UNIVERSIDAD DE EXTREMADURA

TESIS DOCTORAL

DESARROLLO Y OPTIMIZACIÓN MECÁNICA DE ANDAMIAJES HÍBRIDOS COAXIALES CERÁMICO/POLÍMERO PARA APLICACIONES BIOMÉDICAS

CLAUDIA ISABEL PAREDES SÁNCHEZ

PROGRAMA DE DOCTORADO EN CIENCIA Y TECNOLOGÍA DE NUEVOS MATERIALES

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La conformidad de los directores de la tesis (Pedro Miranda González y Francisco Javier Martínez Vázquez) consta en el original en papel de esta Tesis Doctoral

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ESCUELA DE INGENIERÍAS INDUSTRIALES

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DEVELOPMENT AND MECHANICAL OPTIMIZATION OF HYBRID CERAMIC / POLYMER COAXIAL SCAFFOLDS FOR BIOMEDICAL APPLICATIONS

PhD dissertation submitted by Claudia Isabel Paredes Sánchez to apply for the degree of Doctor at University of Extremadura

Badajoz, 2021

Resumen

Esta tesis persigue el desarrollo y optimización de nuevos andamiajes bioactivos porosos para su aplicación en ingeniería de tejido óseo, combinando de forma óptima un material cerámico y otro polimérico para potenciar sus propiedades mecánicas y biológicas. En concreto, los andamiajes híbridos desarrollados en esta tesis están compuestos por barras cuya vaina externa es cerámica y cuyo núcleo es polimérico. Esta combinación y disposición de materiales asegura que las estructuras fabricadas cuenten con una macroporosidad abierta e interconectada y presencia de iones calcio y fosfato en la superficie del andamiaje para garantizar la colonización celular y su capacidad osteoconductora. Para su fabricación se emplean técnicas de manufacturación aditiva (moldeo robotizado y procesado por luz directa) y procesos de infiltración en fase líquida de polímeros biodegradables. Una vez fabricadas, las estructuras son caracterizadas microestructuralmente y evaluadas mecánicamente, bajo esfuerzos de compresión y flexión. Cálculos por elementos finitos de los campos de tensión producidos bajo esfuerzos de compresión se utilizan para el análisis y la ampliación de los resultados experimentales. En los andamiajes desarrollados, a la rigidez y resistencia del esqueleto biocerámico se suma el efecto de las fibras poliméricas, que proporcionan tenacidad, dificultando la propagación de las fisuras, e integridad mecánica a la estructura incluso a altas deformaciones.

Abstract

This thesis pursues the development and optimisation of novel bioactive porous scaffolds for bone tissue engineering applications combining a biodegradable ceramic and polymer to enhance their mechanical and biological properties. Specificaly, the hybrid scaffolds developed in this thesis are comprised of struts with ceramic outer shells and polymeric cores. This combination and arrangement of materials ensures, firstly, that the structures have an open and interconnected macroporosity and, secondly, the presence of calcium and phosphate ions on the surface of the scaffold, to guarantee cell ingrowth and its osteoconductive capacity, respectively. Additive manufacturing techniques (Direct Ink Writing and Direct Light Processing) are used for their fabrication along with liquid polymer infiltration processes. After fabrication, the scaffolds are characterised microstructurally and evaluated mechanically, under compressive and bending loads. FEM numerical calculations of the stress fields developed in compression are used in the analysis and extension of the experimental results. In the developed scaffolds, the effect of the polymeric core fibres is added to the stiffness/strength of the bioceramic skeleton, providing toughness, by hindering the propagation of the cracks, and mechanical integrity to the structure even at large strains.

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Cuando pienso en mi corta vida laboral la palabra suerte es la primera que viene a mi mente. Nunca planeé ser investigadora, pero he encontrado en esta profesión una pasión, y más que un trabajo una afición. Quiero agradecer sinceramente al maravilloso equipo con el que he tenido el placer de trabajar estos cuatro años, a Toni y mis dos directores de tesis, Chico y Pedro. Muchas gracias por ser siempre tan agradables conmigo, desde el primer día hasta el último. Toni, gracias siempre por tu amabilidad y tu paciencia a la hora de explicarme tan bien las cosas. Por compartir tu conocimiento con tanta sencillez y facilidad. Chico, gracias por tu paciencia y tu ayuda tan fundamental en los inicios. Por explicarme una y mil veces las cosas, por tu preocupación y tus consejos. Pedro, gracias por tener siempre palabras de aliento, por enfrentarte de forma tan positiva a cualquier contratiempo, con esa actitud siempre hay ganas de seguir adelante. Por tu indudable ayuda y capacidad, tan efectiva y admirable. Gracias a los tres por vuestra mirada crítica y vuestras correcciones (no puedo creer que esté escribiendo esto XD), que me han ayudado a aprender tanto. A todos, gracias por vuestra implicación, por confiar en mí y escucharme siempre como a uno más. Por ofrecerme vuestra ayuda y apoyo en todo lo que he necesitado, no me he sentido sola o perdida en ningún momento. Habéis sido unos guías extraoridinarios. He disfrutado mucho haciendo este trabajo, desde el momento en el que empecé, he trabajado muy a gusto. He tenido una suerte enorme de haber formado parte de vuestro equipo. Este trabajo no es más mío que vuestro.

Gracias al Dipartimento di Ingegneria Industriale de la Universita' di Padova, donde tuve el placer de realizar una enriquecedora estancia. En especial a Paolo y Hamada, gracias por dedicarme gran parte de vuestro tiempo, por acogerme tan bien y por compartir conmigo tanto.

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Table of content

1. Motivation, Objectives and Chapter Layout

The development of bone-like biomaterials is essential to solve the limitations associated with the currently available procedures for treating bone lesions. Autografts remain the gold standard for this purpose, but their use is limited by the amount of available material and the need for secondary surgical sites to harvest them. And while allografts may solve most of these limitations, their biological performance is not as good, and they intrinsically involve a risk of immunogenic response and disease transmission. Artificial ‘bioinert’ prosthesis can be a solution when restoring functionality is the only goal, but they often cause bone resorption, which leads to implant loosening and the need for subsequent replacement surgeries. Indeed, bone replacement surgical interventions are among the most frequent ones in the European Union (EU) and their number is only expected to increase as the population ages, with an ever-increasing economic impact on the healthcare systems. A better alternative would be to not just repair but seek a complete regeneration of the damaged tissue instead. This is the goal of tissue engineering, where scaffolds, porous structures fabricated from ‘bioactive’ materials, are used to promote bone ingrowth and to boost the organism self-healing capabilities.

Bioactive ceramics, in particular calcium phosphates, are an excellent choice for the fabrication of scaffolds due to their similar composition to the inorganic-phase of bone. Unfortunately, they are also mechanically weak and extremely brittle materials, especially when used to fabricate porous structures. Not even the use of additive manufacturing (AM), which provides exceptional control on external shape and pore architecture, allows the fabrication of scaffolds with sufficient strength, and they remain far from cortical bone performance. Such low strength, together with their intrinsic brittleness, relegates their application to regions of the skeleton exposed to low loads.

Adding a biodegradable polymeric phase, either through a complete impregnation (Fig. 1.1a) or as a superficial coating (Fig. 1.1b), has been recently pursued as a suitable alternative to improve the toughness of bioceramic scaffolds. However, while these strategies unquestionably enhance the mechanical properties of the scaffolds, especially in terms of toughness, they also involve evident drawbacks in terms of the biological performance: namely, the loss of the bioceramic osteoconductive surface and, in the case of full impregnation, the blockage of the porosity required for tissue ingrowth. Even if those characteristics could be eventually recovered after implantation as the biodegradable polymer is resorbed, they will undoubtedly delay scaffold’s osteointegration, tissue regeneration and the overall healing process.

As an alternative approach, potentially capable of overcoming the aforementioned limitations, this work pursued the fabrication of porous structures with composite struts consisting of an outer bioactive ceramic shell and a tough, biodegradable polymeric core (Fig. 1.1c) and the optimization of their mechanical performance. This novel arrangement should still combine the best mechanical features of both materials, the stiffness of the bioceramic and the toughness of the polymer, without losing the osteoconductive surface of the bioceramic nor sacrificing the interconnected porosity required for bone ingrowth.

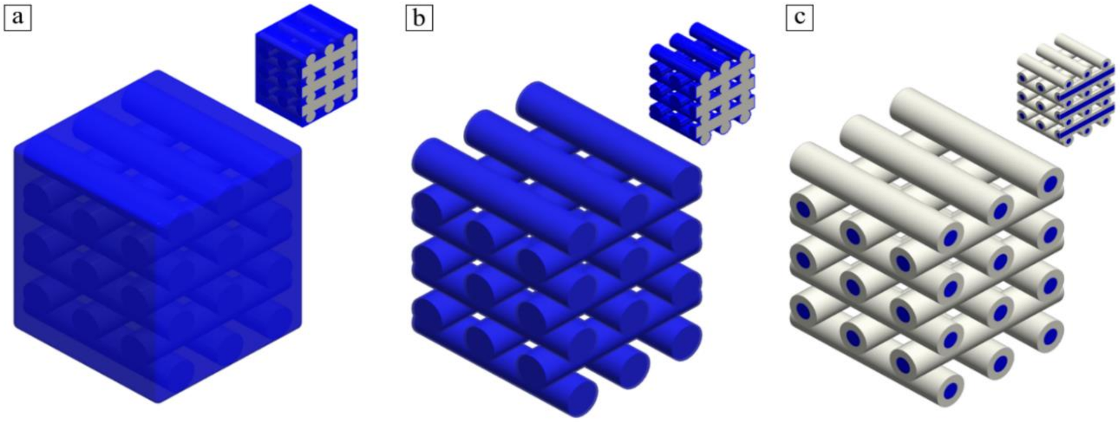


Figure 1.1. Schematic of different alternatives for the fabrication of ceramic/polymer (grey/blue) composites through polymer impregnation: (a) fully impregnated, (b) coated and (c) core/shell scaffolds.

Consequently, this study seeks to provide a proof-of-concept for this novel hybrid ceramic/polymer scaffold architecture by fabricating simple calcium phosphate scaffolds with hollow rods through additive manufacturing—using two techniques: Direct Ink Writing (DIW) and Digital Light Processing (DLP)— and then impregnating the intra-strut channels with a ductile polymer like polycaprolactone (PCL). Moreover, the impact on mechanical performance of parameters such as strut architecture, channel size or the concentration of polymer within the cores are analysed, both experimentally and with the aid of numerical simulations using finite element modelling (FEM).

This work is organized, in addition to this chapter, in 6 more:

In Chapter 2, the state of the art is described, providing a brief background on current strategies used in bone substitutive medicine and current biodegradable materials used in bone tissue engineering for the development of scaffolds. Besides, the main strategies that are being pursued for improving the deficient mechanical performance of bioceramic scaffolds, namely the use of additive manufacturing and the impregnation with biodegradable polymers, are discussed. This serves as justification of the need for hybrid structures like those to be developed in this work and, finally, all existing literature in this specific area, most of it loosely related or contemporary to the execution of this study, is presented.

Chapter 3 deals with the development of polymer/ceramic core/shell scaffolds through robocasting (a.k.a. direct ink writing, DIW). The methods for producing bioceramic structures with hollow struts by extrusion through coaxial nozzles are described in detail, together with a comparison of two different methods for the infiltration of the polymer: injection of a PCL solution and suction of the molten polymer. The advantages and limitations of the developed techniques, together with the mechanical enhancement achieved by impregnation are conveniently discussed. All the results are presented as in the two publications derived from this first part of the work:

- *Development by robocasting and mechanical characterization of hybrid HA/PCL coaxial scaffolds for biomedical applications, Journal of the European Ceramic Society* 39 (2019), *4375-4383.*
- *Novel strategy for toughening robocast bioceramic scaffolds using polymeric* cores, *Ceramics International 45 (2019) 19572–19576.*

Chapter 4 similarly describes the results corresponding to the development of core/shell hybrid scaffolds by DLP. The general limitations of this AM technique in terms of the fabrication of bioceramic porous structures and their resulting mechanical properties are first discussed. Their suitability for the fabrication of core/shell scaffolds through melt suction impregnation is subsequently demonstrated. Besides, the versatility of this technique is exploited to analyse, with the complementary aid of FEM simulations, the effect of scaffold design parameters like channel size and interconnectivity, and the modulus ratio of the selected materials, on the mechanical performance of such hybrid structures. Again, the results are shown as in the following publications:

- *Evaluation of direct light processing for the fabrication of bioactive ceramic scaffolds: Effect of pore/strut size on manufacturability and Mechanical performance. Journal of the European Ceramic Society 41 (2021) 892–900.*
- *Using ductile cores for enhancing the mechanical performance of hollow strut β-TCP scaffolds fabricated by Digital Light Processing. Ceramics International (2021). In press.*
- *Co-continuous calcium phosphate / polycaprolactone bone scaffolds fabricated by Digital Light Processing and polymer melt suction. To be submitted.*

In Chapter 5, a structured global summary of the main results arising from these publications and their discussion are included, in accordance with the provisions of the Regulations for Doctoral Studies.

Finally, in Chapter 6, the main conclusions derived from this work are summarized. First, the general features, advantages and weaknesses of the different fabrication routes developed in this study are analysed. Then, the effects on scaffold mechanical behaviour produced by polymer infiltration and their implications for the fabrication of hybrid organic/inorganic scaffolds for bone tissue engineering with improved mechanical performance are discussed.

The manuscript ends with Chapter 7 providing a brief outlook into future lines of work that could be explored with the aim of further enhancing the promising mechanical performance that the novel bioactive structures developed in this thesis already provide.

2. State of the art

As an introduction to the topic of this thesis, an overview of the state of the art in the field of biomaterials science and engineering for bone repair is presented in this chapter, with an especial focus on the mechanical aspects involved but without losing the biological perspective. First, the structure and composition of bone tissue are briefly described, along with the currently available solutions in substitutive medicine for bone repair. Then the difficulties found for the selection of optimal biomaterials candidates for bone replacement are highlighted. The role of biodegradable materials as a potential solution to the current drawbacks of inert implants, as a means to enable bone tissue regeneration rather than just repair, are discussed; and the members of the family selected as candidates for this thesis are described in detail. Lastly, the two main strategies followed by researchers seeking to maximize the mechanical performance of bioceramic scaffolds are described: the use of additive manufacturing to maximize the strength of the ceramic skeleton through a greater control of its microstructure; and the addition of a polymeric phase to the bioceramic skeleton as a way to overcome their intrinsic brittleness. Finally, the proposed concept of core-shell hybrid structures is introduced and the few precursor studies existing in the literature are presented.

2.1. Bone tissue and current repair alternatives

Bone is a living tissue, organized in a complex hierarchical structure and with a very singular mechanical behaviour [1,2]. There are two types of bone tissue: trabecular and cortical. Trabecular tissue is found in small bones and at the epiphysis of the long ones, and it is responsible of distributing and absorbing stresses since its interconnected cavities confer it a great ductility [2]. Cortical bone constitutes most of the skeleton and is compact and resistant. The hierarchical organisation of bone is schematically represented in Figure 2.1.1. Its basic unit is the osteon, where a number of concentric lamellae build a channel that hosts blood vessels and nerves [2]. The vessels irrigate the tissue and provide nutrients to its cells: osteoblasts, bone lining cells, osteocytes and osteoclasts. Bone is capable of repairing itself when suffering small fissures, thanks to a complex process of continuous remodelling in which osteoclasts are responsible for bone resorption and osteoblasts for the formation of the new tissue [1,3]. Two different phases can be found in the composition of osteons: an organic network of collagen fibrils and inorganic apatite crystals. The first one confers the tissue tensile strength and ductility and the second resistance to compressive loads [2]. As a consequence of all this complexity, bone can exhibit a broad range of mechanical properties, varying among different individuals and also along the different regions of the skeleton, which enables it to cope with the different solicitations to which it is normally exposed.

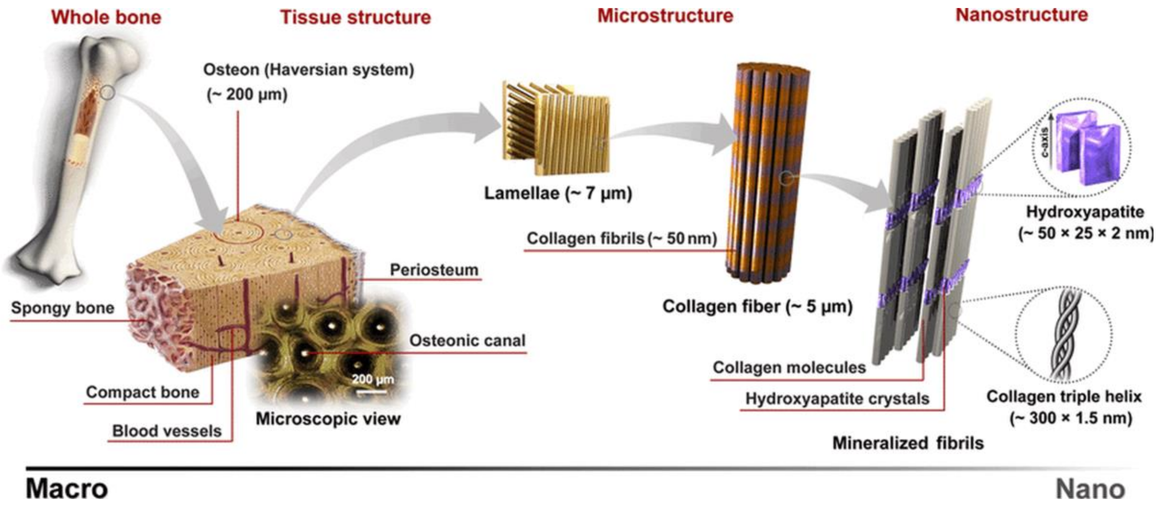


Figure 2.1.1. Hierarchical structure of bone, from macro to nanostructure [4].

When bone tissue is damaged beyond the organism's natural capacity for self-regeneration, the use of substitutive medicine is unavoidable. Bone grafts, either autologous or heterologous are currently considered the ideal solution in bone healing, with autografts being the gold standard against which any other alternatives are pitched. However, it is not always possible to use autografts due to the limited material availability and ensuing donor site morbidity. Similarly, in the case of allografts limitations may come from lack of donors and its associated risks of disease transmission and immunological rejection [1]. This situation unavoidably leads to the use of artificial implants, typically manufactured from synthetic bioinert materials with high mechanical performances in order to survive the demanding loading conditions they have to withstand during and after implantation. This is the case of metallic alloys (Ti- and Co-based or steels) as well as certain polymers (PEEK, UHMWPE, etc) or polymer matrix composites (e.g., carbon fibre reinforced PEEK) [5].

