gives stronger cement (less liquid in the putty composition) since the porosity is reduced. Controlling the mechanical properties with particle size is more complicated since it will need optimization of the size distribution of all powders used in the formulation.[30]

Figure 24 shows the compressive strength results of the putty after immersion in PBS for 1, 7 and 28 d. It is clear that the CS increases with prolonged time during immersion, especially from 1 to 7 d. The difference between 7 and 28 d is minimal or non-existent, therefore it is possible to conclude that the putty reaches its maximum compressive strength on the 7th day.

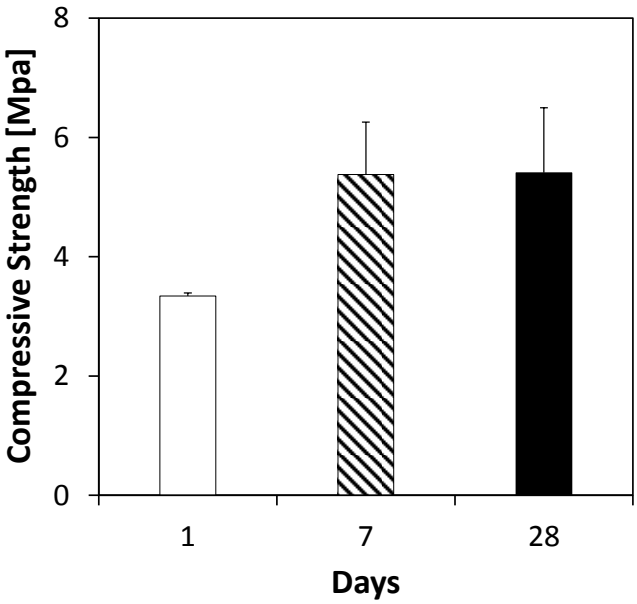

Figure 24 – *Compressive strength results of the putty after immersion in PBS for different time periods.*

5.2.5. Bioactivity and Degradation

The samples were prepared by injecting the putty into several moulds with the same size and format as used before. The filled moulds were immersed for 1, 7 and 28 d in PBS and SBF solutions. The degradation rates were characterized by the weight losses undergone by the samples along the immersion periods. The results are presented in Figures 25 and in Tables 12, 13, 11 and 14. Figure 25 shows that degradation rate in PBS steadily increases with increasing immersion time, oppositely to what happened upon immersing the samples in SBF where an overall decreasing trend was observed.

The lower degradation rate in SBF is not surprising considering that this solution is close to saturation. Moreover, with the gradual dissolution of solid particles the solution is likely to reach a supersaturation degree. Under these conditions an apatite layer tends to precipitate at the surface of the solid components.[4] The deposited surface layer is expected to exert a gradual hindrance towards further dissolution of solid components. This helps explaining the divergence observed in the degradation profiles in PBS and SBF (Figure 25). However, the weight variations in SBF show high deviation values between immersion time intervals, making it difficult to draw firmer conclusions.

Despite the slower degradation rates in SBF, the samples immersed in SBF were much more damaged than the putty samples immersed in PBS.

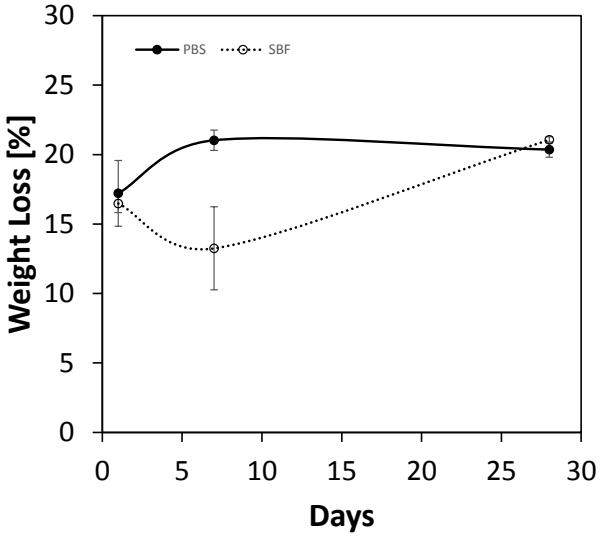

Figure 25 – *Evolution of weight loss of the putty samples upon immersion in PBS and in SBF solution solutions for various periods.*

Table 12 – *Degradation rates after 24 h of putty immersion in PBS and SBF solutions (starting pH = 7.4). T (d) – Time of incubation in days; N – Sample Number; M – Mould weight (g); M + S – Mould + Sample weight (g); W_0 – Dry weight of initial sample; W_T – Dry weight after degradation; D – Weight loss (%); Mean (D) – Mean weight loss (%)*

Medium	T (d)	N	M (g)	M + S (g)	pH	W_0 (g)	W_T (g)	D (%)	Mean (D)
PBS	1	2-1P	0.96	3.16	7.37	2.20	1.86	15.45	17.21
PBS	1	2-2P	0.97	3.12	7.33	2.15	1.80	16.28	
PBS	1	2-3P	0.87	2.88	7.29	2.01	1.61	19.90	
SBF	1	2-1S	0.90	2.91	7.50	2.01	1.69	15.92	16.48
SBF	1	2-2S	0.84	2.74	7.54	1.90	1.59	16.32	
SBF	1	2-3S	0.95	3.16	7.37	2.21	1.83	17.19	

Table 13 – *Degradation rates after 7 days of putty immersion in PBS and SBF solutions. T (h) – Time of incubation in hours; T (d) – Time of incubation in days; N – Sample Number; M – Mould weight (g); M + S – Mould + Sample weight (g); W_0 – Dry weight of initial sample; W_T – Dry weight after degradation; D – Weight loss (%); Mean (D) – Mean weight loss (%)*

Medium	T (d)	N	M (g)	M + S (g)	pH	W_0 (g)	W_T (g)	D (%)	Mean (D)
PBS	7	2-1P	0.84	2.83	7.45	1.99	1.56	21.61	21.03
PBS	7	2-2P	0.87	2.75	7.41	1.88	1.48	21.28	
PBS	7	2-3P	0.88	2.71	7.28	1.83	1.46	20.22	
SBF	7	2-1S	0.9	2.78	7.50	1.88	1.69	10.11	13.25
SBF	7	2-2S	0.84	2.74	7.54	1.90	1.59	16.32	
SBF	7	2-3S	0.95	3.16	7.37	2.21	1.83	17.19	

Table 14 – *Degradation rates after 28 days of putty immersion in PBS and SBF solutions. T (h) – Time of incubation in hours; T (d) – Time of incubation in days; N – Sample Number; M – Mould weight (g); M + S – Mould + Sample weight (g); W_0 – Dry weight of initial sample; W_T – Dry weight after degradation; D – Weight loss (%); Mean (D) – Mean weight loss (%)*

Medium	T (d)	N	M (g)	M+S (s)	pH	W_0 (g)	W_T (g)	D (%)	Mean (D)
PBS	28	2-1P	0.98	3.27	7.32	2.29	1.83	20.09	20.36
PBS	28	2-2P	0.90	2.90	7.37	2.00	1.58	21	
PBS	28	2-3P	0.95	2.95	7.41	2.00	1.60	20	
SBF	28	2-1S	0.88	2.81	7.57	1.93	1.52	21.24	21.06
SBF	28	2-2S	0.90	2.87	7.53	1.97	1.56	20.81	
SBF	28	2-3S	0.90	2.84	7.53	1.94	1.53	21.13	

5.2.6. Structural Analysis

The hardened cements were characterized by different techniques (XRD, FTIR and SEM) to assess their microstructural and morphological features. The smaller fragments of the samples used in the CS tests were submitted to XRD analysis.

The XRD diffractogram of the specimens immersed in PBS for 1, 7 and 28 d are shown in Figure 26. It can be seen that the phase crystalline assemblage gradually changed along the immersion time. β-TCP is the main phase in all the tested samples, but monetite phase was also identified after 1, 7 and 28 d of immersion in PBS.