Unlike natural bone, bioinert implants cannot regenerate or adapt to body changes and they are typically much stiffer than bone, which leads to stress-shielding of the tissue surrounding the implant. This effect is responsible for the implants becoming loose with time, since the lack of stress alters the balance of the remodelling process, inducing bone resorption. These fixation problems limit the long-term use of the bioinert implants, and replacement surgeries are frequently necessary. Thus, more adequate materials, similar to bone from both the biological and also the mechanical viewpoints would be extremely beneficial, but unfortunately, as illustrated in the Figure 2.1.2, no existing synthetic material can match the outstanding combination of mechanical properties exhibited by bone [6].

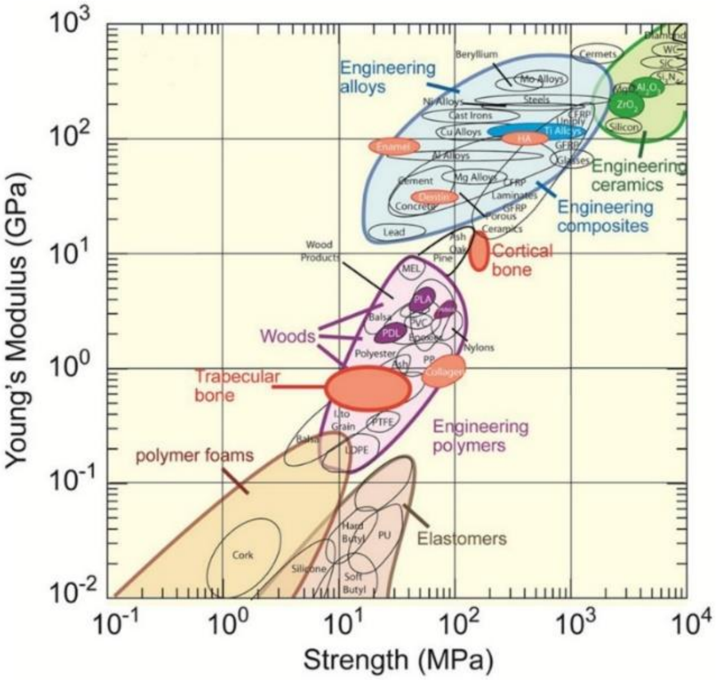


Figure 2.1.2. Ashby diagram comparing Young's modulus and strength of different materials to those of bone tissue. [7].

2.2.Biodegradable materials for bone tissue engineering scaffolds

As discussed in the preceding section, an optimal solution for treating bone lesions does not exist so far, and it urges to find an alternative based on more suitable materials, capable of adapting to the bone defects and actively induce bone regeneration. Many studies have been devoted to the development of bioactive scaffolds, structures that meet specific requirements in order to promote tissue regeneration, using materials capable of forming a secure bond with the surrounding tissue (Fig. 2.2.1). Bone scaffolds are structures consisting of biodegradable, osteoinductive or osteoconductive materials with an interconnected porosity, which serves as vehicle to cell proliferation and tissue ingrowth. Once implanted, these scaffolds should progressively degrade while cells and vessels proliferate towards their interior [2,8]. In order to allow this cell ingrowth, it is essential to preserve a minimum pore size of around 100 µm, while pores larger than 500 µm tend to compromise the scaffold's mechanical integrity [2,8–10]. Nevertheless, from a biological point of view, the co-existence of different pore sizes is found to have an important impact in the osteogenic response [2]. As described in detail in next two sub-sections, these scaffolds are typically made either from biodegradable ceramics or polymers.

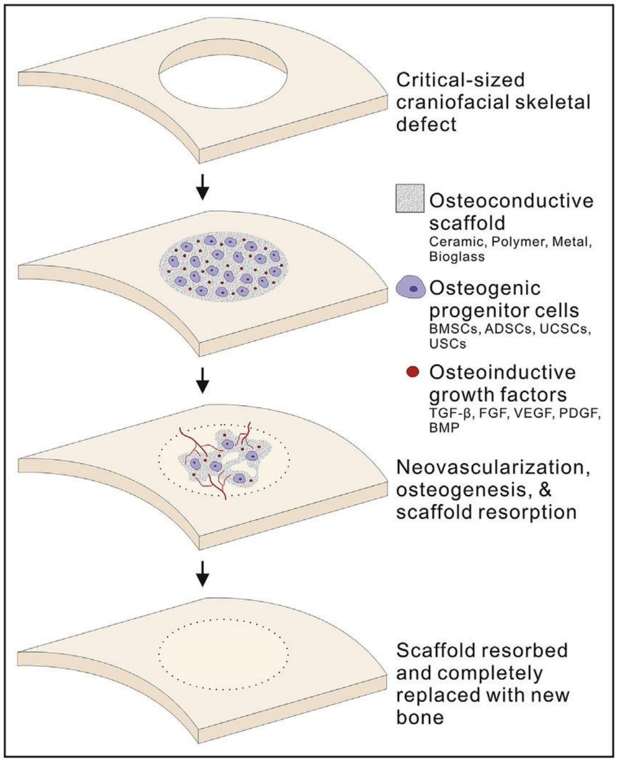
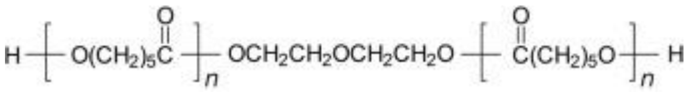


Figure 2.2.1. Ideal bone regeneration process using a scaffold for the reparation of a bone defect [11].

2.2.1. Biopolymers: Polycaprolactone

The use of biodegradable polymers for the fabrication of scaffolds is very extended due to their ability to degrade in biological environments without causing a toxic reaction [12–14]. Among the most used biopolymers with a natural origin are alginate, collagen and chitosan, while the most frequent synthetic compositions are polylactic and polyglycolic acids, and polycaprolactone (PCL) [10,11,15]. These polyesters are widely used in multiple biomedical applications ranging from drug delivery systems to scaffolds and films for tissue engineering [12]. Nonetheless, mechanically speaking, the main problem of biodegradable polymers in their application as bone scaffolds is their elastic moduli, which is too low compared to that of cortical bone. This issue restricts their application to non-load bearing regions of the skeleton or to soft tissue engineering. What is more, these materials are unable to actively induce bone formation [10,15,16] a feature that also limits their biological performance. Nonetheless, as will be discussed in detail later, the combination of these materials with bioactive ceramics offers interesting possibilities and constitutes the main objective of this thesis. In particular, the polymer selected for this thesis is PCL, whose chemical formula can be written as follows:



$$H\left[O(CH_2)_5\overset{O}{\overset{\|}{C}}\right]_n OCH_2CH_2OCH_2CH_2O\left[\overset{O}{\overset{\|}{C}}(CH_2)_5O\right]_n H$$

PCL can be synthesized by ring opening polymerization of ε-caprolactone at low temperatures and mild chemical conditions [17] and is soluble in common organic solvents. Its glass transition temperature occurs at -60 °C, and it melts at 60 °C. At temperatures above 200 °C it starts to degrade [18]. Typical commercial PCL have molecular weights in the range of 42.5-64 kDa (M_n) and 50.4-124 kDa (M_w), and a density of 1.145 g/cm^3. In terms of mechanical performance, it has a relatively low Young's modulus of 252-440 MPa [12] but an excellent ductility, which was the main reason for its selection. Regarding its biological performance, the degradation of PCL, induced via hydrolysis of the ester bonds, is relatively slow (up to 3 years) compared to other biodegradable polymers. This makes it appropriate for long-term implants in many biomedical applications [12,19]. Nonetheless, the stability of PCL is affected by its molecular weight, shape and residual monomer content, and also by other factors related to the physiological conditions (temperature and pH) [12,18]. Moreover, this polymer has the ability to mix readily with other faster degrading polymers in order to tune its physicochemical properties.

2.2.2. Bioceramics: Calcium phosphates

Unlike most biopolymers, biodegradable ceramics, thanks to their compositional similarities with the inorganic-phase of bone, exhibit an excellent osteoconductivity and are capable of bonding directly with the surrounding tissue. During their degradation in contact with the physiological fluids they can release calcium and phosphate ions, triggering and aiding the bone remodelling process so that the ceramic matrix is progressively replaced by healthy bone [10,20]. To this group of materials belong calcium phosphates (hydroxyapatite, tricalcium phosphate, etc.), calcium sulphates and numerous bioglasses (45S5, 13-93, 6P53B, etc.). Scaffolds fabricated from these materials exhibit proper new bone formation and have much higher elastic moduli than those fabricated from biodegradable polymers, but they are brittle and far from matching the mechanical performance of natural bone [2,10,11]. Another important feature of these bioceramics is their solubility within the body fluids, which is beneficial provided the speed of degradation that matches the requirements for each particular application: a slow degradation rate can result in the undesired persistence of foreign material within the body, while an excessively fast one does not ensure the scaffold maintains its mechanical integrity long enough to fulfil its objective [19].

Among all these bioactive ceramics compositions, β-tricalcium phosphate (β-TCP) and hydroxyapatite (HA) have been selected for this study. Both are calcium orthophosphates that have been widely employed as bone substitute due to their excellent osteoconductivity and bioactive properties [21]. There are several parameters that affect the solubility and stability of calcium phosphates, such as purity, crystal size and specific surface area. But one decisive factor is the Ca/P ratio which is inversely proportional to their solubility [22,23].The term hydroxyapatite is generally applied to calcium phosphates with Ca/P ratios of around 1.67, right within the range of values for the natural mineral component of bone (1.63-1.71) [21,24]. Synthetic stoichiometric hydroxyapatite ($Ca_{10} (PO4)_6 (OH)_2$) is considered a suitable option for bone cements and replacement, as a stable osteoconductive and bioactive alternative that induces a cellular response very similar to the natural bone extracellular matrix. When implanted, no inflammatory reactions are induced, and bone tissue binds directly to its surface, supporting cell adhesion, growth and differentiation [25]. However, HA has a much slower solubility than other calcium phosphates and, thus, lower ion exchange with their surroundings. Synthetic HA can be found with monoclinic and hexagonal structure, while natural HA crystals always belong to the most stable, hexagonal phase [21]. The melting point of this material is above 1550 °C, and the most commonly used sintering temperature is 1300 °C [26,27]. Stochiometric HA has a theoretical density of 3.16 g/cm^3 and a Young's modulus typically between 80--110 GPa [28].

Tricalcium phosphate (TCP) is not a natural component of bone, and it presents a much faster solubility in the organism than HA, as a consequence of a lower Ca/P ratio of 1.5 [24]. It can be found in several allotropic forms. The most stable phase at room temperature is β-TCP, with rhombohedral structure [29]. β-TCP exhibits excellent biomineralization and cell adhesion, and can promote the proliferation of cells, so it has been widely used both as bone cements and in bone substitution [21]. A transformation to monoclinic α-TCP occurs over 1135 °C [29], a phase which readily hydrolyses and transforms into calcium deficient hydroxyapatite in a biological environment [30]. A further transformation to α’-TCP takes place above 1470 °C, before its melting point at 1810 °C, but this phase cannot be stabilized at room temperature [29]. β-TCP presents a theoretical density of 3.07 g/cm^3, and a lower Young's modulus than HA, typically between 33-90 GPa [28].

The main drawback of these materials is that, as most ceramics, they are characterized for failing by brittle fracture, with a very low fracture toughness and tensile strength [22,23]. Crack propagates from pre-existing defects, where the stresses are concentrated, and therefore greater than in rest of the structure [31]. Thus, fracture depends on the defect population, which varies between samples in different modes depending on the orientation of the defect, although the most frequent situation in brittle materials is to fracture in mode I, where the two surfaces of the crack displace perpendicularly to each other, driven by tensile stresses. Nonetheless, fracture with tangential displacements between the walls of the crack can also occur due to shear stresses [31,32]. Because of the brittleness and mechanical weakness of these materials, calcium phosphate implants cannot withstand the mechanical solicitations inherent to many dental and orthopaedic applications, and the use of external metallic fixations is necessary (Fig. 2.2.2). And even when a high mechanical performance is not essential for a specific biomedical application, implants are subjected to stresses that can compromise their structural integrity already during surgery.

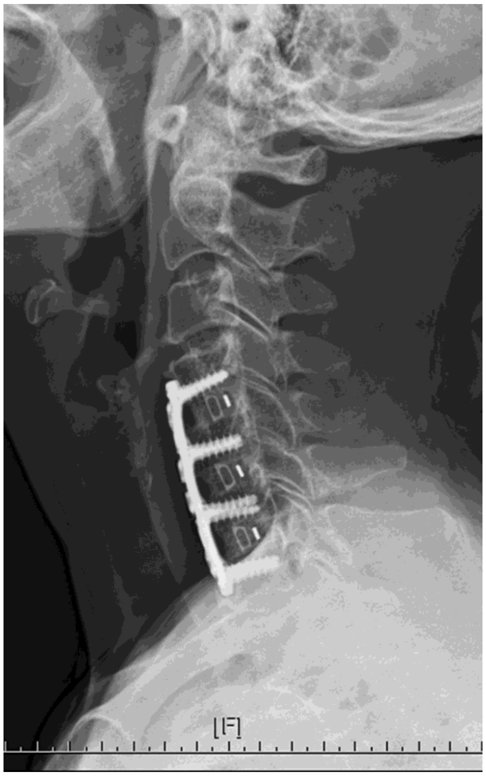


Figure 2.2.2. Example of external metallic fixation to reinforce biodegradable intervertebral cages in cervical spine surgery [33].

Therefore, it is essential to continue the development of structures with adequate mechanical response that can constitute an alternative to traditional bioinert implants in clinical applications subjected to stresses, without requiring the use of bioinert external fixations that either undesirably remain in the organism or must be removed in secondary surgical interventions.

2.3. Strategies for improving the mechanical performance of bioceramic scaffolds.

Various strategies have been proposed for enhancing the mechanical performance of scaffolds for bone tissue engineering. The optimization of the fabrication process (e.g., debinding, sintering, etc.) to eliminate the defects responsible for fracture is an obvious approach to enhance strength of the structures, but once full densification is achieved there is no more room for improvement. However, the pore architecture can also be optimized to minimize the total porosity required to achieve the desired biological performance (i.e. tissue ingrowth). In this regard, the use of additive manufacturing (AM) techniques provides significant benefits compared to traditional fabrication methods of porous substrates.

Another alternative to enhance the mechanical performance of bioactive ceramic scaffolds is to combine them with other materials to form composites. The addition of particulate reinforcements is an option but although some promising results have been obtained particularly with graphene and other carbonaceous species [34], there are concerns about the long-term effect of the release of such particulates to the biological media as the biodegradable ceramic matrix resorbs. An alternative that has received increasing attention lately by the biomaterials research community is the addition of a polymeric phase to the bioceramic `skeletons´. Since both the use of AM techniques and the addition of a polymeric phase are central to the present study, in this section the basic concepts and most relevant literature reports regarding both mechanical optimization strategies are briefly reviewed.

2.3.1. Additive Manufacturing of ceramics

The use of Additive Manufacturing (AM) techniques to fabricate bioceramic scaffolds allows the creation of structures with any desired external shape that could adapt perfectly to the patient's requirements [10]. But more importantly, by means of AM, porous objects with high degrees of interconnectivity can be obtained while maintaining the overall total porosity reduced. Controlling the pore architecture and reducing the porosity have as a consequence an improvement in the mechanical properties over porous structures produced using traditional fabrication methods [35] (gas foaming, foam replication, etc.), where high porosity levels are required to provide sufficient pore interconnectivity [10]. This is illustrated in Figure 2.3.1, where the compressive strength is represented as a function of the material density for glass and ceramic scaffolds fabricated by both AM technologies (in particular, Direct Ink Writing) and traditional fabrication methods. The compressive strength of scaffolds fabricated by AM beat, in most cases, that of structures fabricated by conventional methods with the same density (or even slightly greater). The few exceptions correspond to structures fabricated by freeze drying tested in the direction of the lamellae, which is not the weakest direction of such structures, while DIW scaffolds were all tested on their weakest orientation. The strength, and also the reliability, of AM-scaffolds is significantly greater because their struts and the pores are more homogeneous in size and shape: a chain with no weak links. Therefore, AM should be the preferred means to produce mechanically-sound porous bioceramic scaffolds.

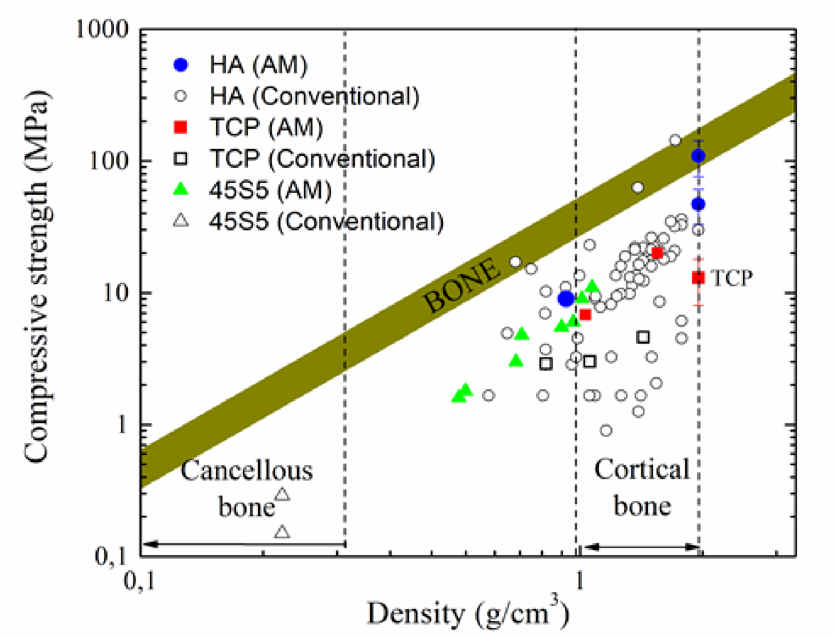


Figure 2.3.1. Comparative plot of compressive strength as a function of density for HA (circles) [35], TCP (squares) [36–38] and 45S5 bioglass scaffolds (triangles) [39,40] from different literature sources (not distinguished). Coloured symbols correspond to structures fabricated by DIW, empty symbols to conventionally fabricated structures. Shaded band represents bone properties (including data dispersion) as a function of apparent bone dry density [41]

Although the use of AM technologies for ceramic materials is growing, their maturation has been slower than in the case of polymers and metals. Nevertheless, they offer clear benefits in the production of complex bioceramic objects, such as those required for biomedical applications, since layer by layer fabrication avoids the arduous machining of such extremely fragile and hard materials [42]. These technologies employ a 3D model data, obtained either by means of CAD or 3D scanning, as an input to produce any complex structure with great accuracy [43,44]. The main struggle of AM techniques is in obtaining dense ceramics, but many biomedical applications can benefit from some residual microporosity.

There are numerous AM technologies classified in seven standard categories: binder jetting, direct energy deposition[1], material extrusion, material jetting, powder bed fusion, sheet lamination and vat polymerization [43]. In the case of ceramics, it seems convenient to separate them also in categories according to the feedstock they use, namely: powder, bulk-solid or slurry [45], as shown in Table 2.3.1. It should be pointed out that new technologies are continuously emerging to overcome the limitations of existing methods, and therefore only the most common technologies within each group are included.

Table 2.3.1. Summary of common technologies in additive manufacturing of ceramics

Material feedstock	Name	Abbreviation	Technology
Powder	Binder Jetting	BJ	Binder Jetting
	Selective Laser Sintering Selective Laser Melting	SLS SLM	Powder Bed Fusion
Bulk-solid	Laminated Object Manufacturing	LOM	Sheet Lamination
	Fusion Deposition of Ceramics	FDC	Material Extrusion
Slurry	Direct Inkjet printing	DIP	Material Jetting
	Direct Ink Writing	DIW	Material Extrusion
	Stereolithography Digital Light Processing Two-photon polymerisation	SL DLP TPP	VAT - polymerisation

2.3.1.1. Powder - based technologies

Powder based technologies, as the name indicates, use a bed of ceramic powder as a substrate and feedstock. Objects are built layer by layers through the action of a binder (e.g. Binder Jetting, BJ) or thermal energy (e.g. Selective Laser Sintering, SLS) [42,45,46]. Thermal post treatments are usually required to eliminate the binders, remove residual stresses or simply to improve densification of the parts. The quality of the print is influenced by various factors, such as the flow-ability of the powder, its interaction with the binder or energy beam and also the post-processing [38]. Thanks to the powder bed, that is constantly surrounding the printed structure, it is possible to create complex features (e. g. overhanging) without the need of additional supports. But the same fact limits certain shapes, as for example in the case of cavities where the powder would stay trapped inside [45]. Main drawbacks

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[1] Direct energy deposition is not usually employed with ceramic materials as feedstock.

are that generally objects fabricated with powder-bed technologies exhibit poor densification and rough surface finishing (Fig. 2.3.2), due to the low initial green density [42,45].

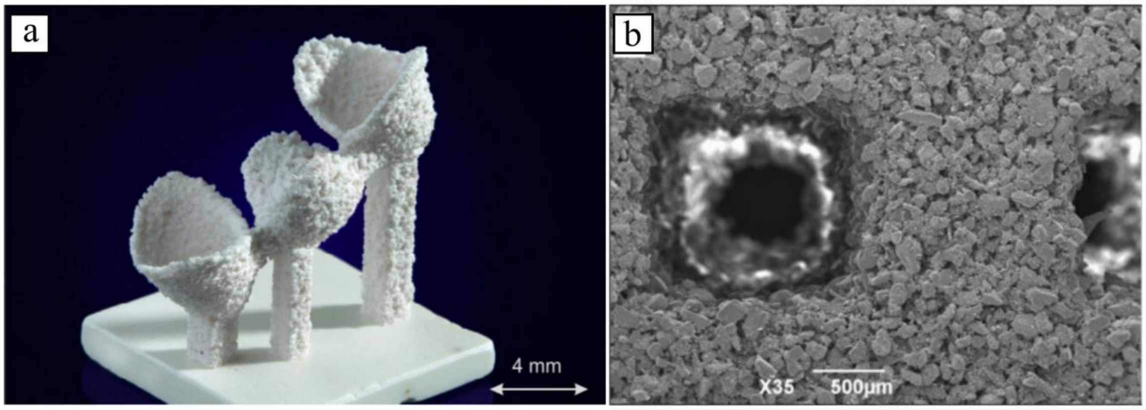

Figure 2.3.2. (a) Parts for dental restoration produced by SLS from a mix of Al_2O_3 *and* ZrO_2 *powders* [47] *and (b) SEM micrograph of a LAS glass - ceramic scaffold produced by Binder Jetting* [48].

2.3.1.2. Bulk - solid based technologies

Solid organic material containing ceramic particles is used as feedstock in this group of techniques. The most common members of this family are Laminated Object Manufacturing (LOM), which uses polymer/ceramic composite sheets and Fusion Deposition of Ceramics (FDC, a version of Fused Deposition Modelling, FDM, for ceramics), which uses ceramic-filled thermoplastic filaments. Layers are built by the action of a laser and press-heating in the first case, while thermal energy is used to melt the filament and deposit the material by extrusion through a nozzle in the second. In both cases, after the green part is obtained, a debinding step to burn out the organics and subsequent sintering at high temperature is performed. LOM is fast and convenient for the fabrication of large parts (Fig. 2.3.3a) but certain limitations are found in the case of cavities and resolution is limited [42,45,49]. In FDC, the resolution of the printing depends on the diameter of the nozzle, which can be as small as 80 µm, but it is a slower process, more adequate for the fabrication of 3D objects (Fig. 2.3.3b) with medium sizes [44,50]. Both technologies can start with high ceramic contents (up to 60 vol.%) which allows a full densification of the parts after sintering [45,51]. However, complications can emerge during the debinding treatments with the appearance of cracking and other defects [42].

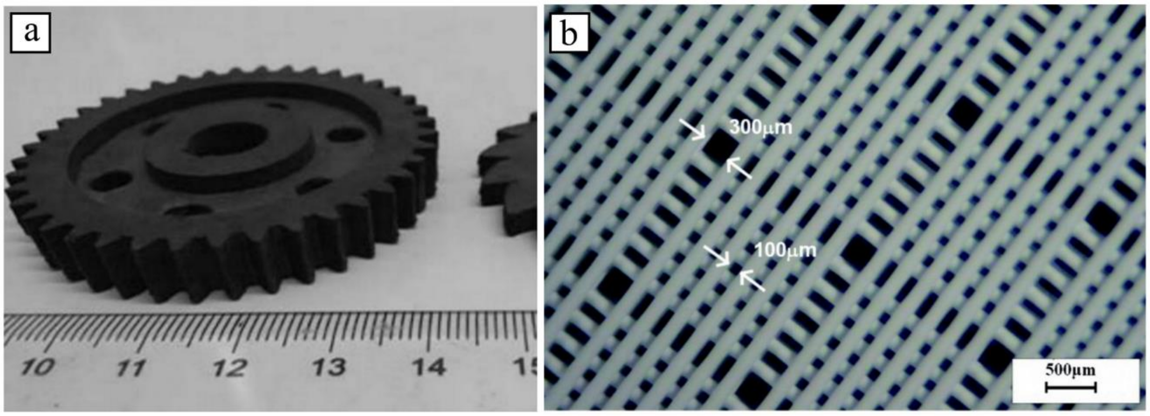

Figure 2.3.3. *(a) SiC gear wheel fabricated by LOM* [52] *and (b) calcium phosphate scaffolds fabricated by FDC* [51].

2.3.1.3. Slurry - based technologies

Slurry-based technologies work with uniform suspensions of ceramic powder in a suitable dispersion medium. Different additives can be used to obtain an adequate rheology (viscosity, storage modulus, etc.) and stability of the dispersion, in order to prevent the agglomeration and sedimentation of the particles [44]. After they are built, green parts must undergo a debinding step to burn out any organics and subsequently sintered at high temperature to produce the final ceramic part [42]. Among the various technologies that use ceramic slurries as feedstock, Direct Ink Writing and Digital Light Processing will be described in detail in this section justifying their adequacy for the fabrication of the hollow struts ceramic scaffolds developed in this work.

Direct Ink Writing

The main example of material extrusion technologies using ceramic slurries is Direct Ink-Writing (DIW), also often referred to as Robocasting, Robotic Molding, Direct Write Fabrication or Robot Assisted Deposition. DIW was developed in 1997 at Sandia National Laboratories and unlike other AM techniques it was originally devised for ceramic materials [53]. DIW uses viscous and highly solid loaded slurries or pastes, typically water-based with a minimal organic content (< 1%) [53]. This technique allows to quickly create complex 3D objects, with or without the help of fugitive pastes to provide support, using a wide range of materials. Robocast parts achieve very high levels of densification and optimal surface finish after sintering thanks to the high solid loading - of the pastes. The high powder concentration in the feedstock minimizes the shrinkage during drying and sintering [54], and the typically low concentration of organics in the formulation greatly simplifies the debinding process, which all contributes to reducing the generation of flaws and increasing the dimensional accuracy of the part. Part resolution and surface finish depends mostly on the diameter of the nozzle, which is typically larger than 100 µm to prevent clogging during printing. In Figure 2.3.4, a typical calcium phosphate scaffold fabricated by DIW is displayed.

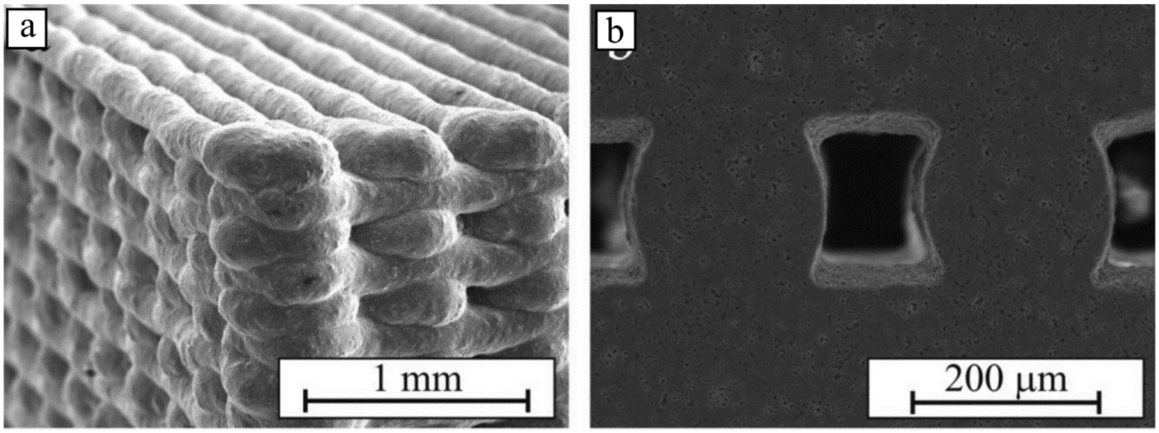

Figure 2.3.4. SEM micrographs of DIW sintered scaffolds, fabricated with a 250 µm nozzle and a slurry with 35 vol.% HA content [55].

In order to achieve a paste suitable for DIW a precise control of the rheological behaviour is needed. The consistency of the paste can be influenced either by ionic forces, pH or by the addition of a solvent [56]. Commonly, water-based pastes are prepared as follows: First, the powder is dispersed in a stable suspension, to avoid agglomerates that could cause the clogging of the nozzles during the process. For this, a polyelectrolyte is adsorbed onto the particle surfaces (Fig. 2.3.5a) to produce a repulsion between them. Then, a viscosifier is added to provide plasticity, avoiding the separation of phases that the pressure of extrusion may cause [53]. Finally, a flocculant is added to the paste to induce its gelation, thus increasing the storage modulus of the mixture. This is an essential feature since the paste must maintain its shape after leaving the nozzle in order to support the weight of the subsequent layers (Fig. 2.3.5b).

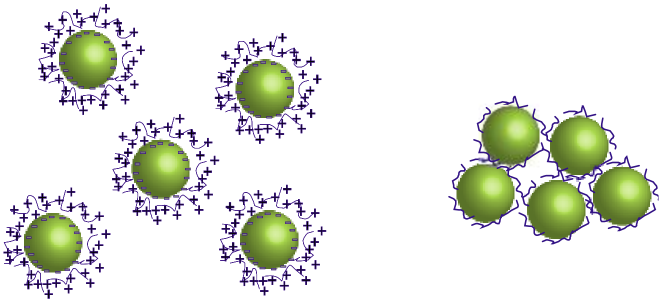

Figure 2.3.5. Paste formulation: schematic illustration of the fluid-to-gel transition [56].

The resulting homogeneous paste (ink) is used to fill a syringe, which is then vigorously shaken to eliminate any bubbles. For a successful fabrication, the ink should exhibit a shear-thinning rheological behaviour with a yield point: it must be able to flow through the tip when extruded, retain the shape once it leaves the nozzle and resist the stresses that the following layers induce [54]. The shape and dimension of the extruded rods is determined by the nozzle features, which are normally cylindrical with a diameter between 0.20 to 1.60 mm. These features also determine the selection of the powder in terms of particle shape and, especially, size. Since clogging of the tip during extrusion should be prevented.

Deposition of the ink onto a flat substrate surface is made layer-by-layer by a computer-controlled robotic system capable of precisely moving in the three cartesian axes [35] . The extrusion is made by applying pressure on the syringe using either a hydraulic system or a piston. Controlling the drying kinetics upon deposition is crucial. Typically, the deposition is made within an oil bath rather than in air to avoid the uneven drying of each deposited layer, which prevent the appearance of cracks and other printing defects [35] when the water evaporates. A schematic of the process is represented in Figure 2.3.6.

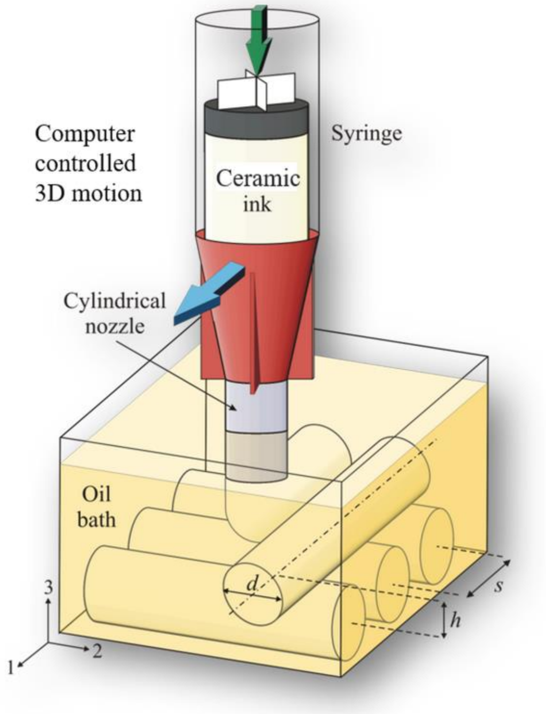

Figure 2.3.6. Schematic of Direct Ink Writing fabrication process.

Typical scaffolds fabricated by robocasting consist of 3D-networks of cylindrical rods in a log-pile architecture [56]. The geometrical features defining such patterns (Fig. 2.3.6) are the diameter size of the nozzle *(d)*, the spacing between adjacent rods in a layer *(s)* and the layer height *(h)*. Layer height is smaller than the diameter of the rods since some overlapping between layers is necessary to facilitate the printing. Robocast scaffolds with hollow struts, such as those required to build the core/shell hybrid structures that are the main objective of this thesis, could be readily obtained using a coaxial nozzle.

Digital Light Processing

Vat polymerization technologies use a light source to produce selectively the polymerization of a photosensitive polymer contained on a transparent vat [43]. In the case of ceramics, an UV-photocurable slurry is prepared filling the polymer with ceramic particles or preceramic polymers [42,57]. To this group belong the Stereolitrography (SL), since 1986, and since 1997, Digital Light Processing (DLP) and Two Photon Polymerization (TPP) [45]. In order to shape a complete three-dimensional structure, a 3D-model corresponding to the object must be previously sliced. In SL and TPP each layer is cured point to point either using a UV-light or a laser. In the case of DLP the whole layer is exposed to the UV-light at once [45].

These versatile technologies allow the fabrication of complex scaffolds with almost any external shape, disposition and geometry of struts with a good surface finish [42]. They provide different levels of resolution. While TPP allows to fabricate objects from micro to millimetre scale, using SL and DLP objects with sizes varying from micro to centimetre scale can be manufactured. Costs of production and fabrication speed also varies between techniques, being especially affordable and fast in the case of DLP [45]. The difference and possibilities of fabricating complex structures, with a different level of detail, using either DLP or TPP is illustrated in Figure 2.3.7 through an example.

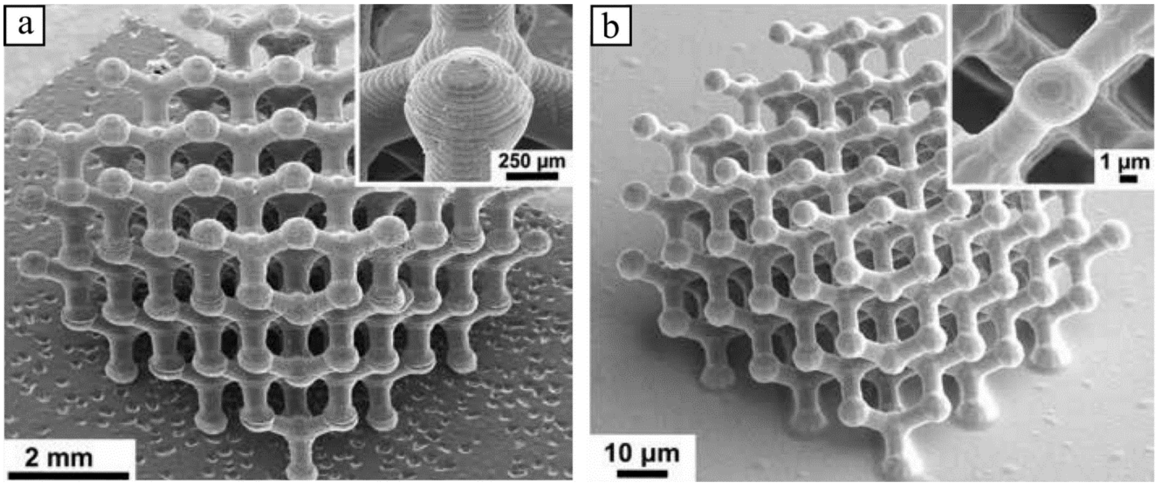

Figure 2.3.7. (a) DLP sintered and (b) TPP unpylorized diamond structures using SiOC powders [57].

The fabrication of ceramics using Digital Light Processing has achieved great acceptance in many different applications. Recent commercial solutions have been demonstrated capable of producing ceramic structures with excellent densification and surface finish from highly loaded slurries. This is the case of the printers and feedstocks developed and commercialized by Lithoz GmBH since 2014, Admatec BV in 2016, and Prodways TECH since 2017 [42,58].