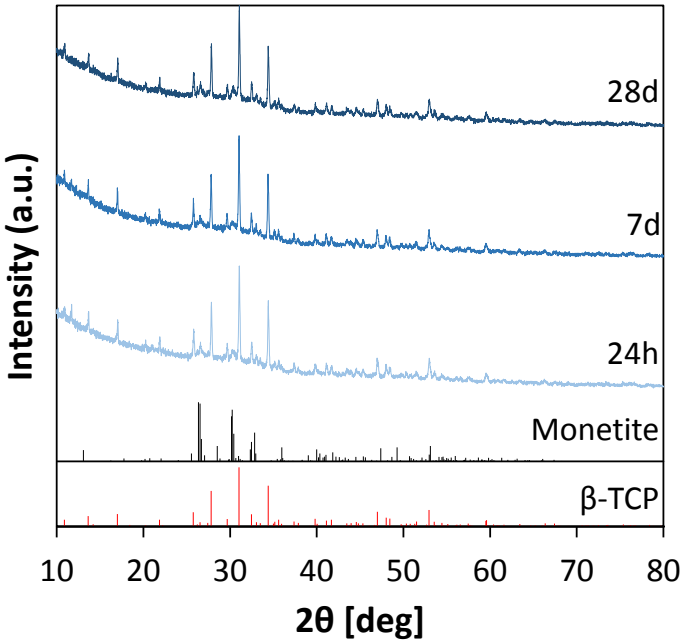

Figure 26 – *XRD of the putty after immersion in PBS for different time periods.*

As mentioned by Aberg[30], there are plenty of experimental evidences reported in literature that monetite is formed when β-TCP and MCPM are prepared as a premixed non-aqueous CPC. This is likely due to the shortage of water molecules upon starting hydration reactions. The monetite formation is also favoured at higher temperatures, being therefore fostered at the body temperature in comparison to the room temperature.[30] Therefore, only monetite was detected as a new formed during the different immersion times, together with the some unreacted β-TCP.

MCPM and FastOs®BG were not expected to be identified by XRD, the first for being highly soluble, and the second one for being amorphous. As seen from the diffractogram, no apatite could be identified in the incubated samples. This might be caused by the presence of calcium pyrophosphate, which has been shown to inhibit apatite crystal formation[42]. To dispel the doubts and to be sure about the formed crystalline phases, the putty samples were also submitted to FTIR analysis.

FTIR spectra of the same samples used for the XRD analysis are presented in Figure 27. From a general point of view, the spectra of all samples are similar, varying only in the number of identified peaks and transmittance [%], the latter increasing with higher immersion time in PBS. Curiously, the sample immersed for 1 day has more identified peaks than the others. Only the main peaks found in all samples are presented. The peaks and the associated functional groups are identified according to the literature.[43–49]

The monetite bands detected and their corresponding assignments are shown in Table 15.

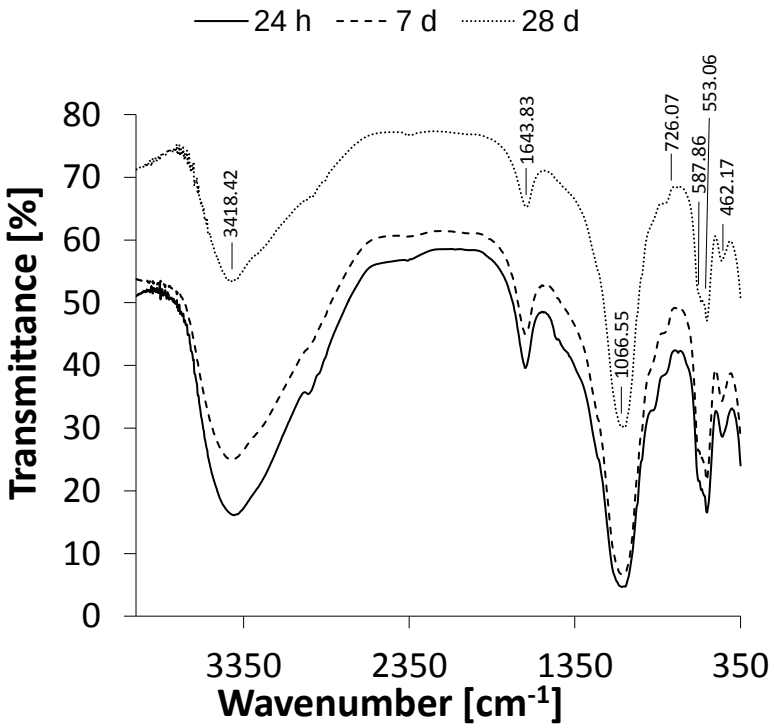

Figure 27 – *FTIR spectra of the putty after immersion in PBS for different time periods.*

Table 15 – *IR monetite wavenumbers and its corresponding assignments (Adapted from Salimi et.al.[45]).*

Monetite Wavenumbers [cm^{-1}]	Assignments
3461	O–H stretching of residual free water
2921	C–H stretching
2852	C–H stretching
1637	H–O–H bending and rotation of residual free water
1419	Carbonate (from atmosphere)
1400	P–O–H in plane (bending)
1130	P–O stretching
1064	P–O stretching
902	P–O(H) stretching
583	O–P–O(H) bending mode
530	O–P–O(H) bending mode

Considering the information available in the literature (Table 15), the bands around 587.86, 1066.55, 1643.83 and 3418.42 cm^{-1} can be associated with monetite.[45] Therefore the FTIR spectra of the putty samples also confirm that monetite has been formed during the immersion period. Due to small available amounts of water within the putty during immersion, the MCPM concentration becomes high, causing lower pH, which leads to more monetite formation. Monetite cements have shown the potential to be osteoconductive, with the amount of bone formation being highly dependent on the site of implantation and the vascular supply, as an adequate blood supply can quicken cement resorption and replacement by new woven bone.[50]

The band at around 462.17 cm^{-1} can be attributed to Si–O–Si bending modes of BG, while the band at around 726.07 cm^{-1} may be due to Si–O–Si symmetric stretching with simultaneous Si cation motions. Further, the band centred at around 553.06 cm^{-1} corresponds to P–O bending modes. In addition to that, the band appearing at 1066.55 cm^{-1}, can also be assigned to Si–O–Si stretching vibrations. These bands indicate the

development of interfacial high-area silica gel layer, as postulated in Hench's inorganic reactions set.[46–49]

The peaks at around 553.06, 587.86 and 1066.55 cm^{-1} are present in all samples and are evidences of the formation of hydroxyapatite and other crystalline phosphate species, as these bands correspond to P–O bending vibrations in a PO_4^{-3} tetrahedron.[43,44,48]

Putty samples after immersion in water, and in PBS were observed by SEM. Figure 28 presents the SEM images of putty samples immersed in water and PBS for 1 d and 28 d.

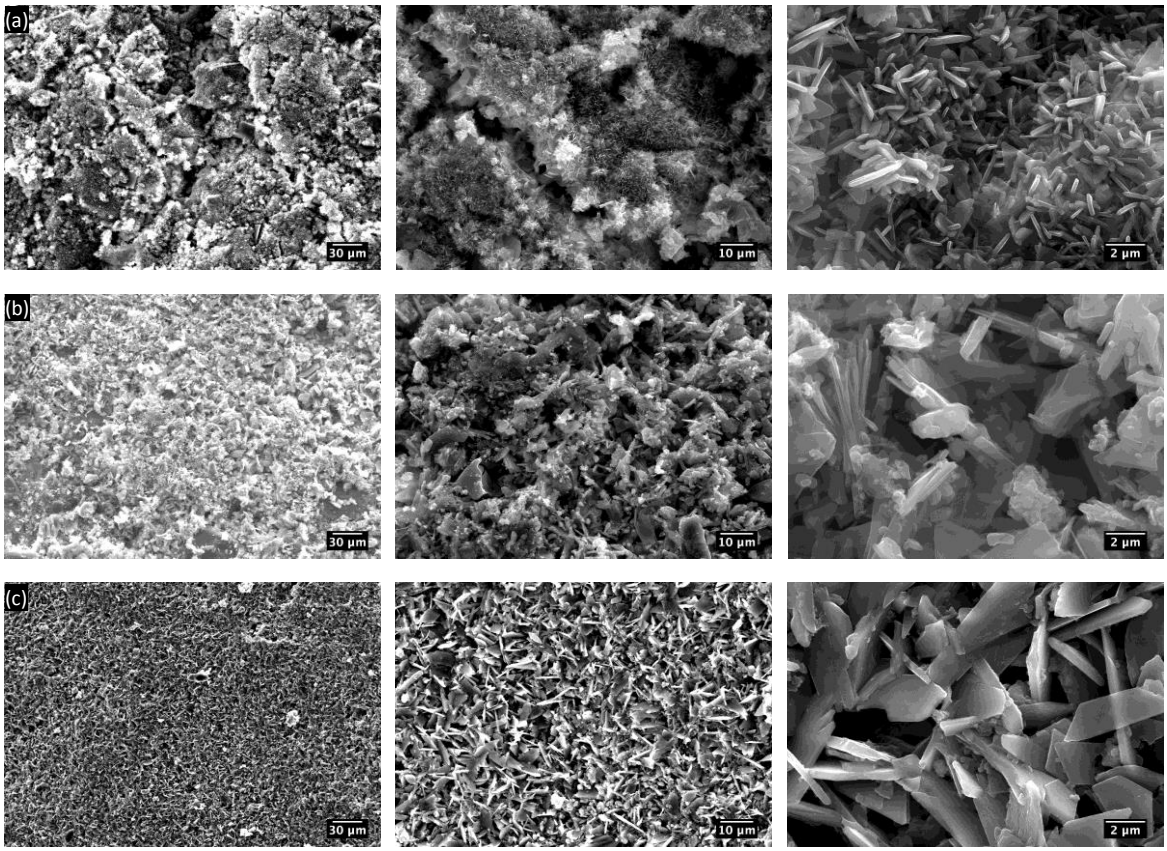

Figure 28 – *(a) SEM images of the putty after 1 d immersion in distilled water; (b) 1d in PBS and (c) 28 d in PBS.*

The aim was to investigate the interaction differences between the putty and the immersion media. FastOs®BG, β-TCP, and MCPM particles could be easily identified in Figure 28. Figure 28 (a) shows the microstructure of the putty after 1 d of immersion in water. In the presence of water the putty sets quickly, due to the presence of MCPM (it quickly dissolves in water). The fluid exchange between putty and water is also faster under these conditions, which led to the formation of an apatite layer well visible on the surface of the hardened cement sample.

Figure 28 (b and c) show the microstructure of the putty samples after 1 d and 28 d of immersion in PBS, respectively. Since PBS has higher ionic concentration, the fluid exchange is slower and therefore the presence of the apatite layer is not so clear. However there is a noticeable evolution of the putty microstructures from 1 d to 28 d, with the latter showing better developed crystals and larger pore sizes.

5.2.7. Shelf life

After 6 weeks of storage at room temperature the putty could not be injected, since it had undergone partial hardening of drying. This might be due to the insufficient dehydration of MCPM. The stoichiometric water carried by MCPM and water vapor from the atmosphere during the preparation process is likely to undertake partial setting reactions. When the putty is stored in prefilled sealed syringes the total amount of water present in the cement will be determinant for the shelf life. It was also found that shelf life can be improved by lowering the temperature. Therefore the syringe filling method and the storage conditions are important for the shelf life. Shelf life needs to be further investigated in order to extend it enough to allow an industrial design. Similar findings have been reported by Gbureck.[28]

5.3. Legislation of Medical Devices

In accordance with Rule 8 of Annex IX to Directive 93/42/EEC:

“All implantable devices and long-term surgically invasive devices are in Class IIb unless they are intended:

— To be placed in the teeth, in which case they are in Class IIa,

— To be used in direct contact with the heart, the central circulatory system or the central nervous system, in which case they are in Class III,

— To have a biological effect or to be wholly or mainly absorbed, in which case they are in Class III,

— Or to undergo chemical change in the body, except if the devices are placed in the teeth, or to administer medicines, in which case they are in Class III.’

Considering this, the developed putty is considered a class III MD. In order to meet the essential requirements set out in Annex I of the Directive 93/42/EEC, the clinical evaluation carried out has given rise to the documents annexed in this thesis: Clinical evaluation report (CER) 03_01, Instructions for Use (IFU) 03_01, Datasheet (DS) 03_01 and Product Description (PD) 03_01.

CHAPTER V

CONCLUSION AND PROSPECTS

6. Conclusion and prospects

6.1. Conclusion

Putties constitute a recent innovation in the market of medical devices. The results obtained in this work are encouraging and support the conclusion that the handling is where the ready-to-use injectable bone substitutes have their biggest advantages. A wide use of putties in the clinics would shorten operation times and reduce infection rates to the benefit of both patients and medical staff and at the same time, it would cut the costs for these procedures.

The obtained putty has a set of interesting features, including a relatively high solid/liquid weight ratio of 3.3, mean ST of 25.33 min, ~96% of injectability coupled with the absence of FPE, and a maximum CS of 6 MPa, which is closer to normal mixed cements with the same composition and similar to that of trabecular bone. Therefore, the putty has an excellent handling ability and the strength after hardening competes with that of normal mixed cements. The structural analyses (XRD and FTIR) of the putty after immersion in PBS and water show the formation of monetite in the hardened cements and apatitic layer at the solid/liquid interface (SEM). These findings are indicators that the putty has the potential to be osteoconductive, being appropppriate for bone regeneration.

There are still a few shortcomings related to the following key aspects: setting time and shelf life. Both are interrelated and there are ways to enhance these properties, either by using MCPA instead of MCPM and/or by the storage at lower temperatures. The syringe filling method (under vacuum conditions) also needs to be improved to grant an efficient degassing of the putty. The setting time should desirably be shortened from the current ~25 min to 15-20 min without deteriorating the shelf life (~6 weeks).

The clinical evaluation report, CER_03_01, and other documents, DS_03_01, IFU_03_01 and PD_03_01 are settled. The product name of the developed putty is BonActive®Putty.

6.2. Prospects

In order to cope with the above referred shortcomings, the future work prospects should address the effects of adding acidic compounds or other type of additives to decrease the ST of the putty without shortening its shelf life and the use of MCPA instead of MCPM, in order to verify if the shelf life increases are also planned.

The putty was characterized regarding its performance only in simulated body fluids. Having that in mind, *in vitro* cell culture and *in vivo* studies are the next key steps to push this project forward.

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ANNEX

Clinical Evaluation Report

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BonActive®Putty

Index

0. About this document

Structure of this document is supported on document "MEDDEV. 2.7.1 Rev.3[1]: CLINICAL EVALUATION: A GUIDE FOR MANUFACTURERS AND NOTIFIED BODIES" and Reg4life procedure WP 15 - Clinical Evaluation.

3.1 0.1 Edition History

Edition nº	Reason for this edition	Date
1	First publish	2016/10/28

3.2 0.2 Team Identification

Name	Function	Responsibility	Relevant Training and Experience please see CV attached	Signature
José Maria Ferreira	Administration RDI Representative	Assign team members. CER approval.	BSc in Ceramic and Glass Engineering. PhD on Materials Science and Engineering. 30 years of experience in research and teaching.	
José Maria Ferreira	MD RDI Researcher	Technical characteristics expertise. Provides to all team members, all the design characteristics, specifications, physiochemical properties including energy intensity, deployment methods, critical performance requirements, principles of operation.		
Ana Brito	Coordinator	Coordinates activities of clinical evaluation. Keep CE report and plan updated. Receive information from PMS, Vigilance, Risk Management and determine the need for CER review. Identifies standards and other relevant documents and determine CE procedures.	MSc in Biomedical Engineering. PhD in Biomedical Sciences. 8 years of experience in research.	
Luís Gonçalves	Clinical Researcher	Determine and specify device application, including foreseeable misuse. Identifies Clinical Data and applies CE procedures, including: • Identify and analysis of Pre-Clinical studies ; • Identify Clinical Data; • Appraisal of Clinical Data; • Analysis of relevant Clinical Data. Elaborates CER	BSc in Biomedical Sciences. 2 years of experience in research.	

3.3 0.3 Product

Product Reference	**03**
Product name	**BonActive®Putty**
Generic Medical Device Name	**Synthetic Bone Graft**
MDD 93/42/EEC Ann. IX classification rule	**R8**
Risk classification acc. to Directive 93/42/EEC	**Medical Device class III**

Is the device placed on the market in sterile condition?	**Yes. The product is sterilized using gamma radiation**
Single use device?	**Yes**

1. General details

Device name: BonActive®Putty

Device manufacturer: Reg4life – Regeneration Technology, S.A.

2. Description of the device and its intended application

BonActive®Putty is a synthetic, bioactive and osteoconductive bone graft substitute. It is supplied sterile and is ready-to-use. This medical device consists of a mixture between a solid phase (FastOS®BG, β-TCP and MCPM) and a liquid phase (Glycerol). It is composed solely of elements that exist naturally in normal bone (Ca, P, Na, Si, O).

This medical device can be clinically applied for the regeneration of hard tissues. The device is injectable, fast absorbed and replaced by new bone tissue during the healing process. BonActive®Putty is indicated to be injected into bone voids or gaps to fill and/or augment orthopaedics, oral, dental intraosseous and cranio-maxillofacial defects.

BonActive®Putty is sterilized by gamma irradiation and is available in syringes, in different unit sizes.

For more information please see Product Description (PD 03_01), Datasheet (DS 03_01) and Instructions for Use (IFU 03_01).

4. Intended therapeutic indications and claims

BonActive®Putty is indicated for use as an osseous defect filler for bone regeneration in dentistry, cranio-maxillofacial surgery and orthopaedics. Thus, BonActive®Putty indications for use are:

Dentistry

- Correction of periodontal and furcation defects.
- Socket regeneration – ridge preservation.
- Graft material for bone regeneration to be used during oral implant surgery.

Cranio-maxillofacial surgery

- Bone cavity filling in cranio-maxillofacial area including the jaw.
- Frontal or maxillary sinus obliteration.

Orthopaedics

- Bone cavity filling.

- Bone cavity filling in the extremities and pelvis.
- Bone cavity filling in the treatment of bone tumours.

Instruction For Use (IFU 03_01) describe the correct use and application.