During the fabrication process, a UV-light source projects consecutively different images, corresponding to the layers in which the object to print is previously divided. For this purpose, the light is first directed to a micromirror device and then projected onto the transparent VAT containing the resin [59]. DLP exists in two modalities: bottom-up and down. The main differences are in the disposition of the building platform, the direction of the light and the quantity of resin necessary for the printing [42,45]. Bottom-up is the technology employed in this work. The light irradiates the vat containing the suspension from below, and the build platform stands opposite to the vat, moving towards and away from it for every exposition. A simple schematic of the process is included in Figure 2.3.8.

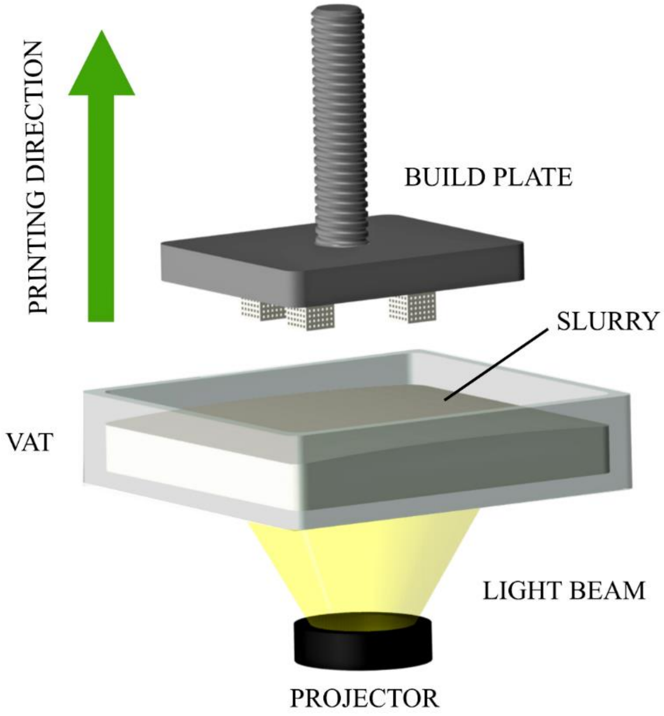

Figure 2.3.8. Schematic of a bottom-up Digital Light Processing fabrication process.

Ceramic suspensions used as feedstock for DLP consist of a photopolymerizable medium filled with ceramic particles that do not directly participate in the polymerization reaction but can definitely influence the process. The dispersion medium consists of three main components: either acrylic or epoxy monomers as the main component, photoinitiators and photoinhibitors. When the medium is exposed to an UV radiation, the photoinitiators generate free radicals reacting with the monomers and starting the polymerization reaction. The photoinhibitors reduce both the penetration depth and the lateral scattering of the UV light [60]. The polymerization depends on the properties of the mentioned photoactive components and their sensibility to certain wavelengths, which normally vary between 385 and 405 nm for different DLP machines. The cure depth (C_d) is the distance the resin gets cured when it is exposed to the light during a certain exposure time (e_t), after receiving a certain energy dose (U). Jacob´s equation [61] expresses the relation between these two parameters, C_d and U, for a given suspension, which depends on two parameters: a critical energy dose (U_c) and the light penetration depth (D_p) [59] for that suspension:

$$C_d = D_p \ln \frac{U}{U_c}$$

To avoid two undesired defects during fabrication by DLP, overexposure and delamination (Figure 2.3.9), the layer thickness selected for the process must match the cure depth for the selected exposure time. In this regard, evaluating the influence of the ceramic fillers on the curing process is critical. First, their presence reduces the volumetric concentration of photoactive medium, which would require a lower energy dose for a given cure depth target, but at the same time increases the light scattering [42,45,60] and, thus, reduces its penetration depth, $D_{p.}$ In the end, longer exposure times are typically needed to produce a target depth of penetration. In turn, this derives in a more accused light scattering [60] that reduces the in-plane spatial resolution of the printing process, as it increases the area illuminated by each pixel in the projector. In order to minimize all these issues and optimize the printing results, the refractive indexes of the powder and the photosensitive medium should be as similar as possible. Besides, UV-transparent ceramics are preferred over UV-light absorbing compositions since they further complicate the process [45,60].

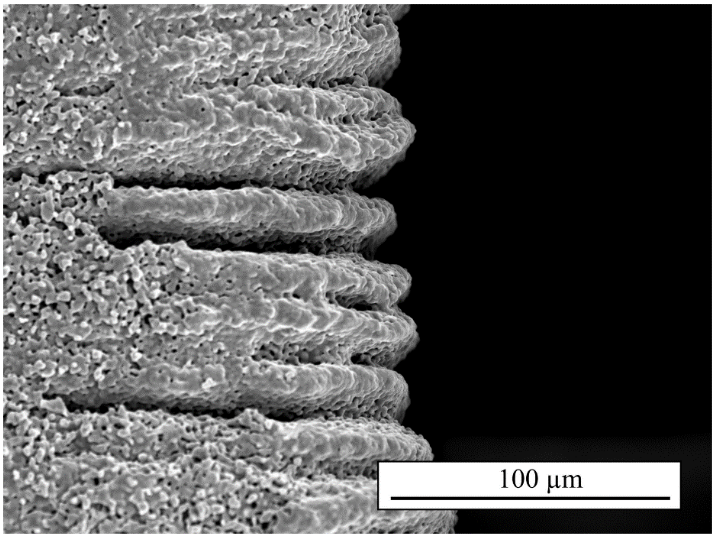

Figure 2.3.9. Example of a fabrication defect typical in DLP: strut exhibiting delamination due to insufficient UV radiation exposure.

Another factor that essentially affects the fabrication by DLP is the fluidity of the suspension. Once the powder is dispersed, suspensions are generally stable thanks to the relatively high viscosity of the resins (compared to water). However, the addition of the ceramics inevitably increases the viscosity, lowering the fluidity of the mixture, a problem that increases with the solid loading. Flowability of the slurry is essential to guarantee replenishing the feedstock material between exposures and, thus, avoid printing errors. Fortunately, the viscosity can be lowered either by raising the temperature of the suspension and/or adding diluents [42,45,60,62].

After the printing process, the green part has to be cleaned to eliminate any residual uncured suspension and, typically, subject to further UV-curing to improve polymer consolidation. The part has to be then heat-treated to carefully burn out the polymeric binder without introducing undesired defects or microcracks, prior to the final sintering of the ceramic parts. Although the debinding process is typically harder than in DIW due to the higher organic content in the feedstock, the high nominal spatial resolution (in the tens of microns range) of the DLP technique makes it well suited to the production of hollow-strut scaffolds since the intra-strut channels can be directly incorporated into the initial CAD design of the part.

2.3.2. Ceramic / polymer hybrid scaffolds

Although AM technologies can provide a substantial increase in the strength of bioceramic scaffolds, their performance is still far from matching that of bone with similar density values (see Fig. 2.3.1), and they remain as fragile. The addition of a secondary phase, preferably a biodegradable polymer to avoid severely compromising the biological performance, appears as a complementary strategy to further enhance the strength and, especially the toughness of the bioceramic scaffolds. The improvements achieved by polymer coating and impregnation are first reviewed in this section, followed by a short overview of the few precursor works for the proposed approach of incorporating the polymer internally, through the fabrication of hybrid core-shell scaffolds.

2.3.2.1. Coated and fully-impregnated hybrid scaffolds

The combination of biodegradable ceramics and polymers is excellent for bone replacement applications, with the ceramic providing sufficient rigidity and the polymer increasing its defect tolerance and mechanical performance, by hampering crack initiation and subsequent propagation. Consequently, multiple studies from diverse authors have focused on impregnating or coating bioceramic scaffolds with degradable polymers (Figs. 1.1a and 1b), observing in both cases a clear improvement in strength and toughness [8,16,36,63–65]. Strengthening occurs mainly through a defect healing mechanism, where the polymer bonds together the walls of pre-existing flaws increasing the stress needed to initiate a crack from them [36,64]. A secondary mechanism, observed only when sufficiently rigid polymers impregnate completely the scaffold porosity, is a limited reduction of the stresses in the bioceramic part due to the presence of the polymer. This effect is often referred to as stress shielding [36,64]. Increasing the scaffold’s strength immediately increases the fracture energy of the structure. But more importantly, fibrils of the polymer increase the toughness of the structure further by bridging together the fracture surfaces of any cracks that finally pop-in.

This significantly increases the energy necessary for crack propagation and provides mechanical integrity and residual load-bearing capacity after the ceramic skeleton fails. Crack bridging mechanism provides an outstanding contribution to the strain energy density especially in fully impregnated structures where large polymeric fibres occupy the scaffold macropores, greatly hindering crack propagation and holding the structure together even at large deformations [16,36,63,64]. The enhancement in the fracture energy of the hybrid structures can even surpass that of cortical bone in compression for some cases (Fig. 2.3.10a). And although the measured performances are a little bit poorer in bending (Fig. 2.3.10b), fully impregnated structures come substantially closer to natural bone values even under this most deleterious loading mode [64,65].

However, despite the significant benefits in terms of mechanical performance demonstrated in these preceding works, all the aforementioned hybrid scaffolds have a common drawback: their biological performance has been compromised. Indeed, a complete polymeric impregnation of the scaffold implies the loss of the interconnected porosity necessary for cell colonization and proliferation, for angiogenesis and tissue regeneration. Even a simple polymeric coating would, as well, retard the remodelling process as the osteoconductive or osteoinductive capacity of the bioceramic will be diminished as it is no longer directly in contact with the physiological medium, at least until the polymer has been degraded. Even if those characteristics might be eventually recovered after implantation as the biodegradable polymer is resorbed, its presence will obviously retard the scaffold's osteointegration, tissue regeneration and the overall healing process for some essential time. The present study seeks to provide a solution to this issue by inversing the distribution of phases on the scaffold struts, keeping the ceramic exposed to the physiological medium and moving the polymer underneath, at the strut cores. The few existing literature reports that could be considered as precursors of this strategy are summarized in the next section.

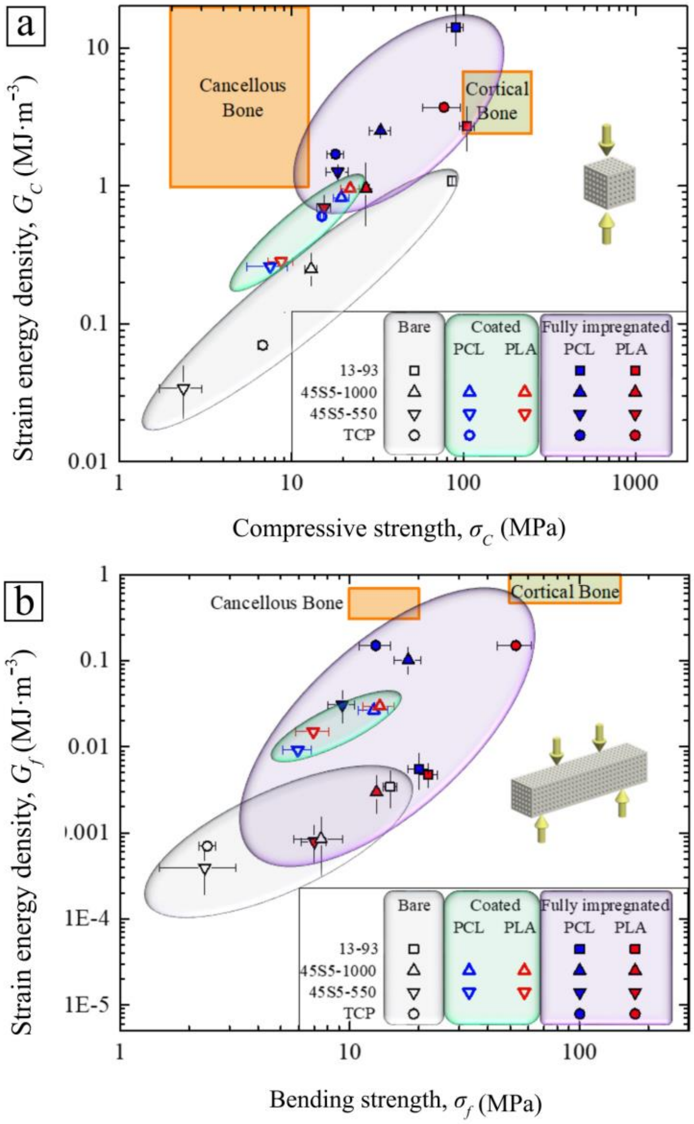

Figure 2.3.10. Comparative plots of strain energy density as a function of strength in (a) compression and (b) bending for bare, coated, and fully-impregnated bioceramic scaffolds fabricated by DIW [37,64–68]. *Shaded rectangular areas correspond to natural bone properties* [69–71].

2.3.2.2. Hybrid core-shell scaffolds

Hybrid porous scaffolds must further enhance their functionality to be considered an optimal solution for bone regeneration. The improvements in mechanical performance achieved by the conventional hybridization strategies described in the previous section are very positive but they involve a cost in terms of biological performance. Hybrid scaffolds comprised of struts with bioactive ceramic outer shells and ductile polymeric cores could provide a suitable mechanical enhancement over all-ceramic structures without jeopardizing their biological behavior.

Indeed, such core-shell design (Figure 1.1c) where the tougher (but non-bioactive) phase is located internally and the bioactive ceramic stays at the surface, where scaffold/tissue interaction will eventually take place, seems a more appropriate alternative [72]. Such design should ensure: first, the existence of an open and interconnected macroporosity that allows a cellular proliferation. Second, the presence of calcium and phosphate ions on the surface of the scaffold that guarantees its osteoconductive capacity. And third, the existence of continuous polymeric fibres capable of hindering the propagation of cracks, increasing the material toughness and providing mechanical integrity to the structure even at high deformations.

Although this approach is new, some studies have focused on improving vascularization of all-ceramic scaffolds by varying the geometry of their struts to include internal channels. According to these authors the tubular struts imitate the natural disposition of channels and promotes cell ingrowth and vascularization as a consequence of a more effective transport of nutrients [72]. Recently other authors have successfully fabricated structures with hollow channels and also core-shell structures for different purposes motivated by the possibilities that such composite structures offer in different fields, including drug release and tissue engineering. First reports dealt with two-polymer structures fabricated by electrospinning [73] but many others employed extrusion-based techniques, in particular DIW with the help of coaxial nozzles, for the fabrication of the core-shell structures.

Already in 2006, Moroni et al. explored the fabrication of structures comprised of hollow polymeric fibres (Fig. 2.3.11a), with controlled diameter and thickness, by the co-extrusion of two polymers and subsequent dissolution of the core material [74]. With the aim of enhancing vascularization of tissue-engineered structures, in 2013 Luo et al. developed hollow fibre scaffolds using alginate pastes [75] (Fig. 2.3.11b) using a simple fabrication method that avoided the use of organic solvents that may cause toxicity problems in biomedical applications. Later on, they successfully used the same method in the fabrication of scaffolds for bone regeneration using calcium phosphate ceramics [76] (Fig. 2.3.11c). Their *in vitro* and *in vivo* tests showed a clear improvement in cell attachment and proliferation in the scaffolds with hollow struts compared to their dense counterparts, and also a superior capacity for bone formation *in vivo* [76].

Similarly, in 2014, Cornock et al. obtained coaxial scaffolds with PCL shells and alginate cores (Fig. 2.3.11d) through coaxial melt extrusion, developing for this purpose a coaxial nozzle equipped with a temperature control system [77]. Later, DIW was employed by Schlordt et al. and Moon et al. to obtain alumina hollow struts scaffolds with very porous shells (Fig. 2.3.11e,f). The first authors made the deposition in an oil bath, using a nozzle with an occluded centre tip [78], while in the second case a co-extrusion of alumina paste and a camphene fugitive ink was made through a coaxial nozzle [79]. Contemporary to the present work, in 2018 Mueller et al. developed flexible epoxy core – brittle epoxy shell lattices (Figure 2.3.11h) by DIW, showing the mechanical benefit in terms of toughness of using these coaxial structures even in all-organic systems [80].

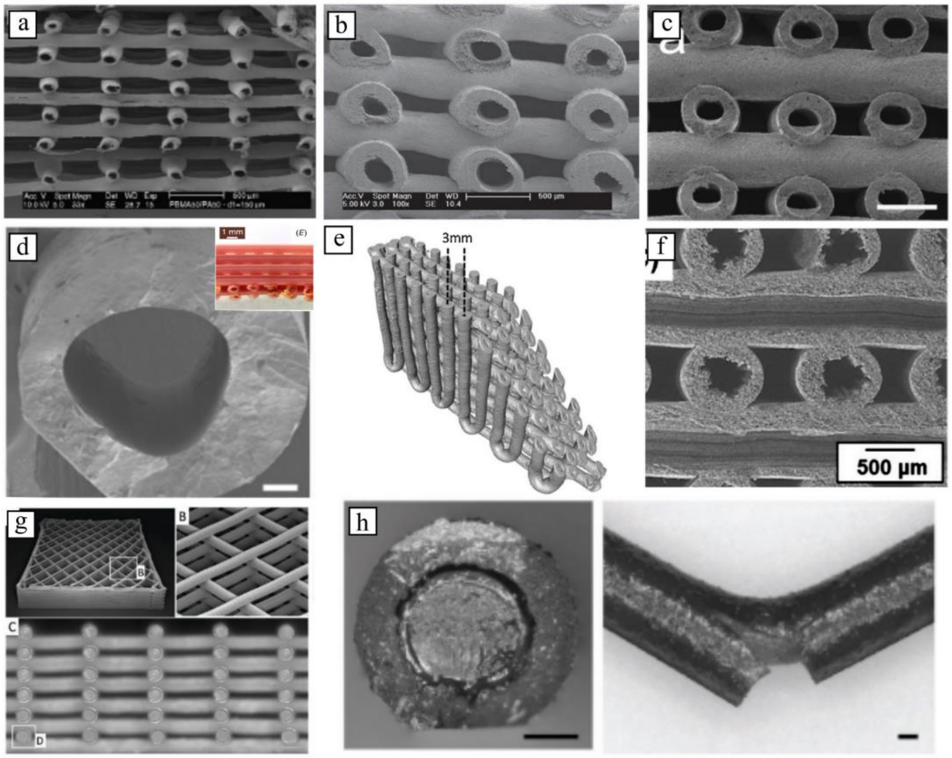

Figure 2.3.11. Overview of structures consisting of hollow and core/shell struts developed by different authors from: (a) polymeric fibers [70], (b) alginate [71], (c) calcium phosphate [72], (d) PCL, (e,f) alumina, (h) flexible epoxy core – brittle epoxy shell

However, as already mentioned, most of these studies have focused on demonstrating the hypothesis that tubular structures present important advantages in the regeneration of tissues, promoting cell proliferation, tissue ingrowth and vascularization and also providing a functional drug delivery system [72,81,82]. As a good example, Figure 2.3.12 shows hollow-strut scaffolds developed by Zhang et al. by DIW using bioceramic pastes, and their osteogenic response when implanted. Hollow struts scaffolds presented there a path to shorten the regeneration time and good potential for the repair of large defects [82]. However, not many authors have focused in the mechanical optimization of these types of coaxial scaffolds for bone regeneration through the combination of bioceramics and polymers. The only exception is a contemporary work from Jo et al. which showed that the channels of calcium phosphate ceramic scaffolds with hollow struts could be successfully infiltrated by biopolymers using in-situ polymerization [83]. Nonetheless this study concluded that further improvements should be done towards the control of the core channel size and homogeneity and evidenced a need for more extensive mechanical characterization, including flexural behaviour.