4. Context of the evaluation and choice of clinical data types

In the last years, a large number of commercial ceramic based cements and putties have been introduced as bone graft substitutes[2]. Putties, also called premixed/ready-to-use cements or non-setting pastes, are a particularly strong and recent trend. Its production is less tricky than of cements and their biological response is often better[2]. These bone substitutes are also easier to use because they do not require any mixing or any transfer to an appropriate delivery system. Therefore, the handling of these bone substitutes is its biggest advantage[2].

A wide use of putties in the clinical practice would shorten operation times and reduce infection rates to the benefit of both patients and medical staff and at the same time, it would cut the costs for these procedures[3].

BonActive®Putty is a bone graft substitute with several applications and consists of a mixture between a solid phase (FastOS®BG, β-TCP and MCPM) and a liquid phase (Glycerol).

It can be delivered to the operating room in a prefilled, sealed and ready-to-use syringe. When injected, the putty will be exposed to the body fluid present in the bone, where the non-aqueous liquid (glycerol), which is easily soluble in water, diffuses out from the cement while body fuild (mainly composed by water) diffuses into the cement causing it to set [3].

Table 1 describes the compositions (in vol. %) of BonActive®Putty and its commercial equivalent medical device, NovaBone Putty ®.

Table 1 – Solid and liquid phases composition and the S/L ratio in BonActive®Putty and in its commercial equivalent medical device Novabone Putty®.

	Solid Phase				Liquid Phase		S/L ratio (vol. %)
	45S5 Bioglass®	FastOs®BG	β-TCP	MCPM	Gly	PEG	
Novabone® Putty	100 %	0 %	0 %	0 %	60 %	40 %	70/30
BonActive® Putty	0 %	40 %	30 %	30 %	100 %	0 %	60/40

According to Table 1, BonActive®Putty is equivalent to the commercialized putty. The changes in composition are related to:

- Components, namely :
  - Different bioactive glass;
  - Absence of PEG in the liquid phase;
  - Higher Glycerol concentration;
  - β-TCP addition;
  - MCPM addition;
  - Different S/L ratio.

Differences in composition of the bioactive glasses are displayed in Table 2.

Table 2 – Comparison between FastOs®BG and 45S5 Bioglass® (in mol %).

Bioactive glass	SiO_2	P_2O_5	CaO	Na_2O	MgO	CaF_2
45S5 Bioglass®	46.10	2.60	26.90	24.40	0.00	0.00
FastOs®BG	38.49	5.61	36.07	0.00	19.24	0.59

Considering Table 2, FastOs®BG has some compositional differences when compared with 45S5 Bioglass®. These changes are related to: MgO and CaF_2 addition, raise in CaO and P_2O_5 concentration, reduction in SiO_2 and absence of Na_2O. All these changes were introduced to overcome some shortcomings of 45S5 Bioglass®. For example, high amounts of Na_2O make the glass surface highly reactive in physiological environments, leading to fast degradation rates not matching the new bone ingrowth, loosening of the bonding strength to living tissues and some cytotoxicity effects[4–7].

The incorporation MgO and CaF_2 of in the constitution of bioactive glasses is based on a biological and medical explanation. Regarding magnesium (Mg) this is one of the most important mineral elements in the bone matrix, and more than half of the total physiological magnesium are stored in bone tissues[8]. A relevant role of Mg in the development of bone tissue is related to adhesion and growth of osteoblasts[8]. Therefore Mg have a key role in bone tissue development, and the

ability of MgO to modify the physical, thermal, and mechanical properties of silicate systems, stimulated the incorporation of Mg in the bioactive glasses compositions[8].Regarding fluoride, in dentistry it is well known that this component prevent dental cavities by inhibiting enamel and dentine demineralisation, enhancement of remineralisation and inhibition of bacterial enzymes[9]. Furthermore, an important factor in this context is the formation of fluorapatite in physiological solutions, as fluorapatite is more acid resistant than carbonated hydroxyapatite, the main component of enamel and dentine. In orthopaedics fluoride is also known to increase bone density and despite a narrow therapeutic window and some dispute on its effectiveness in preventing bone fractures, fluoride-releasing implants might be of interest for patients suffering from osteoporosis[9]. Thus, osteoblasts exposed to the dissolution products of bioactive glasses that contain fluoride or early doses of sodium fluoride showed increased alkaline phosphatase activity, a bone mineralization marker, suggesting that fluoride can target osteoblast differentiation[9]. Regarding CaO and P_2O_5, although they are present in FastOs®BG in a larger percentage than in equivalent medical devices, as can be calculated from Table 2, FastOs®BG CaO/P_2O_5 ratio is 6.43, i.e., it is smaller than 45S5 Bioglass® (10.34) ratio. Relatively high CaO/P_2O_5 ratio make the glass surface highly reactive in physiological environments, , leading to fast degradation rates not matching the new bone ingrowth, loosening of the bonding strength to living tissues[6,7]. Thus, the FastOs®BG CaO/P_2O_5 may offer advantages in relation to the equivalent medical device. In relation to SiO_2, some studies show that the critical feature for bioactivity is a SiO_2 content < 60% in weight[10]. Thus, the bioactivity of FastOs®BG is assured since its SiO_2 content in weight is 38.49 (Table 2).

In vitro and *in vivo* tests were performed on FastOs®BG bioactive glass using 45S5 Bioglass® as a control. *In vitro*, in a study performed by in human mesenchymal stem cells (hMSC), there was no difference between the effect that 45S5® Bioglass and FastOs®BG have on cell proliferation. *In vivo*, a recent study shown that bone remodelling process for FastOs®BG is safe and presents faster bone regeneration than 45S5 Bioglass®[11]. Due to its composition, mechanism of action and intended use, FastOs®BG is equivalent to 45S5 Bioglass®.

Regarding β-TCP and MCPM, they are also already widely used in clinical practice. In biomedicine, β-TCP is widely used in calcium orthophosphate bone cements and other types of bone substitution bioceramics. Dental applications of β-TCP are also known, for instance, pure β-TCP is added to some brands of toothpaste as a gentle polishing agent [12]. β-TCP first contributes to the core of the calcification in the bone defect and then promotes osteogenesis around β-TCP, after which the β-TCP is resorbed and replaced by bone. Resorption only occurs by a fluid contributed process, which can be justified by the absence of phagocytic cells. This occurs due to acute resorption when the blood flow attaches to the surface of the β-TCP and is then surrounded by bony granulation tissue. A slow resorption of bone probably occurs afterwards by cells which have a phagocytic capability [13].

On the other hand MCPM is also used in biomedicine as a component of several self-hardening calcium orthophosphate cements and it is commonly added to several tooth pastes [12].

Due to its composition, mechanism of action and intented use, BonActive®Putty is equivalent to to NovaBone Putty®. Therefore, no issues regarding safety or effectiveness are raised by the changes in composition between BonActive®Putty and its commercial equivalent NovaBone®Putty.

6. Summary of the clinical data and appraisal

A bibliographic research was conducted by the clinical Researcher in PubMed in order to identify clinical studies performed using bioactive glass putties in different situations. The following strategy was used in the research: putty, bioglass putty, NovaBone®, or NovaBonePutty®, or 45S5 putty, or dental putty. NovaBone®, structurally corresponds to 45S5 Bioglass®.

Pubmed was chosen because is a search engine accessing primarily the MEDLINE database of references and abstracts on life sciences and biomedical topics. It includes bibliographic information for articles from academic journals covering medicine, nursing, pharmacy, dentistry, veterinary medicine, and health care.

On the other hand, NovaBone Putty® was chosen because it is substantially equivalent to BonActivePutty®, since both products are injectable putties with of bioactive glasses e«in their composition (Table 1) and are intended to be used as bone cavity filling materials .

The clinical studies performed feature the following aspects:

• Medical specialties in which the medical devices were applied and clinical conditions for their use;

• Methodological features of the studies;

• Evaluated medical devices.

The selection process of publication, as well as the number of publications included and excluded from this report are presented in Figure 1.

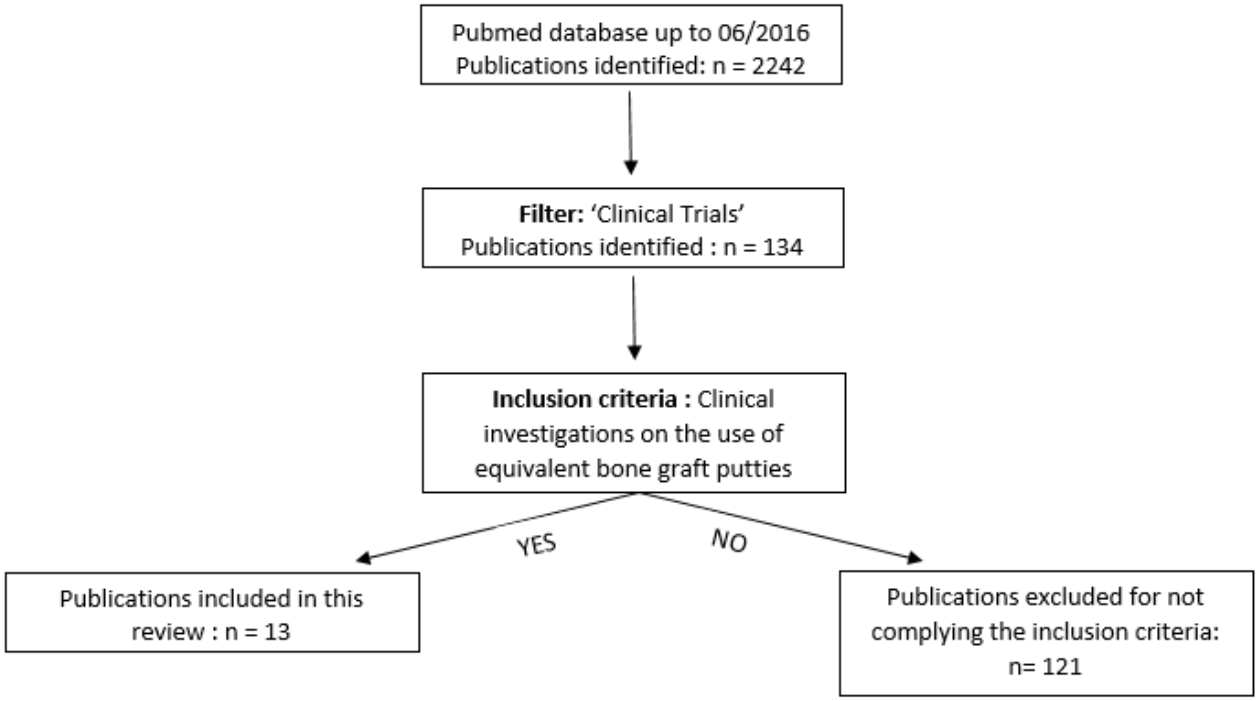

Figure 1 - Identification of publications relating to clinical investigations on the use of putties as bone graft substitutes using the search strategy: putty, bioglass putty, or NovaBone®, or NovaBone Putty®, or 45S5 Bioglass® putty, or dental putty. NovaBone® it structurally corresponds to 45S5 Bioglass®.

The 13 articles selected from PubMed were subsequently subjected to a criteria analysis for suitability according to Table D1 (in the annex).

According to our internal criteria, all the items that were considered "other device" (D3), with "major deviation" on device application (A3), to consider "different population" group (P3) or with "insufficient information" (R3) were considered unsuitable.

This analysis is present in Table 3.

Table 3: Publication suitability evaluation

Article	Appropriate device	Appropriate device application	Appropriate patient group	Acceptable report / data collation	Suitability
Evaluation of the efficacy of a bioactive synthetic graft material in the treatment of intrabony periodontal defect[14]	D2	A1	P1	R2	YES
Implants placed simultaneously with lateral window sinus augmentation using a putty alloplastic bone substitute for increased primary implant stability: A retrospective study[15]	D2	A1	P2	R1	YES
Socket preservation with alloplast: Discussion and a descriptive case[16]	D2	A1	P2	R3	NO
A randomized, blinded, controlled clinical study of particulate anorganic bovine bone mineral and calcium phosphosilicate putty bone substitutes for socket preservation[17]	D2	A1	P1	R1	YES
Human histologic evaluation of the use of the dental putty for bone formation in the maxillary sinus: Case series[18]	D2	A1	P1	R1	YES
Clinical and cone beam computed tomography comparison of NovaBone Dental Putty and PerioGlas in the treatment of mandibular Class II furcations[19]	D2	A1	P2	R1	YES
Comparative Evaluation of Coronally Advanced Flap with and without	D2	A2	P1	R1	YES

Bioactive Glass Putty in the Management of Gingival Recession Defects: A Randomized Controlled Clinical Trial[20]					
Socket Grafting with Calcium Phosphosilicate Alloplast Putty: A Histomorphometric Evaluation[21]	D2	A1	P1	R1	YES
Periotest Values of Implants Placed in Sockets Augmented with Calcium Phosphosilicate Putty Graft: A comparative Analysis against Implants Placed in Naturally Healed Sockets[22]	D2	A1	P1	R1	YES
Treatment of high-energy tibial shaft fractures with internal fixation and early prophylactic NovaBone grafting[23]	D3	A1	P1	R1	NO
Clinical evaluation of 262 oseeointegrated implants placed in sites grafted with calcium phosphosilicate putty: a retrospective sudy[24]	D2	A1	P1	R1	YES
Alveolar Ridge Preservation With the Socket-Plug Technique Utilizing an Alloplastic Putty Bone Substitute of a Particulate Xenograft[25]	D2	A1	P1	R1	YES
Histomorphometric Evaluation of a Calcium-Phosphosilicate Putty Bone Substitute in Extraction Sockets[26]	D1	A1	P1	R1	YES

Thus, according to Table 3, 2 articles from the initial 13 were considered unsuitable for this study. Subsequently, the data contribution of each of 11 articles selected from Table 3 was evaluated according to Table D2 (in the annex). This analysis is present in Table 4.

According to our internal criteria, when the design of the study was considered inappropriate (T2), the outcome measures reported did not reflect the intended performance of de device (O2), the follow-up was considered inappropriate (F2), when it was not provided statistical analysis or the same was inadequate (S2) or the magnitude of the treatment effect observed was clinically insignificant (C2) it was considered that the data did not contribute to the study.

Table 4: Data contribution analysis

Article	Data source type	Outcome measures	Follow up	Statistical significance	Clinical significance	Data Contribution
Evaluation of the efficacy of a bioactive synthetic graft material in the treatment of intrabony periodontal defect[14]	T1	O1	F1	S1	C1	YES
Implants placed simultaneously with lateral window sinus augmentation using a putty alloplastic bone substitute for increased primary implant stability: A retrospective study[15]	T1	O1	F1	S1	C1	YES
A randomized, blinded, controlled clinical study of particulate anorganic bovine bone mineral and calcium phosphosilicate putty bone substitutes for socket preservation[17]	T1	O1	F1	S1	C1	YES
Human histologic evaluation of the use of the dental putty for bone formation in the maxillary sinus: Case series[18]	T1	O1	F1	S3	C1	NO
Clinical and cone beam computed tomography comparison of NovaBone Dental Putty and PerioGlas in the treatment of mandibular Class II furcations[19]	T1	O1	F1	S1	C1	YES
Comparative Evaluation of Coronally Advanced Flap with and without Bioactive Glass Putty in the Management of Gingival Recession Defects: A Randomized Controlled Clinical Trial[20]	T1	O1	F1	S1	C1	YES
Socket Grafting with Calcium Phosphosilicate Alloplast Putty: A Histomorphometric Evaluation[21]	T1	O2	F1	S3	C1	NO
Periotest Values of Implants Placed in Sockets Augmented with Calcium Phosphosilicate Putty Graft: A comparative Analysis against Implants Placed in Naturally Healed Sockets[22]	T1	O1	F1	S1	C1	YES
Clinical evaluation of 262 oseeointegrated implants placed in sites grafted with calcium phosphosilicate putty: a retrospective sudy[24]	T1	O1	F1	S1	C1	YES
Alveolar Ridge Preservation With the Socket-Plug Technique Utilizing an Alloplastic Putty Bone Substitute of a Particulate Xenograft[25]	T1	O1	F1	S1	C1	YES
Histomorphometric Evaluation of a Calcium-Phosphosilicate Putty Bone Substitute in Extraction Sockets[26]	T1	O1	F1	S1	C1	YES

According to the previous analysis (Table 4) 9 articles were selected to contribute significantly to this study.

7. Data analysis

The selected studies studies[14,15,17,19,20,22,24–26], resulting from previous analysis will be described below, according to performance (6.1) and safety (6.2). Subsequently, a performance and safety analysis summary is present in Table 5. According to these reports, NovaBonePutty®is used in the following medical specialties: dentistry, cranio-maxillofacial surgery and orthopaedics.

7.1 Performance

A brief description of the results obtained in the selected studies[14,15,17,19,20,22,24–26], and its clinical significance regarding the performance is present at this point. These studies were selected as those that most contribute to the clinical evaluation of BonActive®Putty and are analyzed in chronological order.

The first clinical research study that relates the treatment of intrabony periodontal defects using a commercially available bioactive glass putty (NovaBone Dental Putty®), was published in 2013[14]. The purpose of this study was to evaluate the efficacy of a bioactive synthetic graft material (NovaBone Dental Putty®) in the treatment of intrabony periodontal defects. Mean radiographic defect fill, with the putty, of 64.76% (2.49 ± 0.5 mm) was observed in 6 months, which was statistically significant. A statistically significant relative attachment level gain of 2.71 ± 1.13 mm and probing pocket depth reduction of 4.21 ± 1.18 mm was recorded at the end of the study. A significant decrease in mobility and gingival index was observed. The clinical results of the use of bioactive glass putty shows that this biomaterial is an efficacious treatment option for the reconstruction of intrabony periodontal defects [14].