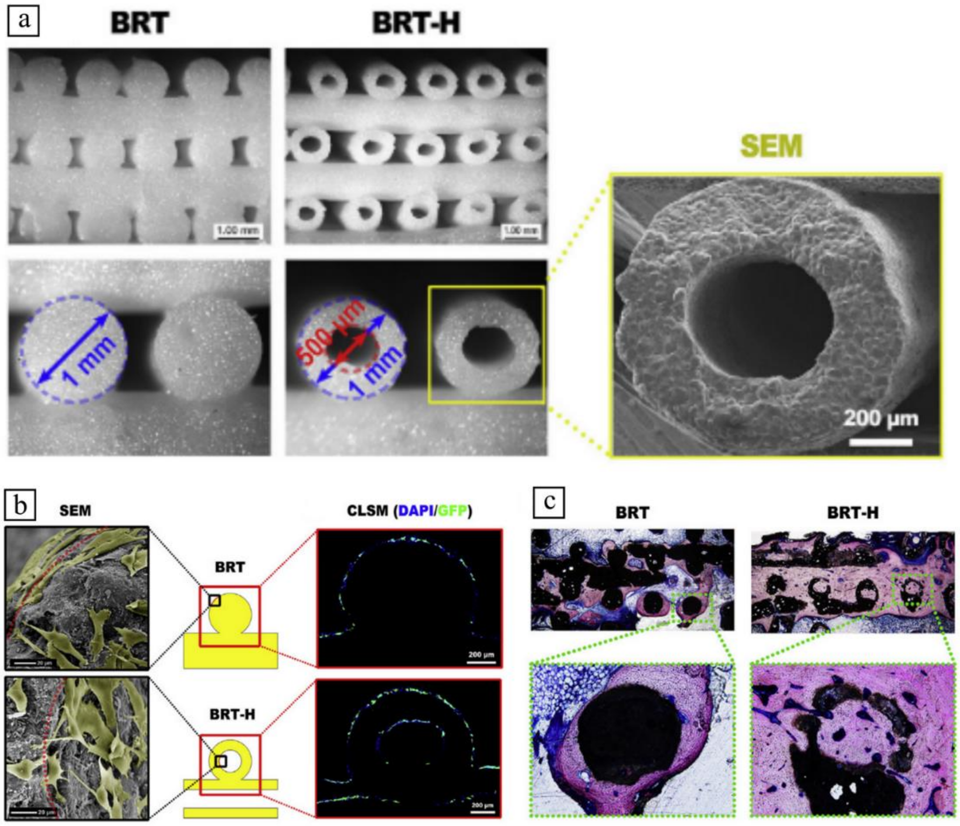

Figure 2.3.12. Cell proliferation and new bone formation in scaffolds with dense and vs. hollow struts [82].

All these previous studies are very encouraging and demonstrate the viability of the proposed strategy to obtain novel versatile candidates for the intended application. If these innovative core/shell ceramic/polymer hybrid scaffolds can be successfully optimized, they have the potential to become ideal scaffolds for the clinical treatment of orthopaedic bone defects. And the present work constitutes a solid step toward accomplishing such a long sought-after goal.

2.4. References

[1] W. Wang, K.W.K. Yeung, Bioactive Materials Bone grafts and biomaterials substitutes for bone defect repair : A review, Bioact. Mater. 2 (2017) 224–247. doi:10.1016/j.bioactmat.2017.05.007.

[2] J. Henkel, M.A. Woodruff, D.R. Epari, R. Steck, V. Glatt, I.C. Dickinson, F.M. Choong, M.A. Schuetz, D.W. Hutmacher, Bone Regeneration Based on Tissue Engineering Conceptions – A 21st Century Perspective, Nat. Publ. Gr. (2013) 216–248. doi:10.4248/BR201303002.

[3] R. Florencio-Silva, G.R. da S. Sasso, E. Sasso-Cerri, M.J. Simões, P.S. Cerri, Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells, Biomed Res. Int. 2015 (2015) 17. doi:10.1155/2015/421746.

[4] M. Sadat-Shojai, M.T. Khorasani, E. Dinpanah-Khoshdargi, A. Jamshidi, Synthesis methods for nanosized hydroxyapatite with diverse structures, Acta Biomater. 9 (2013) 7591–7621. doi:10.1016/j.actbio.2013.04.012.

[5] E. Provaggi, C. Capelli, J.J.H. Leong, D.M. Kalaskar, A UK-based pilot study of current surgical practice and implant preferences in lumbar fusion surgery, Med. (United States). 97 (2018). doi:10.1097/MD.0000000000011169.

[6] U.G.K. Wegst, M.F. Ashby, The mechanical efficiency of natural materials, Philos. Mag. 84 (2004) 2167–2186. doi:10.1080/14786430410001680935.

[7] Qiang Fu, E. Saiz, M.N. Rahaman, A.P. Tomsia, Bioactive glass scaffolds for bone tissue engineering: state of the art and future perspectives, Mater. Sci. Eng. C. Mater. Biol. Appl. 31 (2011) 1245–1256. doi:10.1016/j.msec.2011.04.022.

[8] A.J. Wagoner Johnson, B.A. Herschler, A review of the mechanical behavior of CaP and CaP / polymer composites for applications in bone replacement and repair, Acta Biomater. 7 (2011) 16–30. doi:https://10.1016/j.actbio.2010.07.012.

[9] D.W. Hutmacher, J.T. Schantz, C. Xu, F. Lam, K.C. Tan, T.C. Lim, State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective, (2007) 245–260. doi:10.1002/term.

[10] B. Thavornyutikarn, N. Chantarapanich, K. Sitthiseripratip, G.A. Thouas, Q. Chen, Bone tissue engineering scaffolding: computer-aided scaffolding techniques, 2014. doi:10.1007/s40204-014-0026-7.

[11] V. Tollemar, Z.J. Collier, M.K. Mohammed, M.J. Lee, G.A. Ameer, R.R. Reid, Stem cells, growth factors and scaffolds in craniofacial regenerative medicine, Genes Dis. 3 (2016) 56–71. doi:10.1016/j.gendis.2015.09.004.

[12] E. Malikmammadov, T.E. Tanir, A. Kiziltay, V. Hasirci, N. Hasirci, PCL and PCL-based materials in biomedical applications, J. Biomater. Sci. Polym. Ed. 29 (2018) 863–893. doi:10.1080/09205063.2017.1394711.

[13] M. Abrisham, M. Noroozi, M. Panahi-Sarmad, M. Arjmand, V. Goodarzi, Y. Shakeri, H. Golbaten-Mofrad, P. Dehghan, A. Seyfi Sahzabi, M. Sadri, L. Uzun, The role of polycaprolactone-triol (PCL-T) in biomedical applications: A state-of-the-art review, Eur. Polym. J. 131 (2020). doi:10.1016/j.eurpolymj.2020.109701.

[14] M.A. Woodruff, D.W. Hutmacher, The return of a forgotten polymer - Polycaprolactone in the 21st century, Prog. Polym. Sci. 35 (2010) 1217–1256. doi:10.1016/j.progpolymsci.2010.04.002.

[15] B. Dhandayuthapani, Y. Yoshida, T. Maekawa, D.S. Kumar, Polymeric Scaffolds in Tissue Engineering Application : A Review, 2011 (2011). doi:10.1155/2011/290602.

[16] M. Peroglio, L. Gremillard, J. Chevalier, L. Chazeau, C. Gauthier, T. Hamaide, Toughening of bio-ceramics scaffolds by polymer coating, J. Eur. Ceram. Soc. 27 (2007) 2679–2685. doi:http://10.1016/j.jeurceramsoc.2006.10.016.

[17] A.J. Sawdon, C.A. Peng, Ring-opening polymerization of ε-caprolactone initiated by ganciclovir (GCV) for the preparation of GCV-tagged polymeric micelles, Molecules. 20 (2015) 2857–2867. doi:10.3390/molecules20022857.

[18] M. Bartnikowski, T.R. Dargaville, S. Ivanovski, D.W. Hutmacher, Degradation mechanisms of polycaprolactone in the context of chemistry, geometry and environment, Prog. Polym. Sci. 96 (2019) 1–20. doi:10.1016/j.progpolymsci.2019.05.004.

[19] L. Yildirimer, A.M. Seifalian, Three-dimensional biomaterial degradation - Material choice, design and extrinsic factor considerations, Biotechnol. Adv. 32 (2014) 984–999. doi:10.1016/j.biotechadv.2014.04.014.

[20] L.E. Rustom, M.J. Poellmann, A.J. Wagoner, Mineralization in micropores of calcium phosphate scaffolds, Acta Biomater. 83 (2019) 435–455. doi:10.1016/j.actbio.2018.11.003.

[21] J. Jeong, J.H. Kim, J.H. Shim, N.S. Hwang, C.Y. Heo, Bioactive calcium phosphate materials and applications in bone regeneration, (2019) 1–11.

[22] S. V. Dorozhkin, Multiphasic calcium orthophosphate (CaPO4) bioceramics and their biomedical applications, Ceram. Int. 42 (2016) 6529–6554. doi:10.1016/j.ceramint.2016.01.062.

[23] S. Ramesh, C.Y. Tan, M. Hamdi, I. Sopyan, W.D. Teng, The influence of Ca/P ratio on the properties of hydroxyapatite bioceramics, Int. Conf. Smart Mater. Nanotechnol. Eng. 6423 (2007) 64233A. doi:10.1117/12.779890.

[24] S. V Dorozhkin, Functional Biomaterials Calcium Orthophosphates as Bioceramics : State of the Art, (2010) 22–107. doi:10.3390/jfb1010022.

[25] V.S. Kattimani, S. Kondaka, K.P. Lingamaneni, Hydroxyapatite–-Past, Present, and Future in Bone Regeneration, Bone Tissue Regen. Insights. 7 (2016) BTRI.S36138. doi:10.4137/btri.s36138.

[26] G. Muralithran, S. Ramesh, The Effects of sintering temperature on the properties of hydroxyapatite, Ceram. Int. 26 (2000) 221–230. doi:10.1016/S0272-8842(99)00046-2.

[27] C. Shuai, Y. Zhou, Y. Yang, P. Feng, L. Liu, C. He, M. Zhao, S. Yang, C. Gao, P. Wu, Biodegradation resistance and bioactivity of hydroxyapatite enhanced Mg-Zn composites via selective laser melting, Materials (Basel). 10 (2017). doi:10.3390/ma10030307.

[28] M. Ceramics, V.A. Dubok, Bioceramics - yesterday, today, tomorrow, 39 (2001) 381–394.

[29] I.S. Cho, H.S. Ryu, J.R. Kim, D.W. Kim, K.S. Hong, Sintering behavior and microwave dielectric properties of tricalcium phosphate polymorphs, Japanese J. Appl. Physics, Part 1 Regul. Pap. Short Notes Rev. Pap. 46 (2007) 2999–3003. doi:10.1143/JJAP.46.2999.

[30] A. Barba, A. Diez-escudero, Y. Maazouz, K. Rappe, E.B. Montufar, M. Bonany, J.M. Sadowska, J. Guillem-marti, O. Caroline, C. Persson, M. Manzanares, J. Franch, M. Ginebra, Osteoinduction by Foamed and 3D-Printed Calcium Phosphate Scaffolds : Effect of Nanostructure and Pore Architecture, (2017). doi:10.1021/acsami.7b14175.

[31] B.R. Lawn, Fracture of Brittle Solids, 2nd-ed ed., 1993. doi:https://doi.org/10.1017/CBO9780511623127.

[32] P. Miranda, A. Pajares, F. Guiberteau, Finite element modeling as a tool for predicting the fracture behavior of robocast scaffolds, Acta Biomater. 4 (2008) 1715–1724. doi:https://10.1016/j.actbio.2008.05.020.

[33] H.K. Chang, W.C. Huang, T.H. Tu, L.Y. Fay, C.H. Kuo, C.C. Chang, C.L. Wu, J.F. Lirng, J.C. Wu, H. Cheng, S.M. Hsu, Radiological and clinical outcomes of 3-level cervical disc arthroplasty, J. Neurosurg. Spine SPI. 32 (n.d.) 174–181. doi:10.3171/2019.8.SPINE19545.

[34] S. Eqtesadi, A. Motealleh, R.W. Wendelbo, A.L. Ortiz, P. Miranda, Reinforcement with reduced graphene oxide of bioactive glass scaffolds fabricated by robocasting, J. Eur. Ceram. Soc. 37 (2017) 3695–3704. doi:10.1016/j.jeurceramsoc.2016.12.047.

[35] P. Miranda, A. Pajares, E. Saiz, A.P. Tomsia, F. Guiberteau, Mechanical properties of calcium phosphate scaffolds fabricated by robocasting, J. Biomed. Mater. Res. Part B Appl. Biomater. 85 218–227. (2007). doi:10.1002/jbm.a.31587.

[36] F.J. Martínez-Vázquez, F.H. Perera, P. Miranda, A. Pajares, F. Guiberteau, Improving the compressive strength of bioceramic robocast scaffolds by polymer infiltration, Acta Biomater. 6 (2010) 4361–4368. doi:10.1016/j.actbio.2010.05.024.

[37] F.H. Perera, I. Van Der Meulen, A. Heise, F.J. Martínez-Vázquez, A. Pajares, P. Miranda, Impregnation of β-tricalcium phosphate robocast scaffolds by in situ polymerization, J. Biomed. Mater. Res. A. (2013) 3086–3096. doi:https://10.1002/jbm.a.34609.

[38] F.J. Martínez-Vázquez, A. Pajares, P. Miranda, Effect of the drying process on the compressive strength and cell proliferation of hydroxyapatite-derived scaffolds, (2017). doi:10.1111/ijac.12755.

[39] S. Eqtesadi, A. Motealleh, P. Miranda, A. Pajares, A. Lemos, J.M.F. Ferreira, Robocasting of 45S5 bioactive glass scaffolds for bone tissue engineering, J. Eur. Ceram. Soc. 34 (2014) 107–118. doi:10.1016/j.jeurceramsoc.2013.08.003.

[40] Q. Chen, D. Mohn, W.J. Stark, Optimization of Bioglass® scaffold fabrication process, J. Am. Ceram. Soc. 94 (2011) 4184–4190. doi:10.1111/j.1551-2916.2011.04766.x.

[41] T.S. Keller, Predicting the compressive mechanical behavior of bone, J. Biomech. 27 (1994) 1159–1168. doi:10.1016/0021-9290(94)90056-6.

[42] Y. Lakhdar, C. Tuck, J. Binner, A. Terry, R. Goodridge, Additive manufacturing of advanced ceramic materials, Prog. Mater. Sci. 116 (2021) 100736. doi:10.1016/j.pmatsci.2020.100736.

[43] M. Technologies, Additive Manufacturing Technologies 1,2, (2013) 2–4. doi:10.1520/F2792-12A.2.

[44] N. Travitzky, A. Bonet, B. Dermeik, T. Fey, I. Filbert-Demut, L. Schlier, T. Schlordt, P. Greil, Additive manufacturing of ceramic-based materials, in: Adv. Eng. Mater., 2014: pp. 729–754. doi:10.1002/adem.201400097.

[45] Z. Chen, Z. Li, J. Li, C. Liu, C. Lao, Y. Fu, C. Liu, Y. Li, P. Wang, Y. He, 3D printing of ceramics : A review, J. Eur. Ceram. Soc. 39 (2019) 661–687. doi:10.1016/j.jeurceramsoc.2018.11.013.

[46] B. Derby, 3D Printing — Review Additive Manufacture of Ceramics Components by Inkjet Printing, Engineering. 1 (2015) 113–123. doi:10.15302/J-ENG-2015014.

[47] H. Yves-Christian, W. Jan, M. Wilhelm, W. Konrad, P. Reinhart, Net shaped high performance oxide ceramic parts by Selective Laser Melting, Phys. Procedia. 5 (2010) 587–594. doi:10.1016/j.phpro.2010.08.086.

[48] A. Zocca, C.M. Gomes, E. Bernardo, R. Müller, J. Günster, P. Colombo, LAS glass-ceramic scaffolds by three-dimensional printing, J. Eur. Ceram. Soc. 33 (2013) 1525–1533. doi:10.1016/j.jeurceramsoc.2012.12.012.

[49] C. Griffin, J. Daufenbach, S. McMillin, Solid Freeform Fabrication of Functional Ceramic Components Using a Laminated Object Manufacturing Technique, Solid Free. Fabr. Conf. (1994) 17–25.

[50] H. Yang, S. Yang, X. Chi, J.R.G. Evans, Fine ceramic lattices prepared by extrusion freeforming, J. Biomed. Mater. Res. - Part B Appl. Biomater. 79 (2006) 116–121. doi:10.1002/jbm.b.30520.

[51] S. Onagoruwa, S. Bose, A. Bandyopadhyay, Fused deposition of ceramics (FDC) and composites, Proc. Solid Free. Fabr. Symp. (2001) 224–231. http://edge.rit.edu/edge/P10551/public/SFF/SFF 2001 Proceedings/2001 SFF Papers/26-Onagoruwa.pdf.

[52] H. Zhong, X. Yao, Y. Zhu, J. Zhang, D. Jiang, J. Chen, Z. Chen, X. Liu, Z. Huang, Preparation of SiC ceramics by laminated object manufacturing and pressureless sintering, J. Ceram. Sci. Technol. 6 (2015) 133–140. doi:10.4416/JCST2014-00049.

[53] J. Cesarano, A review of robocasting technology, Mater. Res. Soc. Symp. - Proc. 542 (1999) 133–139. doi:10.1557/proc-542-133.

[54] J.E. Smay, J. Cesarano, J.A. Lewis, Colloidal inks for directed assembly of 3-D periodic structures, Langmuir. 18 (2002) 5429–5437. doi:10.1021/la0257135.

[55] P. Miranda, A. Pajares, E. Saiz, A.P. Tomsia, F. Guiberteau, Fracture modes under uniaxial compression in hydroxyapatite scaffolds fabricated by robocasting, Wiley Intersci. (2007). doi:https://10.1002/jbm.a.

[56] J.A. Lewis, J.E. Smay, J. Stuecker, J. Cesarano, Direct ink writing of three-dimensional ceramic structures, J. Am. Ceram. Soc. 89 (2006) 3599–3609. doi:10.1111/j.1551-2916.2006.01382.x.

[57] B.P. Colombo, J. Schmidt, G. Franchin, A. Zocca, Additive manufacturing techniques for fabricating complex ceramic components from preceramic polymers, (n.d.).

[58] M. Schwentenwein, J. Homa, Additive manufacturing of dense alumina ceramics, Int. J. Appl. Ceram. Technol. 12 (2015) 1–7. doi:10.1111/ijac.12319.

[59] H. Gojzewski, Z. Guo, W. Grzelachowska, M.G. Ridwan, M.A. Hempenius, D.W. Grijpma, G.J. Vancso, Layer-by-Layer Printing of Photopolymers in 3D: How Weak is the Interface?, ACS Appl. Mater. Interfaces. 12 (2020) 8908–8914. doi:10.1021/acsami.9b22272.

[60] J.W. Halloran, V. Tomeckova, S. Gentry, S. Das, P. Cilino, D. Yuan, R. Guo, A. Rudraraju, P. Shao, T. Wu, T.R. Alabi, W. Baker, D. Legdzina, D. Wolski, W.R. Zimbeck, D. Long, Photopolymerization of powder suspensions for shaping ceramics, J. Eur. Ceram. Soc. 31 (2011) 2613–2619. doi:10.1016/j.jeurceramsoc.2010.12.003.

[61] Rapid prototyping & manufacturing— Fundamentals of stereolithography: Paul F. Jacobs, principal author © 1992 Society of Manufacturing Engineers xiv + 434 pp. $76.00 ($65.00 to SME members), J. Manuf. Syst. 12 (1993) 430–433. doi:https://doi.org/10.1016/0278-6125(93)90311-G.

[62] V. Tomeckova, J.W. Halloran, Flow behavior of polymerizable ceramic suspensions as function of ceramic volume fraction and temperature, J. Eur. Ceram. Soc. 31 (2011) 2535–2542. doi:10.1016/j.jeurceramsoc.2011.01.019.

[63] M. Peroglio, L. Gremillard, C. Gauthier, L. Chazeau, S. Verrier, M. Alini, J. Chevalier, Mechanical properties and cytocompatibility of poly(e-caprolactone)-infiltrated biphasic calcium phosphate scaffolds with bimodal pore distribution, Acta Biomater. 6 (2010) 4369–4379. doi:http://10.1016/j.actbio.2010.05.022.

[64] S. Eqtesadi, A. Motealleh, A. Pajares, F. Guiberteau, P. Miranda, Improving mechanical properties of 13-93 bioactive glass robocast scaffold by poly (lactic acid) and poly (ε-caprolactone) melt infiltration, J. Non. Cryst. Solids. 432 (2016) 111–119. doi:10.1016/j.jnoncrysol.2015.02.025.

[65] F.J. Martínez-Vázquez, A. Pajares, F. Guiberteau, P. Miranda, Effect of Polymer Infiltration on the Flexural Behavior of β-Tricalcium Phosphate Robocast Scaffolds, Materials (Basel). 7 (2014) 4001–4018. doi:https://10.3390/ma7054001.

[66] F.J. Martínez-Vázquez, P. Miranda, F. Guiberteau, A. Pajares, Reinforcing bioceramic scaffolds with in situ synthesized e -polycaprolactone coatings, (2013) 3551–3559. doi:https://10.1002/jbm.a.34657.

[67] S. Eqtesadi, A. Motealleh, A. Pajares, F. Guiberteau, P. Miranda, Influence of sintering temperature on the mechanical properties of ε-PCL-impregnated 45S5 bioglass-derived scaffolds fabricated by robocasting, J. Eur. Ceram. Soc. 35 (2015) 3985–3993. doi:10.1016/j.jeurceramsoc.2015.06.021.

[68] S. Eqtesadi, A. Motealleh, F.H. Perera, A. Pajares, P. Miranda, Poly-(lactic acid) infiltration of 45S5 Bioglass® robocast scaffolds: Chemical interaction and its deleterious effect in mechanical enhancement, Mater. Lett. 163 (2016) 196–200. doi:10.1016/j.matlet.2015.10.073.

[69] L.L. Hench, Biomaterials, Artificial Organs and Tissue Engineering, in: L.L. Hench, J.R.B.T.B. Jones (Eds.), Woodhead Publ. Ser. Biomater., 2005: pp. 79–89. doi:https://doi.org/10.1533/9781845690861.2.79.

[70] D. Taylor, J.H. Kuiper, The prediction of stress fractures using a “stressed volume” concept, J. Orthop. Res. 19 (2001) 919–926. doi:https://10.1016/S0736-0266(01)00009-2.

[71] T. M. Keaveny, W.C. Hayes, T.M. Keaveny, W.C. Hayes, Mechanical properties of cortical and trabecular bone, in: Bone, 1993: pp. 285–344.

[72] T. Yu, Q. Liu, T. Jiang, X. Wang, Y. Yang, Y. Kang, Channeled β-TCP Scaffolds Promoted Vascularization and Bone Augmentation in Mandible of Beagle Dogs, Adv. Funct. Mater. 26 (2016) 6719–6727. doi:10.1002/adfm.201602631.

[73] D. Han, A.J. Steckl, Coaxial Electrospinning Formation of Complex Polymer Fibers and their Applications, Chempluschem. 84 (2019) 1453–1497. doi:10.1002/cplu.201900281.

[74] L. Moroni, R. Schotel, J. Sohier, J.R. de Wijn, C.A. van Blitterswijk, Polymer hollow fiber three-dimensional matrices with controllable cavity and shell thickness, Biomaterials. 27 (2006) 5918–5926. doi:10.1016/j.biomaterials.2006.08.015.

[75] Y. Luo, A. Lode, M. Gelinsky, Direct plotting of three-dimensional hollow fiber scaffolds based on concentrated alginate pastes for tissue engineering, Adv. Healthc. Mater. 2 (2013) 777–783. doi:http://10.1002/adhm.201200303.

[76] Y. Luo, D. Zhai, Z. Huan, H. Zhu, L. Xia, J. Chang, C. Wu, Three-Dimensional Printing of Hollow-Struts-Packed Bioceramic Scaffolds for Bone Regeneration, ACS Appl. Mater. Interfaces. 7 (2015) 24377–24383. doi:https://10.1021/acsami.5b08911.

[77] R. Cornock, S. Beirne, B. Thompson, G.G. Wallace, Coaxial additive manufacture of biomaterial composite scaffolds for tissue engineering, Biofabrication. 6 (2014). doi:10.1088/1758-5082/6/2/025002.

[78] T. Schlordt, S. Schwanke, F. Keppner, T. Fey, N. Travitzky, P. Greil, Robocasting of alumina hollow filament lattice structures, J. Eur. Ceram. Soc. 33 (2013) 3243–3248. doi:10.1016/j.jeurceramsoc.2013.06.001.

[79] Y. Moon, I. Choi, Y. Koh, H. Kim, Macroporous alumina scaffolds consisting of highly microporous hollow filaments using three-dimensional ceramic / camphene-based, J. Eur. Ceram. Soc. 35 (2015) 4623–4627. doi:http://10.1016/j.jeurceramsoc.2015.08.017.

[80] J. Mueller, J.R. Raney, K. Shea, J.A. Lewis, Architected Lattices with High Stiffness and Toughness via Multicore – Shell 3D Printing, Adv. Mater. 1705001 (2018) 1–8. doi:10.1002/adma.201705001.

[81] Q. Gao, Y. He, J. zhong Fu, A. Liu, L. Ma, Coaxial nozzle-assisted 3D bioprinting with built-in microchannels for nutrients delivery, Biomaterials. 61 (2015) 203–215. doi:10.1016/j.biomaterials.2015.05.031.

[82] W. Zhang, C. Feng, G. Yang, G. Li, X. Ding, S. Wang, Y. Dou, Z. Zhang, J. Chang, C. Wu, X. Jiang, 3D-printed scaffolds with synergistic effect of hollow-pipe structure and bioactive ions for vascularized bone regeneration, Biomaterials. 135 (2017) 85–95. doi:https://10.1016/j.biomaterials.2017.05.005.

[83] I.H. Jo, Y.H. Koh, H.E. Kim, Coextrusion-based 3D plotting of ceramic pastes for porous calcium phosphate scaffolds comprised of hollow filaments, Materials (Basel). 11 (2018). doi:http://10.3390/ma11060911.

3. Hybrid core-shell structures via DIW and polymer infiltration

This chapter describes the first part of the research carried out in this thesis, dealing with the fabrication by DIW of hollow structures made of calcium phosphate ceramics (β–TCP or HA) (Fig. 3.1a) and their subsequent infiltration by PCL (Fig. 3.1b). Two publications derived from this research are presented in the following sections, with the style adapted to this document (i.e. not using the final format of the journals). In the first work, described in section 3.1, hollow strut HA scaffolds were fabricated using a coaxial nozzle. A peristaltic pump was used to circulate the paraffin oil contained in the printing bath through the internal channel of the nozzle while the extrusion of the ceramic paste is performed to avoid a collapse of the tubes due to differential pressure. Injection of PCL solutions was used as the method to impregnate the resulting strut channels. The effect of design parameters (struts arrangement) and testing conditions (load orientation) on their mechanical performance are evaluated. In the second work (section 3.2), hollow strut TCP scaffolds were fabricated analogously, but using a sacrificial graphite ink for co-extrusion instead. In this case the polymeric cores were produced by suction of molten PCL.

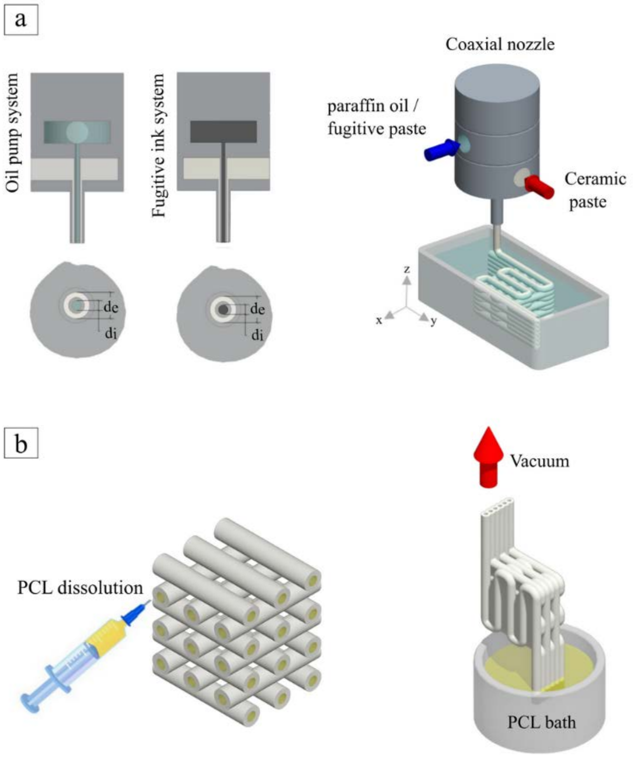

Figure 3.1. Two extrusion systems employing coaxial nozzles. (a) Schematic of the nozzles for both methods and schematic of the printing of a structure in the bath.(b) Schematic of the infiltration by injection of PCL solution and by suction of polymer melt.

3.1. Development by robocasting and mechanical characterization of hybrid HA/PCL coaxial scaffolds for biomedical applications

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Abstract

Porous structures consisting of a tetragonal three-dimensional mesh of interpenetrating coaxial tubes were fabricated by robocasting from hydroxyapatite (HA) inks. After sintering the structures, polycaprolactone (PCL) was infiltrated within the tubes core by injection of a polymer solution. The addition of the polymer enhanced the mechanical performance in terms of toughness over dense- and hollow-strut all-ceramic scaffolds, specially under bending stresses. PCL impregnation improved also the compressive strength over hollow-strut scaffolds —although dense-strut structures remained stronger especially in compression. Thus, this coaxial core-shell strut configuration combines the best features of each material: the necessary stiffness and excellent osteoconductivity of the bioceramic, with the high toughness and ductility of the biopolymer; and allows the fabrication of hybrid scaffolds with the interconnected macroporosity necessary for cell ingrowth. Hence, this work successfully provides a proof-of-concept of this novel strategy for the mechanical enhancement of bioceramic-based scaffolds while preserving their osteoconductive properties.

Keywords: Coaxial scaffolds; Robocasting; Polymer impregnation; Mechanical properties; Hydroxyapatite; Polycaprolactone.

3.2. Novel strategy for toughening robocast bioceramic scaffolds using polymeric cores

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Abstract

A novel method for the fabrication of hybrid polymer/ceramic porous scaffolds with core/shell struts is developed. Robocasting with coaxial needles is used to deposit beta-tricalcium phosphate scaffolds with hollow rods, which are subsequently filled with a polycaprolactone melt by suction. The polymeric core provides outstanding improvement of the toughness of the structure in bending, with strain energy density increasing nearly two orders of magnitude and continuous polymeric fibres holding the structure together even after large deflections. Moreover, flexural strength is not significantly reduced compared to dense-strut structures; and the macroporosity of the scaffold and osteoconductivity of bioceramic surfaces are preserved.

Keywords: Composite scaffolds; ceramic; polymer; mechanical properties; bending test; robocasting; coextrusion

4. Hybrid core-shell structures via DLP and polymer infiltration.

In this chapter the second group of publications derived from this thesis are presented. In these works, DLP was used for the fabrication of the hollow-strut structures as a more versatile process (Fig. 4.1) capable of overcoming some of the inherent limitations of the DIW technique, facilitating changes in the design of the core/shell structure. The first work, described in the section 4.1, evaluated the limitations of the DLP technique in the fabrication of simple porous structures, together with the effect of design parameters (pore and strut sizes) and testing conditions (load orientation) on their mechanical performance. The following studies exploited the versatility of DLP technology to analyse the effect of different strut architectural parameters on the mechanical performance of hybrid scaffolds. In the first one (section 4.2), the effect of the diameter of the internal channels was studied both experimentally and numerically through FEM simulations. In the third, as yet unpublished study (section 4.3) a different architecture with interconnected struts was analysed and the solid loading of the DLP feedstock was improved. Full impregnation of the cores was achieved in these last two studies by suction of a PCL melt (Fig. 19b), a process previously optimized for DIW (Section 3.2).

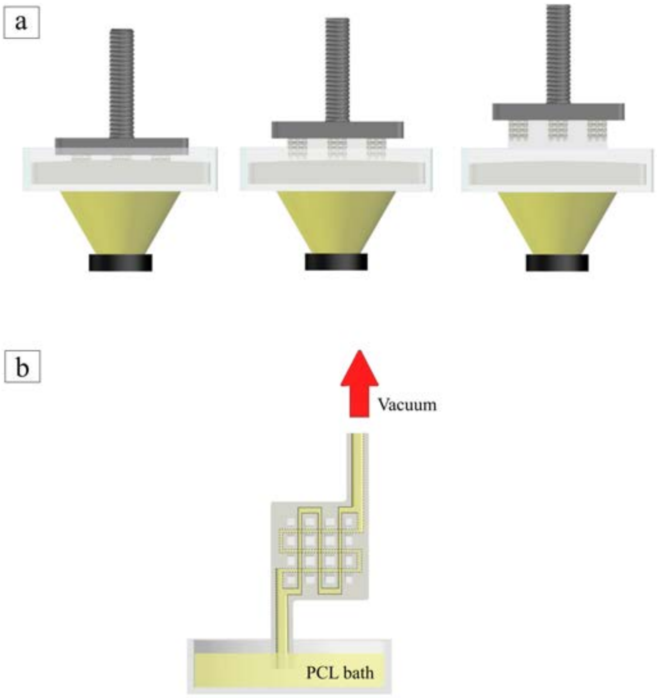

Figure 4.1. Schematics of (a) the printing process of an array of scaffolds by DLP and (b) the infiltration process by suction of a PCL melt.

4.1. Evaluation of Direct Light Processing for the fabrication of bioactive ceramic scaffolds: Effect of pore/strut size on manufacturability and mechanical performance

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Abstract

Bioactive ceramic scaffolds for bone regeneration consisting of a three-dimensional mesh of interpenetrating struts with square section were fabricated via Digital Light Processing (DLP). The ability of the technique to manufacture 3D porous structures from β-tricalcium phosphate (β-TCP) powders with different dimensions of struts and pores was evaluated, and the possibilities and limitations of the manufacturing process are identified. In particular, small pore sizes were found to seriously complicate the elimination of excess slurry from the scaffold's innermost pores. The impact of the variation of the strut/pore size on the mechanical performance of the scaffolds under compressive stresses was also evaluated, but no significant effect was found. Under compressive stresses, the structures were found to be weaker when tested perpendicularly to the printing plane due to interlayer shear failure. Interlayer superficial grooves are proposed as potential failure-controlling defects, which could also explain the lack of a Weibull size effect on the mechanical strength of the fabricated DLP scaffolds.

Keywords: Scaffolds; Ceramic; Mechanical properties; Digital Light Processing

4.2. Using ductile cores for enhancing the mechanical performance of hollow strut β-TCP scaffolds fabricated by Digital Light Processing.

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Abstract

Hybrid scaffolds consisting of a three-dimensional network of hollow struts of β-tricalcium phosphate (β-TCP) with cores filled with polycaprolactone (PCL) were fabricated. For the development of the ceramic structures the additive manufacturing technique known as Digital Light Processing (DLP) was employed. After sintering, cores were infiltrated with molten PCL by suction. The enhancement of the scaffolds´ compressive strength and toughness upon infiltration was evaluated through uniaxial compression tests. The effect of the core diameter on the mechanical performance was also analyzed. Moreover, a comprehensive finite element analysis was carried out to broaden the study on the effect of core/shell respective diameters and to analyze the role of the modulus of the core material on the strength of the hybrid structure.

Keywords: composite scaffolds; ceramic; polymer; mechanical properties; digital light processing, finite element method

4.3. Co-continuous calcium phosphate/polycaprolactone bone scaffolds fabricated by Digital Light Processing and polymer melt suction

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Abstract

Hybrid scaffolds consisting of a cartesian mesh of interpenetrated struts consisting of polycaprolactone (PCL) and β-tricalcium phosphate (β-TCP) in a core-shell configuration were developed in this study. First, photocurable slurries with up to 40 vol.% of TCP powders thanks to the addition of camphor to the mixture, which drastically reduced its viscosity. Using this optimized feedstock, ceramic matrices of hollow struts were fabricated via Digital light processing (DLP), sintered and subsequently infiltrated with molten PCL via suction. A continuous polymeric phase occupying the internal channels of the bioceramic struts was thus obtained throughout the structure. The mechanical performance of scaffolds with this novel architecture was evaluated experimentally under compressive and bending loads, and the results were analysed under the light of the developed stress fields as calculated by finite element numerical simulations. The benefits of incorporating the polymeric core were evidenced as a notable increase in toughness. This novel co-continuous configuration significantly facilitates the impregnation process and should improve the isotropy of the reinforcement provided by the polymeric core, but significantly increases the stresses in the ceramic shell. However, scaffolds strength was not significantly reduced in this study compared to preceding works, thanks to the significantly increased solid loading in the feedstock.