In 2014, Kotsakis and co-workers published several clinical studies using NovaBone Dental Putty® for socket extraction or preservation and sinus augmentation[15,17,25,26].

In the first study, the clinical efficacy of an anorganic bovine bone graft particulate (BOV) and a calcium phosphosilicate putty alloplast (PUT) (NovaBone Dental Putty®), for socket preservation, was compared[17]. Postgrafting radiograph revealed adequate bone fill in all sockets of the tested groups. Both test groups had statistically significant reduction in mean ridge width (BOV: 1.39 ± 0.57 mm; PUT: 1.26 ± 0.41 mm) in comparison to the control group (no graft or suturing) (2.53 ± 0.59 mm). No statistically significant difference was identified between the test groups. The maximum implant insertion torque (MIT) for PUT was ≤ 35 N/cm^2 (MIT grade 4) for seven of the nine implants. MIT values in the BOV group ranged from grade 1 (10 to 19 N/cm^2) to grade 4, which was statistically significantly lower than for the PUT group. The overall implant success rate was 94.1% and no implants were lost in the PUT group (failure of one implant in the BOV group).

According to the authors, both bone substitutes can be recommended for alveolar ridge preservation following extraction. The calcium phosphosilicate putty might be more suitable for achieving primary stability for implants placed at 5 to 6 months postextraction[17].

Later, in another study, was evaluated the bone regeneration in 24 sockets grafted with a calcium phosphosilicate putty (NovaBone Dental Putty®). Radiographic analysis during the postextraction healing period (5 to 6 months) showed radiopaque tissue in all sockets. Histomorphometric analysis revealed a mean vital bone content of 31.76% (± 14.20%) and residual graft content of 11.47% (± 8.99%) after a mean healing period of 5.7 months. The high percentage of vital bone in the healed sites in combination with its timely absorption rate suggest that NovaBone Dental Putty® can be a reliable choice for osseous regeneration in extraction sockets[26].

The same authors published another clinical article[22], whose aim was to measure the implant stability using periotest values of implants placed in sockets augmented with calcium phosphosilicate putty (NovaBone Dental Putty®) as compared with implant stability in naturally healed sockets. Periotest values were significantly lower in the grafted group, indicating a better implant stability in sites grafted with NovaBone Dental Putty®. The results suggests that socket grafting with this putty may enhance the quality of available bone for implantation[22].

In a retrospective clinical study[15], Kotsakis *et al.* evaluated the primary stability of implants (5 mm of bone height were included as controls and NovaBone Dental Putty®) placed in significantly pneumatized maxillary sinuses with minimum residual bone height. A total of 30 (15 controls and 15 NovaBone Dental Putty®) implants were inserted with a maximum insertion torque number ›20 N/cm^2. Logistic regression analysis failed to show any association between residual bone height and primary implant stability. The diminished preoperative vertical dimensions of the residual ridges did not seem to negatively influence the osseointegration of implants placed in this study[15]. Therefore the plenitude of human clinical studies have verified the osseous regenerative potential of this putty[15].

Also in 2014, a bioglass particulate, PerioGlas®, was compared to Novabone Dental Putty® in the treatment of mandibular Class II furcations[19]. There was no significance between group differences in clinical parameters and defect size at the baseline. After 6 months, PerioGlas® showed a mean resolution of 50.48 ± 16.47% and 51.11 ± 9.48%, respectively for vertical defect and horizontal defect while the putty form showed a mean resolution of 43.48 ± 9.33% and 42.88 ± 11.09%, respectively. Mean resolution in furcation width was 40.15 ± 13.00% for particulate form as compared with 36.27 ± 11.41% in putty form. Statistically, there was no significant differences between the two groups except for the horizontal defect fill where PerioGlas® showed statistically significant better results. These results demonstrated that the use of both the forms of bioactive glass, that is putty and particulate, effectively regenerated Class II furcation defects with an uneventful healing of the sites[19].

More recently, in 2015, *Babbush & Kanawati* clinically evaluated 262 osseointegrated implants in sites grafted with a calcium phosphosilicate putty (Novabone Dental Putty®)[24]. The aim of this study was to report the clinical efficacy of calcium phosphosilicate (CPS) putty in a wide variety of indications related to implant reconstruction and to report the survival rate of implants placed in these grafted sites. Treatments were categorized into following groups: extraction graft, extraction with immediate implant placement, all-on-four concept, peri-implantitis treatment, bone augmentation before implant placement, implant replacement graft, and grafting around implant placed in resorbed ridges. Four implants for a total of 266 grafted sites were diagnosed with peri-implantitis and were treated as with peri-implantitis treatment. The implant success rate at 1 year was 98.1%. The use of this putty as a bone graft can simplify bone-grafting procedures and reduce intraoperative time in various grafting indications with efficacy and very high survival rates[24].

Also in 2015, *Kotsakis el al.*, histologically evaluated and compare bone regeneration in extraction sockets grafted with either a putty alloplastic bone substitute (Novabone Dental Putty®) or particulate anorganic bovine xenograft utilizing the socket-plug technique[25]. A bone core was obtained during the implant procedure from each site and was used for histologic analysis. Histomorphometry revealed that residual graft values were significantly higher in the bovine xenograft group (25.60% 6 5.89%) compared to Novabone Dental Putty® (17.40% 6 9.39%) ($p<0.05$). The amount of new bone regenerated was also statistically significant higher in Novabone Dental Putty® group (47.15% 6 8.5%) as compared to the xenograft group (22.2% 6 3.5%) ($P<0.05$). The results suggest that ridge preservation using Novabone Dental Putty® demonstrates more timely graft substitution and increased bone regeneration when compared to an anorganic bovine bone xenograft[25].

The latest clinical study was published in 2016[20]. The authors clinically evaluated the outcomes of Coronally Advanced Flap (CAF) with and without NovaBone® bioactive glass putty, in terms of root coverage, gains of keratinized tissue height and root coverage aesthetic score in multiple gingival recession (GR) defects[20]. Six months post-surgery all clinical parameters showed significant reductions. Gingival recession showed significant reduction both in test and control groups (2.0 ± 0.47 mm and 2.3 ± 0.48 mm, respectively; $p<0.05$) with no intergroup difference. The exposed root was covered by 72% (test) and 79% (control). Clinical attachment level (CAL) gain was also significant in both groups (test: 2.7 ± 0.67 mm; control: 2.8 ± 0.78 mm; $p<0.05$) with no intergroup difference. Keratinized tissue height (KTH) gain was significant in both the groups (test group: 1.2 ± 0.42 mm; control group: 0.9 ± 0.57 mm) with no intergroup difference. Also, the root coverage esthetic score (RES) was significant for both the test and control groups (7.2 ± 2.78 and 7.7 ± 1.41 respectively) with no intergroup differences. In isolated Class I/II GR defects, CAF associated with bioactive glass putty provided no significant difference in root coverage, CAL, KTH or RES compared to CAF alone. However, statistically significant gains were seen in all the parameters in both groups as compared to baseline[20].

7.2 Safety

The safety of each of the selected articles [14,15,17,19,20,22,24–26] is then individually analyzed.

Grover *et al.* [14] studied the efficacy of Novabone Dental Putty® in twelve systemically healthy subjects (fourteen intrabony defects), 8 men and 4 women, having moderate to severe chronic periodontitis. Patients were evaluated after bone grafting with the Novabone Dental Putty® for a period of 6 months. Clinical and radiographic evaluations were made at baseline, and 3 and 6 months following surgery. All the treated sites resulted in uneventful healing. No complications such as allergic reaction, abscesses, or infections were observed throughout the study period, in any of the patients. Safety characteristics and intended purpose of the device does not require specific training in addition to the surgical doctor training.

In their 2014 study, Kotsakis and colleagues[17] extracted thirty teeth from 24 patients (17 men, 9 women). In order to compare the clinical efficacy of an anorganic bovine bone graft particulate to Novabone Dental Putty® for socket preservation, The sockets were debrided and received anorganic bovine bone mineral (BOV, n=12), calcium phosphosilicate putty (PUT, n=12), or no graft (CTRL, n=6). The sockets were assessed clinically and radiographically 5 months later. Eight sockets in the BOV group and nine in the PUT group received implants 5 to 6 months postgrafting. The maximum implant insertion torque (MIT) was measured as an index of primary implant stability. The data were analyzed with the Mann-Whitney test. Intraoral clinical examination revealed healthy peri-implant mucosa without clinical signs of inflammation of the peri-implant tissues. All osseointegrated implants functioned well during the follow-up period, for a cumulative postloading success rate of 100%. The authors did not report the occurrence of any side effects arising from the use of Novabone Dental Putty®. Safety characteristics and intended purpose of the medical device does not require specific training in addition to surgical training[17].