Keywords: composite scaffolds; ceramic; polymer; mechanical properties; digital light processing; finite element simulations.

5. Summary of results and discussion

As required by the Regulations for Doctoral Studies of the University of Extremadura, this section provides a structured summary of the main results derived from this thesis and their discussion. The main conclusions that emanate from the work are subsequently compiled in the following section.

Novel hybrid organic/inorganic core/shell scaffolds for bone tissue engineering applications have been developed in this thesis. Additive manufacturing, either DIW or DLP, have been used for the fabrication of porous bioceramic (HA or TCP) scaffolds with hollow struts which were subsequently impregnated with a biodegradable polymer (PCL). Different methods were evaluated also for the infiltration of the biodegradable polymer into the bioceramic struts' internal channels so that the desired core/shell composite structures could be obtained. The mechanical behavior, under compressive and/or bending loads, of all the materials fabricated was evaluated experimentally. FEM numerical calculations of the stress fields developed in compression were used in the analysis and extension of the experimental results.

First, porous structures consisting of a tetragonal three-dimensional mesh of interpenetrating coaxial tubes were fabricated by Direct Ink Writing (DIW) from hydroxyapatite (HA) inks using the internal pressure of circulating paraffin oil to stabilize the tubular struts during scaffold deposition. After sintering the structures, polycaprolactone (PCL) was infiltrated within the tubes by injection of a polymer solution. Despite the partial occupation of the channels achieved by this procedure, the addition of the polymeric cores significantly enhanced the mechanical performance in terms of toughness over both dense- and hollow-strut all-ceramic scaffolds, especially under bending stresses. PCL impregnation also improved the compressive strength over hollow-strut scaffolds, although dense-strut structures remained stronger especially in compression.

The fabrication process was further optimized in a subsequent study by co-extruding a fugitive graphite ink together with, in this case, a beta-tricalcium phosphate (β-TCP) paste. This eliminated the cumbersome process of adjusting the circulating oil pressure and provided additional support to the tubular log-pile structures during printing, although at the expense of adding an additional debinding step to eliminate the sacrificial graphite cores. Infiltration process was also optimized, and the sintered hollow rods were filled with a PCL melt by suction. The resulting dense polymeric core provided an outstanding improvement of the structure's toughness in bending, with strain energy density increasing by nearly two orders of magnitude and continuous polymeric fibres holding the structure together even after large deflections. Moreover, flexural strength was not significantly reduced compared to dense-strut structures; and the macroporosity of the scaffold and osteoconductivity of bioceramic surfaces was preserved.

Although DIW using coaxial nozzles was demonstrated to be a suitable technique to produce bioactive ceramic scaffolds with hollow struts ready for the subsequent polymer impregnation, several limitations were found regarding the versatility on the design provided by the technique. Not only the geometrical features of the strut and internal channel (size, shape, etc.) are limited by the coaxial nozzles available, but also, the infiltration path is always a single continuous channel that, if blocked by any printing defect, results in an incomplete impregnation. For this reason, Digital Light Processing (DLP), a technique with higher nominal resolution, was evaluated as an alternative for the production of the desired core/shell structures. In a first study, the capabilities and limitations of the technique to manufacture simple 3D porous structures from β-TCP powders consisting of a three-dimensional network of interpenetrating struts with square section. In particular, small pore sizes (under 400 µm) were found to seriously complicate the elimination of any residual slurry from the scaffold's innermost pores, which already implied a limit on the attainable diameters of the internal channel in hollow-struts scaffolds. Aside from this issue, the variation of the strut/pore size had no significant effect on the strength and toughness of the scaffolds tested under uniaxial compression. However, loading direction did have a significant effect, and DLP structures were found significantly weaker when the load was applied perpendicularly to the printing plane. This was explained by the evidence found of interlayer shear failure when testing was performed in this direction, which is not uncommon in laminated structures such as those produced by DLP. Interlayer flaws produced during printing were proposed as potential failure-controlling defects, which could also explain the lack of a Weibull size effect on the strength of the DLP scaffolds.

The same fabrication procedure optimized for that initial work was then used to fabricate hollow-strut β-TCP scaffolds, with two different channel diameters, in a log-pile structure analogous to those produced by DIW. After sintering, the strut channels were impregnated with molten PCL by suction to produce hybrid scaffolds with core/shell ceramic/polymer struts. The fabricated TPC/PCL hybrid structures tested in compression exhibited an order of magnitude enhancement in toughness (strain energy density), without any significant reduction in the compressive strength over dense-strut β-TCP scaffolds. The effect of the polymeric core diameter on the amount of toughening appeared to be minor under this type of mechanical stresses. However, both the experimental results and the FEM simulations, performed to extend the range of diameters explored, indicated that increasing the size of the core severely reduces the strength of the bioceramic skeleton. Besides, FEM simulations performed varying the modulus ratio in the core/shell scaffolds under compression showed that increasing the stiffness of the core material would also contribute to enhancing the strength of the structure. However, this should not be done at the expense of sacrificing the core's ductility, since this would compromise the toughening effect it can provide.

Finally, the solid loading of the DLP feedstock was improved through the addition of camphor and oleic acid to the mixture to reduce the viscosity and avoid the sedimentation of the powder in the concentrated slurry. The improved composition was used to explore novel designs for the core/shell scaffolds. Making use of the greater versatility of DLP technique and PCL melt suction, hybrid scaffolds consisting of a cartesian mesh of interpenetrated struts with co-continuous PCL and β-TCP phases were developed. The improvement on the solid loading of the feedstock (to 40 vol. % of β-TCP powders) facilitated significantly the debinding and sintering process. The scaffold strength was also increased, although this enhancement was less evident due to the change in the structure design, which increases the stresses developed within the structure in compression. However, the new design is still deemed beneficial both in terms of facilitating the polymer infiltration and improving the isotropy of the mechanical enhancement provided by the ductile phase. Additional improvements in the strength should be attainable in future works by reinforcing and optimizing the geometry at the struts' intersections. In any case, the results of this last study demonstrated, once again, the advantages of using polymeric cores for improving the toughness of bioceramic scaffolds with the hybrid structures exhibiting a strain energy density more than one order of magnitude higher than all-ceramic (hollow or dense) counterparts in bending.

The mechanical performance, in compression and bending, of the different hybrid core/shell polymer/ceramic scaffolds developed in this thesis are compared to bone properties in Figure 5.1. Data for all-ceramic structures and conventional hybrid scaffolds produced by polymer coating or full impregnation of DIW structures from previous works by GEMA group in different material systems are included for comparison. The hybrid core-shell scaffolds produced in this work are the only structures squarely within the range of cancellous bone performance in compression (Fig. 5.1a). Nonetheless, their strength, both in compression and bending, is inferior to values achieved in coated and full-impregnated structures, scarcely matching the values of bare structures with dense struts, as already discussed. On the contrary, only fully impregnated scaffolds can compete in terms of toughness with the newly developed structures in both loading configurations, and that comes at the expense of completely sacrificing their macroporosity. Despite their increased strength, simply coated structures do not come close to achieving the same level of strain energy density demonstrated by core-shell architectures neither in compression nor in bending, since they do not have large polymeric fibers hampering crack propagation and holding the structure together at large strains.

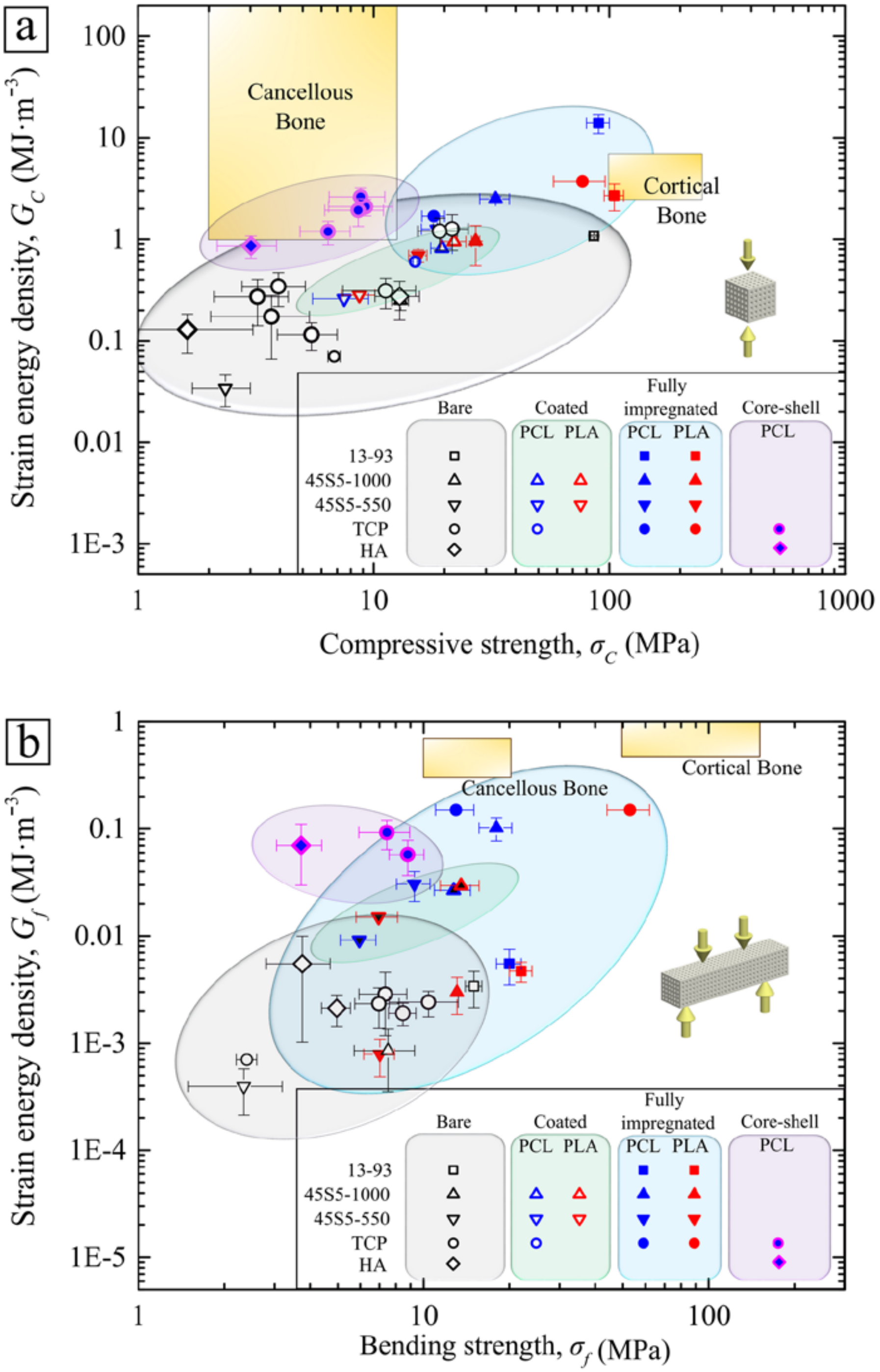

Figure 5.1. Ashby diagrams of strain energy density vs. strength in (a) compression and (b) bending for bare and hybrid scaffolds of different materials. Core-shell structures fabricated in this work (chapter 3 and 4) are compared to conventional coated and fully impregnated scaffolds [1–6] and to human bone values [7–9]. Error bars represent standard deviations.

Thus, the only weak point in terms of mechanical performance of the proposed core-shell architecture is their strength. However, as already discussed, strength of the hybrid core-shell scaffolds still has plenty of room for improvement both through the optimization of the scaffolds design and strut geometry and through appropriate material selection. Therefore, the development of bioactive materials approaching cortical bone performance seems entirely within reach.

All in all, the coaxial core-shell strut configuration has been proved to combine the best features of each material: the necessary stiffness of the bioceramic, with the high toughness and ductility of the biopolymer. Moreover, it preserves the excellent osteoconductivity of the bioceramic surfaces by keeping them directly in contact with the physiological fluids and the interconnected macroporosity of the scaffolds, necessary for cell ingrowth. Hence, this thesis successfully provides a proof-of-concept of this novel strategy for the mechanical enhancement of bioceramic-based scaffolds while preserving their biological performances, paving the way for obtaining reliable scaffolds for the repair and regeneration of bone defects in load-bearing regions of the skeleton.

5.1. References

[1] S. Eqtesadi, A. Motealleh, A. Pajares, F. Guiberteau, P. Miranda, Improving mechanical properties of 13-93 bioactive glass robocast scaffold by poly (lactic acid) and poly (ε-caprolactone) melt infiltration, J. Non. Cryst. Solids. 432 (2016) 111–119. doi:10.1016/j.jnoncrysol.2015.02.025.

[2] F.H. Perera, I. Van Der Meulen, A. Heise, F.J. Martínez-Vázquez, A. Pajares, P. Miranda, Impregnation of β-tricalcium phosphate robocast scaffolds by in situ polymerization, J. Biomed. Mater. Res. A. (2013) 3086–3096. doi:https://10.1002/jbm.a.34609.

[3] F.J. Martínez-Vázquez, A. Pajares, F. Guiberteau, P. Miranda, Effect of Polymer Infiltration on the Flexural Behavior of β-Tricalcium Phosphate Robocast Scaffolds, Materials (Basel). 7 (2014) 4001–4018. doi:https://10.3390/ma7054001.

[4] F.J. Martínez-Vázquez, P. Miranda, F. Guiberteau, A. Pajares, Reinforcing bioceramic scaffolds with in situ synthesized e -polycaprolactone coatings, (2013) 3551–3559. doi:https://10.1002/jbm.a.34657.

[5] S. Eqtesadi, A. Motealleh, A. Pajares, F. Guiberteau, P. Miranda, Influence of sintering temperature on the mechanical properties of ε-PCL-impregnated 45S5 bioglass-derived scaffolds fabricated by robocasting, J. Eur. Ceram. Soc. 35 (2015) 3985–3993. doi:10.1016/j.jeurceramsoc.2015.06.021.

[6] S. Eqtesadi, A. Motealleh, F.H. Perera, A. Pajares, P. Miranda, Poly-(lactic acid) infiltration of 45S5 Bioglass® robocast scaffolds: Chemical interaction and its deleterious effect in mechanical enhancement, Mater. Lett. 163 (2016) 196–200. doi:10.1016/j.matlet.2015.10.073.

[7] L.L. Hench, Biomaterials, Artificial Organs and Tissue Engineering, in: L.L. Hench, J.R.B.T.-B. Jones (Eds.), Woodhead Publ. Ser. Biomater., 2005: pp. 79–89. doi:https://doi.org/10.1533/9781845690861.2.79.

[8] D. Taylor, J.H. Kuiper, The prediction of stress fractures using a “stressed volume” concept, J. Orthop. Res. 19 (2001) 919–926. doi:https://10.1016/S0736-0266(01)00009-2.

[9] T. M. Keaveny, W.C. Hayes, T.M. Keaveny, W.C. Hayes, Mechanical properties of cortical and trabecular bone, in: Bone, 1993: pp. 285–344.

6. Conclusions

The most relevant conclusions of this study can be summarized as follows:

Regarding the additive manufacturing of the bioceramic preforms and their mechanical performance,

1.- Both additive manufacturing techniques analyzed in this work, DIW and DLP, have been proved to be adequate for the fabrication of scaffolds with hollow struts using calcium phosphate ceramics. Specifically, scaffolds with comparable strut dimensions in the millimeter / submillimeter range could be reproducibly obtained by both techniques. Moreover, after a sintering treatment, a successful impregnation of the internal channels with PCL using different methods was possible in both cases.

2.- Employing extrusion through coaxial nozzles, DIW allows to obtain simple log-pile structures with intact intra-strut channels, provided an appropriate material co-extruded through the internal nozzle to aid in stabilizing the tubes during assembly. Both the paraffin oil used for the printing bath or a sacrificial graphite ink can be used for this purpose. However, the oil pressure extruded through the internal channel has to be carefully adjusted for a successful printing and does not provide any support after deposition, so the use of a sacrificial ink is recommended. Nonetheless, the use of a sacrificial ink introduces an additional debinding step for its evaporation which, if not correctly performed, can lead to microcracking of the bioceramic struts.

3.- The main limitations observed in DIW were, on the one hand, the low versatility in the design of the channel/strut architecture. This is limited by the extrusion nozzle, whose shape and dimensions are not easily modifiable. And, on the other hand, more difficulties were encountered during the infiltration in DIW structures: the infiltration paths through the appendages could not be easily optimized and the obstruction of a single channel on a given strut hampered the successful infiltration of the rest of the structure. Also, some cracking could be observed in DIW structures which could in some cases produce leakage or prevent a proper suction, ruining the infiltration process completely.

4.- The higher nominal resolution of DLP and the lack of a printing nozzle, make it a much more versatile technique, which allows one to easily vary the strut shapes and dimensions and the channel architecture and interconnectivity. Scaffolds fabricated with DLP typically exhibited also generally less defects after printing. However, they require long debinding treatments to get rid of the large amounts of organics present in the feedstock without producing cracking in the bioceramic struts. Furthermore, a larger amount of organics to be burned implies greater environmental impact compared to DIW, although this is not the case when a secondary sacrificial ink is used and, subsequently, burned in DIW.

5.- Despite its nominally higher resolution, it is difficult to use DLP to considerably reduce the feature dimensions in the scaffolds. The elimination of the residual uncured resin from the pores in the structure constitutes a challenge when pore dimensions become smaller than a few hundreds of microns, so overall dimensions obtained were similar to those in DIW. Also, it is more difficult to achieve high solid-loading in the DLP resin-based feedstock suspensions without compromising their flowability. For this purpose, the addition of camphor (or another solvent) and oleic acid (as dispersant) to the mixture was proved essential, enabling ceramic concentrations similar or even greater than those used in DIW, an essential requirement to facilitate the debinding and densification processes and, thereby, increase the mechanical performance of the resulting structures.

6.- As could be expected, the strength of hollow-strut scaffolds is significantly reduced compared to the analogous dense-strut structures both for DIW and DLP. Moreover, DLP structures exhibited evidence of shear-driven failure when tested perpendicularly to the printing plane, which is attributed to the existence of interlayer defects, although these were not directly observable and both techniques showed an apparently seamless bonding between layers. The interlayers defects in DLP could further compromise the strength of the structure compared to DIW but is considered a minor drawback for the increased versatility provided by the technique in defining the architecture of the core/shell structures.

Regarding the infiltration of polymer and the mechanical performance of the resulting hybrid core/shell scaffolds,

7.- The two infiltration methods tested, injection of PCL dissolutions and suction of PCL melt, were successful when it comes to introducing the polymeric ductile phase at the strut cores. However, the use of a polymer solution produced only a partial infiltration of the polymer within channels. A polymer melt is, thus, preferable to produce a complete impregnation and obtain dense cores perfectly attached to the bioceramic walls. The high temperatures typically required to melt the suitable biodegradable polymers make the suction process a more convenient alternative to injection since it does not require the use of a heated and heat-resistant injection system. On the other hand, for the suction process to succeed, strut walls with no through-thickness open porosity are essential, so it would not be an option if the bioceramic is hard to densify or large superficial microporosities are sought. Although, in any case, large microporosities or microcracks in the strut walls can lead to polymer leakage even when using an injection process and preclude the attainment of the desired core-shell architecture.

8.- As hypothesized, the hybrid structures obtained upon infiltration exhibited a more than one order of magnitude improvement in toughness over all-ceramic scaffolds, either with hollow or dense struts, when their mechanical performance was analyzed under compressive and, especially, bending stresses. Although this effect was highly noticeable with both infiltration methods, it was more accused—at least for a core/shell diameter ratio of 0.45—when the polymer filled the internal channels, as in the case of infiltrations of polymer melt by suction. However, increasing the amount of polymer by increasing the diameter of the dense organic cores did not seem to improve the toughness of the structures any further.

9.- When analyzing the compressive strength an unquestionable improvement upon infiltration resulted in all studies, regardless of the fabrication method, solid loading in the slurry and struts architecture, in comparison to their analogous ceramic hollow-struts scaffolds. However, at best, the strength of hybrid scaffolds approached that of dense-strut structures. This is due to the severe reduction in compressive strength in hollow *vs*. dense struts scaffolds, which is more severe the larger the diameter of the internal channel is. Strategies to reduce the impact in the compressive strength of moving from dense to hybrid core/shell struts would be to reduce the core diameters, which according to the preceding paragraph might not critically compromise the toughness; and enhancing the stiffness of the core material, so it can more effectively support compressive loading and reduce the stresses in the bioceramic walls.

10.- Fortunately, the flexural strength was never affected by the presence of polymer nor great differences were found between hollow and dense strut scaffolds. Moreover, toughness improvement was even more dramatic under this more severe mechanical loading configuration, with the polymeric fibres being able to hold the scaffold in one piece even at extremely high deflections, long after catastrophic failure occurs in the ceramic structure. This is a major advantage of this novel scaffold architecture that can greatly facilitate handling during implantation and in-service reliability of future bone tissue engineering implants.

11.- The introduction of a co-continuous polymeric phase instead of the disjointed fibres found in the log-pile structures produced by DIW is expected to increase the isotropy of the reinforcement provided by the ductile core and overall integrity of the scaffold under complex mechanical loads. This did not translate, however, to any significant improvement in the strength or toughness of the structure neither in bending nor in compression. Compressive performance was, in fact, reduced since stresses were significantly increased in the bioceramic with the interpenetrated configuration compared to the log-pile structure. Therefore, further work is needed for the optimization of this scaffold architecture that, on the other hand, was found to be beneficial in terms of facilitating polymer infiltration.

All in all, the results of this thesis serve as a validation of the proposed fabrication strategy, the use of additive manufacturing (DIW and, especially, DLP) plus suction impregnation, to create hybrid scaffolds with core/shell organic/inorganic struts; composite structures exhibiting improved mechanical performance, especially in terms of toughness, while preserving the osteoconductive properties of the bioceramic material and the scaffold's pre-designed porosity intact. The mechanical performance of developed hybrid core/shell scaffolds can compete not only with porous coated structures but also with fully impregnated blocks in terms of strain energy density both in compression and in bending. Their Achilles heel is found on the strength, which barely matches that of all ceramic structures, but the performed analysis has enabled the identification of suitable strategies that can potentially strengthen the structures. On the whole, the present study lays the foundation for the future development of bioactive scaffolds capable of sustaining unprecedented levels of mechanical loads, potentially enabling the regeneration of bone defects in load bearing regions of the skeleton without resorting to external metallic fixations.

Conclusiones

Las conclusiones más importantes de este estudio pueden resumirse de la siguiente manera:

En lo referente a la manufacturación aditiva de las preformas biocerámicas y su rendimiento mecánico,

1.- Las dos técnicas de manufacturación aditiva analizadas en este trabajo, DIW y DLP, han demostrado ser adecuadas para la fabricación de andamiajes compuestos por barras huecas utilizando cerámicas de fosfato de calcio. Específicamente, andamiajes con dimensiones de barra comparables en el rango milimétrico/submilimétrico pudieron obtenerse de forma reproducible mediante ambas técnicas. Además, después de un tratamiento de sinterización, fue posible en ambos casos una impregnación satisfactoria de los canales internos con PCL utilizando métodos diferentes.

2.- Mediante la extrusión a través de boquillas coaxiales, DIW permite obtener estructuras simples de tronco-apilado con canales internos intactos, siempre y cuando un material apropiado sea co-extruido a través de la boquilla interna ayudando a estabilizar las barras durante la deposición. Para ello se puede utilizar tanto el aceite de parafina del baño de impresión como una tinta de grafito que es posteriormente eliminada. Sin embargo, la presión del aceite extruido a través del canal interno debe ajustarse cuidadosamente para que la impresión sea satisfactoria y no proporciona soporte después de la deposición, por lo que es preferible el uso de la tinta de sacrificio. No obstante, el uso de una tinta de sacrificio introduce un paso adicional de quemado para su eliminación que, si no se realiza correctamente, puede provocar microfisuras en las paredes biocerámicas

3.- Las principales limitaciones observadas en DIW fueron, por una parte, su baja versatilidad en el diseño de la arquitectura de las barras y sus canales internos que está limitado por la boquilla de extrusión, cuya forma y dimensiones no son fácilmente modificables. Y, por otra parte, las dificultades encontradas durante la infiltración en las estructuras obtenidas mediante DIW, debido a la imposibilidad de optimización de los caminos de infiltración a través de los apéndices y a que la obstrucción de un solo canal en una barra determinada dificulta la infiltración satisfactoria del resto de la estructura. Además, en estas estructuras se observaron algunas grietas que en algunos casos podían producir fugas o impedir una succión adecuada, arruinando completamente el proceso de infiltración.

4.- La mayor resolución nominal de DLP y la falta de boquilla de impresión, la convierten en una técnica mucho más versátil, que permite variar fácilmente las formas y dimensiones de las barras así como la arquitectura de los canales internos y su interconectividad. Los andamiajes fabricados con DLP suelen presentar también, por lo general, menos defectos después de la impresión. Sin embargo, requieren largos tratamientos de quemado para eliminar las grandes cantidades de sustancias orgánicas presentes en la materia prima sin producir grietas en las paredes biocerámicas. Además, una mayor cantidad de productos orgánicos a quemar implica también un mayor impacto ambiental en comparación con DIW, aunque no es así cuando se utiliza una tinta de sacrificio secundaria y que debe ser posteriormente quemada.

5.- A pesar de su resolución nominalmente más alta, es difícil utilizar DLP para reducir considerablemente las dimensiones de las características en los andamiajes. La eliminación de la resina residual no curada de los poros de la estructura constituye un desafío cuando las dimensiones de los poros se reducen a unos pocos cientos de micras, por lo que las dimensiones generales obtenidas fueron similares a las de la DLP. Además, es más difícil lograr una alta carga de sólidos en las suspensiones empleadas en DLP sin comprometer su fluidez. Para ello, se demostró esencial añadir a la mezcla alcanfor (u otro disolvente) y ácido oleico (como dispersante), lo que permite obtener concentraciones cerámicas similares o incluso superiores a las utilizadas en DIW, requisito esencial para facilitar los procesos de quemado de orgánicos y sinterizado y, de ese modo, aumentar el rendimiento mecánico de las estructuras resultantes.

6.- Como era de esperar, la resistencia de los andamiajes de barras huecas se reduce significativamente en comparación con las estructuras análogas de barras densas, tanto empleando DIW como DLP para su fabricación. Además, las estructuras de DLP exhibieron evidencias de fallo por cizallamiento cuando se ensayaron perpendicularmente al plano de impresión, lo que se atribuye a la existencia de defectos en las capas intermedias, aunque éstos no se observaron directamente y ambas técnicas mostraron una unión aparentemente sin fisuras entre capas. Los defectos de las capas intermedias en DLP podrían comprometer aún más la resistencia de la estructura en comparación con la DIW, pero éste se considera un inconveniente menor debido a la mayor versatilidad que ofrece la técnica para definir la arquitectura de las estructuras *core-shell*.

En lo referente a la infiltración del polímero y el rendimiento mecánico de los andamiajes híbridos de núcleo polimérico y vaina biocerámica resultantes,

7.- Los dos métodos de infiltración probados, la inyección de disoluciones de PCL y la succión de PCL fundido, sirvieron para introducir con éxito una fase dúctil polimérica en los núcleos de las barras. Sin embargo, mediante el uso de la solución de polímero se consiguió sólo una infiltración parcial del polímero dentro de los canales. Por lo tanto, resulta preferible el empleo de polímero fundido para conseguir una impregnación completa y obtener núcleos poliméricos densos perfectamente adheridos a las paredes biocerámicas. Las altas temperaturas que se suelen requerir para fundir polímeros biodegradables adecuados hacen que el proceso de succión sea una alternativa más conveniente que la inyección, ya que no requiere el uso de un sistema de inyección calentado y resistente a altas temperaturas. Por otra parte, para que el proceso de succión se realice con éxito, es esencial que las paredes de las barras no tengan una porosidad abierta en todo su espesor, por lo que no sería una buena opción el empleo de un material biocerámico difícil de densificar o si se buscan grandes microporosidades superficiales. Aunque, en cualquier caso, las grandes microporosidades o microfisuras en las paredes de las barras pueden dar lugar a fugas de polímeros, incluso cuando se utiliza un proceso de inyección, e impedir la consecución de la estructura deseada.

8.- Como era esperado, las estructuras híbridas obtenidas mediante infiltración mostraron una mejora de tenacidad de más de un orden de magnitud en comparación con los andamiajes totalmente cerámicos, ya sea con barras huecas o densas, cuando se analizó su rendimiento mecánico bajo esfuerzos de compresión y, especialmente, de flexión. Aunque este efecto fue muy notable empleando ambos métodos de infiltración, fue más acusado (al menos para una relación de diámetro de núcleo/vaina de 0,45) al emplear polímero fundido e infiltrar mediante succión. Sin embargo, el aumento de la cantidad de polímero mediante el incremento del diámetro de los núcleos orgánicos no pareció traducirse en una mayor mejora de la tenacidad de las estructuras.

9.- Al analizar la resistencia a compresión de las estructuras se observó una mejora incuestionable mediante la infiltración de polímero en todos los estudios, independientemente del método de fabricación, el contenido en sólidos de la suspensión cerámica y de la arquitectura de las barras empleados, en comparación con andamiajes análogos de barras huecas cerámicas. Sin embargo, en el mejor de los casos, la resistencia de los andamiajes híbridos solo se aproximó a la de las estructuras de barras densas. Esto se debe a la severa reducción de la resistencia a compresión sufrida por los andamiajes de barras huecas frente a los de barras densas, más severa cuanto mayor es el diámetro del canal interno. Las estrategias para reducir el impacto en la resistencia a compresión con el cambio de barras densas a híbridas pasarían por reducir el tamaño de los diámetros de los núcleos, que según el párrafo anterior no tendría que comprometer críticamente la tenacidad; y mejorar la rigidez del material de los núcleos, para que pueda soportar más eficazmente la carga de compresión y reducir las tensiones en las paredes biocerámicas.

10.- Afortunadamente, la resistencia a flexión nunca se vio afectada por la presencia del polímero ni se encontraron grandes diferencias entre los andamiajes de barras huecas y densas. Además, la mejora en tenacidad fue aún más significativa bajo esta configuración de carga mecánica, donde las fibras poliméricas eran capaces de mantener el andamiaje en una sola pieza incluso con deflexiones muy altas, mucho después del fallo catastrófico en la estructura cerámica externa. Esta es una gran ventaja de esta novedosa arquitectura de andamiajes híbridos que puede facilitar enormemente la manipulación durante la implantación y la fiabilidad en servicio de los futuros implantes de ingeniería de tejidos óseos.

11.- Mediante la introducción de una fase polimérica continua, en lugar de las fibras desarticuladas encontradas en las estructuras de tronco apilado obtenidas mediante DIW, se espera que aumente la isotropía del refuerzo proporcionado por el núcleo dúctil y la integridad general del andamiaje bajo cargas mecánicas complejas. Sin embargo, esto no se tradujo en una mejora significativa de la tenacidad ni resistencia de la estructura ni bajo esfuerzos de flexión ni compresión. En cambio, su rendimiento a compresión se redujo, ya que las tensiones aumentaron significativamente en la parte biocerámica con esta configuración interpenetrada en comparación con la estructura de tronco apilado. Por lo tanto, es necesario seguir trabajando para optimizar esta arquitectura de andamiaje que, por otra parte, se consideró beneficiosa en cuanto a la infiltración del polímero.

En definitiva, los resultados de esta tesis sirven para validar la estrategia de fabricación propuesta, el uso de la manufacturación aditiva (DIW y, especialmente, DLP) junto con la impregnación por succión, para crear andamiajes híbridos compuestos por barras con núcleos orgánicos y vainas inorgánicas; estructuras que exhiben un mejor rendimiento mecánico, especialmente en términos de tenacidad, al tiempo que preservan intactas las propiedades osteoconductivas del material biocerámico y la porosidad prediseñada del andamiaje. El rendimiento mecánico de los andamiajes híbridos de núcleo/vaina desarrollados puede competir no sólo con las estructuras híbridas porosas de barras cerámicas revestidas de polímero, sino también con las estructuras totalmente impregnadas en términos de densidad de energía de tensión tanto en compresión como en flexión. Su talón de Aquiles se encuentra en la resistencia, que apenas alcanza el valor de resistencia de las estructuras cerámicas, pero el análisis realizado ha permitido identificar estrategias adecuadas que pueden fortalecer potencialmente estas estructuras. En general, el presente estudio sienta las bases para el futuro desarrollo de andamiajes bioactivos capaces de soportar niveles de cargas mecánicas sin precedentes, que podrían permitir la regeneración de defectos óseos en las regiones del esqueleto que soportan cargas sin recurrir a fijaciones metálicas externas.

7. Future outlook

As in any scientific work, the present study leaves unanswered questions and opens new paths for exploration. While the performed research serves as a clear proof-of-concept for the core/shell hybrid scaffolds, further work should be done in order to optimize this novel scaffold architecture and translate their use to the clinic. Some of the most urgent topics that should be addressed in future studies on this area are highlighted below:

1.- Suitable biological testing should be performed both *in vitro* (dissolution and mineralization, cell culture, etc.) and *in vivo* to verify the expected improved biological performance of this new architecture compared to other ceramic/polymer hybrid scaffolds obtained by conventional impregnation and coating. Some initial studies on this topic are already underway.

2.- The achievement of stiffer hybrid struts is essential to reduce the loss of compressive strength with respect to scaffolds comprised of dense all-ceramic struts. To this end an optimization of the geometrical design (both internal and external shape) of the struts must be performed with the aim of minimizing the tensile and shear stresses developed in the bioceramic shell during compression. This should be possible through the combination of finite element simulations with the resolution and versatility that DLP can offer.

3.- Moreover, to the same end, a better combination of materials could be explored. Using ductile polymers with higher stiffness would provide an effective stress shielding to the fragile shells. There is, nonetheless, a limited number of compositions that could be used for a fully biodegradable scaffold, so the use of particle- or fibre-reinforced composites for the core material could be an interesting alternative. Nevertheless, composites typically exhibit reduced ductility compared to their matrices and a trade-off between enhanced toughness and stiffness/strength will surely ensue. The use of biodegradable metals as core materials could also be explored, although that will undoubtedly pose their own important technological challenges (thermal expansion mismatch, interfacial adhesion and reactivity, etc.) for the infiltration process.

4.- The same fabrication method, preform fabrication by AM plus polymer melt suction impregnation, could be used to enhance the mechanical performance of bio-inert ceramics. Structures with alumina or zirconia shells with cores occupied by biocompatible inert tough polymers such as UHMWPE or PEEK could be produced to develop porous structures that would facilitate biointegration of load-bearing implants and enhance their damage tolerance. Combination of these strong bioceramics with titanium or other metallic alloys could also be possible provided the impregnation technological challenges can be successfully addressed.

5.- Further work could be done also to improve the densification and intrinsic strength of struts. The strategy followed in this thesis for this purpose was mainly to increase the solid content in the slurries, but there are still open paths, like analyzing novel heat treatments capable of maximizing densification while reducing grain size and any associated microstructural flaws. In this regard, pressure-less spark plasma sintering (PL-SPS) and two-stage treatments could be beneficial in the sintering of calcium phosphate bioceramic scaffolds. Moreover, further improvements in the debinding process could help to minimize the interlayer defects that jeopardize the mechanical performance of scaffolds fabricated by DLP.

6.- Finally, reducing the dimensions of the struts is also a pending work. The difficulties encountered to effectively achieve core-shell scaffolds with small struts using DIW or DLP employing ceramic slurries should be solved if higher resolutions are needed for certain applications, and also for improving their intrinsic strength. Indirect 3D printing of polymeric preforms followed by slip casting of highly loaded ceramic suspensions could be a solution. On the other hand, it is technologically a challenge, since neither the impregnation / casting of the ceramic slurry into a very complex and fine structure nor the subsequent burn-out of the preform without fracturing the ceramic are straight-forward.

Abbreviations

3D	Three-dimensional
AM	Additive Manufacturing
BJ	Binder Jetting
DIP	Direct Inkjet Printing
DIW	Direct Ink Writing / Robocasting
DLP	Digital Light Processing
FDC	Fusion Deposition of Ceramics
FDM	Fusion Deposition Modeling
LOM	Laminated Object Manufacturing
PCL	Polycaprolactone
PEEK	Polyetheretherketone
PL-SPS	Pressure-less spark plasma sintering
TCP	Tricalcium phosphate
TPP	Two photon polymerization
SEM	Scanning Electron Microscope
SL	Stereolitography
SLM	Selective Laser Melting
SLS	Selective Laser Sintering
UHMWPE	Ultra-high-molecular-weight polyethylene
UV	Ultraviolet