Later on, Kotsakis *et al.*[26] have made a histomorphometric evaluation of Novabone Dental Putty® in extraction sockets in twenty-four patients (11 men and 13 women). Patients were scheduled for tooth extraction and ridge preservation. Pre and immediate postoperative radiographs were obtained. Post-operative evaluations were performed at 7 and 14 days and at 4 weeks to assess wound healing and record any adverse events. Following 5 to 6 months of healing, surgical re-entry was performed on patients that decided to proceed with implant placement at the healed sites to clinically evaluate the regenerated bone in the original defect and to retrieve a bone biopsy specimen. All 24 treated sites healed without complications. No incidence of alveolar osteitis was reported in any of the treated sockets. A limitation of this study is the multicenter design. Even though a calibration session was provided to minimize any clinician related variations, the relatively high standard deviation values noted in new bone formation may be attributed to the multiple operators located in different settings. The authors didn't report any complications related with putty utilization. In addition to surgical training, no special training by the user doctor was necessary[26].

In Shukla *et al.* 2014 study[22], twenty two sockets were implanted with Novabone Dental Putty® immediately after extraction. The sockets were re-entered after a healing period at 5 to 6 months (average of 5.3 months) for implant placement. Periotest values were recorded during implant insertion to assess primary stability. These were compared with the Periotest values of 26 implants placed in 22 patients, with naturally healed sockets. Safety characteristics and intended purpose of the device does not require specific training in addition to the surgical doctor training.

In another study, Kotsakis *et al.*[15] also evaluated the primary stability of implants, using Novabone Dental Putty®, in seventeen patients with a median age of 51 years old. All patients contributed with at least 1 direct sinus lift procedure. After implant loading, patients were clinically evaluated after 6 months and then followed up on an individualized basis, which included clinical examinations at least biannually. No patients experienced any complications associated with the sinus surgery or implant placement[15]. Safety characteristics and intended purpose of the device does not require specific training in addition to the surgical doctor training.

The Asmita *et al.* study[19] included 28 patients with 40 Class II furcation defects, that were treated with Novabone Dental Putty® and PerioGlas® particulate. Measurement of defects was done using clinical and cone beam computed tomography (CBCT) methods. The patients were followed-up at 6 months. Intergroup comparisons were done using Mann- Whitney U-test[19] All the patients were compliant and healing was uneventful for both groups. Safety characteristics and intended purpose of the device does not require specific training in addition to the surgical doctor training.

In their retrospective study, *Babbush & Kanawati* [24] reported the efficacy of Novabone Dental Putty® in several treatments: extraction graft, extraction with immediate implant placement, all-on-four concept, peri-implantitis treatment, bone augmentation before implant placement, implant replacement graft, and grafting around implant placed in resorbed ridges. 65 patients were included in this study (36 men, 29 women) with a mean age of 63 ± 12 years. In total, 262 implants were placed. Four implants were diagnosed with peri-implantitis and were treated as, for a total of 266 grafted sites. Two implants from the extraction graft category and 3 implants from the all-on- four group were lost and replaced with successfully osseointegrated implants during a mean study follow-up period of 12.24 6 2.32 months[24]. Safety characteristics and intended purpose of the device does not require specific training in addition to the surgical doctor training.

In their 2015 pilot study, Mahesh *et al.* [25] histologically evaluated and compared the quality of bone formation in extraction sockets following implantation with either Novabone Dental Putty® or Bio-Oss® in nineteen patients. All patients underwent 20 tooth extractions and ridge preservation following a standardized protocol. Patients were recalled after 4–6 months to evaluate the bone regeneration and to proceed with implant placement. A bone core was obtained during the implant procedure from each site and was used for histologic analysis[25]. Safety characteristics and intended purpose of the device does not require specific training in addition to the surgical doctor training.

Latter, in 2016, Bansal *et al.*[20] included in their ten healthy patients (age range 18-45 years) with multiple bilateral (n=40: test 20, control 20) and comparable Miller's Class I or Class II gingival recession defects. The defects were randomly assigned by a computer-generated list to either test (Coronally Advanced Flap + Novabone Dental Putty®) or control (Coronally Advanced Flap alone) groups. Clinical parameters included gingival recession (GR), probing pocket depth (PPD), clinical attachment level (CAL), keratinized tissue height (KTH) and root coverage esthetic score (RES) evaluated at baseline and at 6 months post-surgery CAF with or without bioactive glass putty[20]. Safety characteristics and intended purpose of the device does not require specific training in addition to the surgical doctor training.

7.3 6.2.1 Surveillance safety survey

On October 2016, a survey was carried out on all alerts published during the last 20 years on the FDA MAUDE website. A search was performed using NovaBone Putty® as a term.

Four occurrences concerning NovaBone Putty® were reported:

https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfmaude/detail.cfm?mdrfoi__id=4766623 [27]

https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfmaude/detail.cfm?mdrfoi__id=4766615 [28]

https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfmaude/detail.cfm?mdrfoi__id=4763070 [29]

https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfmaude/detail.cfm?mdrfoi__id=4763064 [30]

In two of the cases referred above [28,30] two patients have experienced a staph infection after surgery to implant pioneer surgical devices as well as NovaBone Putty®. Pioneer surgical has submitted medical device reporting related to these two cases. Both patients contracted staph infections. Site irrigation was performed on each and both patients were placed on antibiotics. Both patients were released after 3 days from the hospital. In each of the other two occurrences [27,29] one patient was admitted with infection after surgery. Surgery was anterior cervical discectomy and fusion backup an up later with metal from Biomet. These complications are directly related to the surgical procedure and not to NovaBone Putty®.

For all these events, the manufacture found that manufacturing, sealing and sterility records verify product was manufactured according to specifications with no deviations or non-conformances associated with the lot.

On October 2016, a search on the MHRA and Infarmed websites was performed, using NovaBone Putty® as a term. No complications were found related to the use of NovaBone Putty® in MHRA and Infarmed databases.

7.4 6.3 Product Literature and Instructions for Use

In addition to this report are available:

- BonActive®Putty Datasheet (DS 03_01);
- BonActive®Putty Instructions for Use (IFU 03_01);
- BonActive®Putty Product Description (PD 03_01).

8. Equivalence statement

BonActive®Putty and NovaBone Putty® use biocompatible materials that fill bone voids and provide an environment for bone regeneration. BonActive®Putty is a bone substitute equivalent to those on the market with minor differences in the composition. For all these medical devices the mechanism of action is the same, i.e. the host bone remodels through an osteoconductive process as new bone grows into the porous matrix of the graft materials. The graft materials are slowly resorbed and replaced by the host bone. BonActive®Putty and NovaBone Putty® have the same mode of action, therefore no new issues of safety or performance are presented.

The following table (Table 5) makes a comparison between BonActive®Putty and NovaBone Putty®.

Table 5: Comparison of BonActive®Putty with the equivalent medical device

Substantial equivalence comparison	**New Medical Device**	**Equivalent Medical Device**
Intended use	Bioactive and osteoconductive bone void filler device for the regeneration of hard tissues.	Same as new device
Indications	Is indicated to be packed into bone voids or gaps to fill and/or augment orthopaedics, oral, dental intraosseous and cranio-maxillofacial defects.	Same as new device

Composition	This is a putty that consists of a mixture between a solid phase (FastOS®BG, β-TCP and MCPM) and a liquid phase (Glycerol). It is composed solely of elements that exist naturally in normal bone (Ca, P, Na, Si, O).	Putty that consists of a mixture between a solid phase (45S5Bioglass®) and a liquid phase (PEG and Glycerol)
Device action	Ion diffusion and exchange at particle surfaces form a calcium phosphate surface layer, which acts as a scaffold for new bone formation throughout the graft site via osteoconduction. Continued ion diffusion and exchange results in material resorption.	Same as new device
Performance	Bone infiltration occurs throughout the graft site via osteoconduction, resulting in increased graft site mechanical stiffness and strength.	Same as new device
Bone remodelling	Bioactive and osteoconductive bone void filler device for the regeneration of hard tissues.	Same as new device
Biocompatibility	Biocompatible	Same as new device

9. Conclusions

BonActive®Putty and NovaBone Putty® are putties that have bioactive glass in their composition. Changes in the composition are related to the use of a different bioactive glass, absence of PEG and higher concentration of glycerol in the liquid phase and β-TCP and MCPM addition in the solid phase. These differences are properly explained above, since all different components are already widely used in clinical practice. Therefore, no issues regarding safety or performance are raised by the changes in composition.

Based on the specifications presented, it can be concluded that the intended use, material composition and scientific technology, degradation properties, bioactive, osteoconductive and osteostimulative properties of BonActive®Putty is substantially equivalent with the equivalent medical device, when used in the indications for use described above and do not raise new questions of safety and performance.

In conclusion, the performance and safety of BonActive®Putty as claimed in Product DataSheet (DS 03_01) and Instructions for Use (IFU 03_01) has been established and no risks associated with the use of the device were detected besides those arising from surgery.

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11.Annex

Table D1: Sample Appraisal Criteria for Suitability

Suitability Criteria	Description	Grading System
Appropriate device	Were the data generated from the device in question?	D1 Actual device D2 Equivalent device D3 Other device
Appropriate device application	Was the device used for the same intended use (e.g., methods of deployment, application, etc.)?	A1 Same use A2 Minor deviation A3 Major deviation
Appropriate patient group	Where the data generated from a patient group that is representative of the intended treatment population e.g., age, sex, etc.) and clinical condition (i.e., disease, including state and severity)?	P1 Applicable P2 Limited P3 Different population
Acceptable report/data collation	Do the reports or collations of data contain sufficient information to be able to undertake a rational and objective assessment?	R1 High quality R2 Minor deficiencies R3 Insufficient information

Table D2: Sample Appraisal Criteria for Data Contribution

Data Contribution Criteria	Description	Grading System
Data source type	Was the design of the study appropriate?	T1 Yes T2 No
Outcome measures	Do the outcome measures reported reflect the intended performance of the device?	O1 Yes O2 No
Follow up	Is the duration of follow-up long enough to assess whether duration of treatment effects and identify complications?	F1 Yes F2 No
Statistical significance	Has a statistical analysis of the data been provided and is it appropriate?	S1 Yes S2 No
Clinical significance	Was the magnitude of the treatment effect observed clinically significant?	C1 Yes C2 No

BonActive®Putty

Instructions for Use

Components of the medical device

Inside the package there is a syringe containing BonActive®Putty. All components inside the package are sterile.

Description

BonActive®Putty is a synthetic, bioactive and osteoconductive bone graft substitute. It is supplied sterile and is ready-to-use. This is a medical device which consists of a mixture between a solid phase (FastOS®BG, β-TCP and MCPM) and a liquid phase (Glycerol).

This medical device can be clinically applied for the regeneration of hard tissues. The device is injectable, fast absorbed and replaced by new bone tissue during the healing process. BonActive®Putty is indicated to be injected into bone voids or gaps to fill and/or augment orthopaedics, oral, dental intraosseous and cranio-maxillofacial defects.

BonActive®Putty is sterilized by gamma irradiation and is available in different syringe units.

Indications for use

Dentistry

- Correction of periodontal and furcation defects.
- Socket regeneration – ridge preservation.
- Graft material for bone regeneration to be used during oral implant surgery.

Cranio-maxillofacial surgery

- Bone cavity filling in cranio-maxillofacial area including the jaw.
- Frontal or maxillary sinus obliteration.

Orthopaedics

- Bone cavity filling.
- Bone cavity filling in the extremities and pelvis.
- Bone cavity filling in the treatment of bone tumors.

Contraindications

BonActive®Putty should **not** be applied:

- In acutely or chronically infected tissues at the surgical site,
- To replace structures that are subjected to strong mechanical stress,
- In patients that need chronic anticoagulant therapy,
- In patients that are taking immunosuppressant therapy,
- In patients who are receiving chemotherapy or radiation therapy,
- When patients have had or are undergoing bone irritation,
- In patients with known allergy to bioactive glasses, β-TCP, MCPM, and/or glycerol,
- In patients with metabolic disease known to adversely affect bone healing and mineralization (diabetes, hyperparathyroidism, osteomalsia…),
- In patients with severe hepatic and renal disfunction,
- In patients that have any existing condition or disease that will interfere with good and bone healing.

Interactions with other agents

None known.

Warnings and precautions

- BonActive®Putty is intended for use only by medical qualified professionals. The professionals must be familiar with bone grafting and internal/external fixation techniques.
- No studies have been conducted on pregnant women or on breastfeeding-women. For safety reasons, the implantation of BonActive®Putty is inadvisable during pregnancy and breastfeeding.
- BonActive®Putty is not intended for use with defects other than those listed in the indications for use.

Administration

Prepare surgical site following standard procedures. Thorough debridement of osseous defect is important.

Step 1. Open BonActive®Putty package and aseptically remove the syringe from the tray.

Step 2. Unscrew the cap and plush the plunger to force the putty to the defect site.

Alternatively, push the plunger to force the putty to a sterile cup and subsequently perform the implantation with a sterile instrument.

Step 3. In small increments gently fill the defect completely with BonActive®Putty. Use spoon end of spatula, do not pack or compress material into the site. Remove any excess material and close as per standard practice. Resorbable or non-resorbable membranes may be used while closing.

Complications

Complications may include normal post-surgical related symptoms like edema, redness, tenderness, swelling and fluid collection in the operation area.

Complications specific to oral/dental use are those as may be typically observed for similar bone grafting procedures and may include: tooth sensitivity, gingival recession, flap sloughing, resorption or ankylosis of the treated root, abscess formation.

In case of any adverse effects occur, report to the doctor and / or manufacturer.

Handling

- Do not use the product if the sterile package is damaged.
- Do not use after the shelf-life.
- The product must be promptly used after opening the package.

•The product is a sterile medical device for single use. Do not re-sterilize or use leftover material as it may lead to contamination and non-functionality of the product.

Storage

It is recommended to store the package in a dry place at room temperature (< 25 °C), away from humidity and sunlight.

Reporting Incidents

We instigate the communication of any suspect incident related with the use of this product. Please see how to contact us on page footer.

Information of raw materials

a. Starting materials used

Calcium fluoride (CaF_2)
Calcium carbonate ($CaCO_3$)
Magnesium carbonate ($MgCO_3$)
Ammonium dihydrogen phosphate ($NH_4H_2PO_4$)
Silicon dioxide (SiO_2)
di-Ammonium hydrogen phosphate $(NH_4)_2HPO_4$
Calcium nitrate tetrahydrate pure $Ca(NO_3)_2.4H_2O$
Calcium phosphate monobasic monohydrate ($H_4CaO_8P_2.H_2O$)
Glycerol

b. Delivery method

The raw materials are delivered by carrier.

c. Packaging

Starting materials are packed in plastic bottles or in poly drums, depending on the purchased amounts.

d. Storage conditions

Materials are stored under a normal temperature and humidity conditions.

e. Preparation before use

No special preparation procedures are required before use.

Information on product

a. Sensitive chemical identified/Biocompatibility

Product is not yet evaluated by in-vitro and in-vivo tests.
Biocompatibility tests weren't yet performed.

b. Storage conditions

It is recommended that material be stored under normal temperature and humidity conditions.

c. Distribution conditions

The medical device is delivered by carrier.

d. Potential users

Dentists, orthopaedists and maxillofacial surgeons.

e. Potential consumers

Patients needing bone regeneration.

f. Especially vulnerable consumers

This type of medical devices should not be used in patients who have a systemic metabolic disorder known to adversely affect bone healing and mineralization, other than primary osteoporosis.

No studies have been conducted on pregnant women or on breastfeeding-women. For safety reasons, the implantation of FastOs®BG is inadvisable during pregnancy and breastfeeding.

g. The intended use

BonActive®Putty is indicated for use as an osseous defect filler for bone regeneration in dentistry, cranio-maxillofacial surgery and orthopaedics. Thus, BonActive®Putty indications for use are:

Dentistry

- Correction of periodontal and furcation defects.
- Socket regeneration – ridge preservation.
- Graft material for bone regeneration to be used during oral implant surgery.

Cranio-maxillofacial surgery

- Bone cavity filling in cranio-maxillofacial area including the jaw.
- Frontal or maxillary sinus obliteration.

Orthopaedics

- Bone cavity filling.
- Bone cavity filling in the extremities and pelvis
- Bone cavity filling in the treatment of bone tumours.

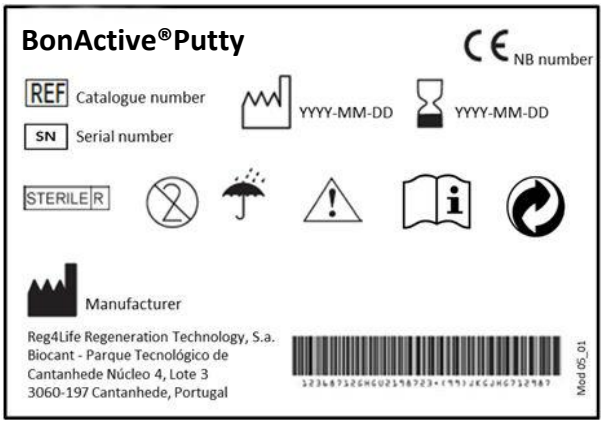
h. Foreseeable misuse

There are no foreseeable misuses outside those resulting from surgical technique.

i. Preparation

BonActive®Putty is supplied sterile in a syringe and is ready-to-use.

j. Labelling [form model example, Mod 05]



k. Specific requirements

Specific requirements do not exist other than those requires for a surgical intervention.

l. Product shelf-life

Product shelf-life is determined by ageing tests.

m. Sterilization

Product is sterilized by gamma irradiation. Sterilization process is validated.