Modulation of release kinetics by plasma polymerization of ampicillin-loaded β-TCP ceramics

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Abstract

Beta-tricalcium phosphate (β–TCP) bioceramics are employed in bone repair surgery. Their local implantation in bone defects puts them in the limelight as potential materials for local drug delivery. However, obtaining suitable release patterns fitting the required therapeutics is a challenge. Here, plasma polymerization of ampicillin-loaded β–TCP is studied for the design of a novel antibiotic delivery system. Polyethylene glycol-like (PEG-like) coating of β–TCP by low pressure plasma polymerization was performed using Diglyme as precursor, and nanometric PEG-like layers were obtained by simple and double plasma polymerization processes. Significant increase in hydrophobicity, and the presence of plasma polymer was visible on the surface by SEM and quantified by XPS. As main consequence of the plasma polymerisation, the release kinetics was successfully modified, avoiding burst release, and slowing down the initial rate of release leading to a 4.5 hours delay in reaching the same antibiotic release percentage, whilst conservation of the activity of the antibiotic was simultaneously maintained. Thus, plasma polymerisation on the surface of bioceramics may be a good strategy to design controlled drug delivery matrices for local bone therapies.

Keywords: Plasma Polymerization, Bioceramics, Drug Delivery, Bone Repair Surgery, β–Tricalcium Phosphate.

1. Introduction

β–Tricalcium Phosphate (β–TCP) is a ceramic biomaterial that has been broadly used as bone graft in reconstructive surgery due to its resorbability and ability to promote new bone formation, especially by releasing a large quantity of inorganic ions such as Ca^{2+}, indispensable for bone regeneration (Wang 1998, Chang 2000, Liu 2012). The possibility to use calcium phosphate materials not only as bone substitutes but also as carriers for local and controlled supply of drugs is very attractive, and can be useful in treatments of different skeletal diseases, such as bone tumours, osteoporosis or osteomyelitis, which normally require long and painful therapies (Ginebra 2012). Specifically, the incorporation of antibiotics in this kind of synthetic bone grafts could allow avoiding post-operatory infections. However, in this type of ceramic drug delivery systems the drugs are usually absorbed on the surface, resulting in a burst release. The development of strategies that enable controlling the drug release beyond the intrinsic properties of the ceramic, facilitating the release of drugs for more prolonged times is of great interest (Canal, 2013).

The main purpose in the design of any implantable drug delivery system consists in obtaining a controlled release of the loaded molecule in the suitable timeframe for each particular therapy; As previously mentioned, one major challenge often consists in overcoming and delaying the burst release of the drug. In this sense, plasma polymerization can be considered as a pertinent tool to obtain a nanometric biocompatible coating on drug-loaded materials, which might act as permissive membrane slowing down the release kinetics. In low pressure plasma polymerization process, the monomer is evaporated or dragged by bubbling with an inert gas, and it is pumped into the vacuum chamber. A glow discharge initiates the polymerization and the monomer molecules break apart creating free electrons, ions, excited molecules and radicals. The radicals absorb or react with the previously activated surface, condense, and polymerize on the substrate creating a thin film coating (Goodman 1960, Williams 1966, Yasuda 1985, Kushner 1987, Hegemann 2006). Plasma-deposited polymers have been extensively studied in the biomedical field for anti-fouling applications (Nisol 2014), to improve biocompatibility, to tailor the physic-chemical properties of the substrates (Yoshida, 2013), to enhance cell/surface or tuneable biomolecule/surface interactions, for tissue engineering applications (Bhatt 2015), for the modification (Cools, 2014) and the patterning (Favia 2003, Sardella 2004, Sardella 2005) of biomedical surfaces or in the design of novel drug delivery systems (Vasilev, 2011, Bhatt 2013, Labay 2015, Canal 2016).

During the plasma polymerization process, complex chemical reactions occur under the influence of the process conditions, the plasma design (De Geyter, 2011), the substrate and the monomer (Yasuda 1977, Friedrich 2011, Whittle 2012, Hegemann 2013, Michelmore, 2013, Li 2014). Selection of monomers for plasma polymerization will determine the polymers produced on the surface (Kelly 2003, Morent 2011, Michelmore, 2013). For biomedical applications, the precursor selected should yield biocompatible plasma polymers. One of the most extensively described polymer for coating of biomaterials is polyethylene glycol (PEG), and its co-polymers, for its well-known biocompatible and biodegradable properties. Plasma polymerization to obtain PEG-like coatings has been already described on various polymeric (Sakthi Kumar 2007, Nisol 2014, Abednejad 2014, Labay 2015) and metallic (Dong, 2004, Buxadera-Palomero 2015) substrates, using monomers such as Diethylene glycol dimethyl ether (Diglyme), Tetraethylene glycol dimethyl ether (Tetraglyme) and diethylene glycol divinyl ether (DEGDVE). In the present case, Diglyme has been selected as monomer for its potential to produce polyethyleneglycol after polymerization. However, since plasma polymerization

involves complex physic-chemical processes (crosslinking, recombination, etching, etc.) and exact composition and structure of the obtained polymer cannot be completely ensured, for the purpose of this work, and according to the literature, the coatings obtained by plasma polymerization will be herein designated as "PEG-like coating".

In the studies mentioned, smooth model surfaces are usually employed to optimize and study the efficiency of the plasma processes for the different applications. β-TCP bioceramics can present a porous structure with complex surfaces, and different reactivity to plasmas than polymers or metals, as shown by their different recombination coefficients (Sarrette 2006).

The behavior of bioceramics for bone regeneration to plasma polymerization processes is uninvestigated, and providing new tools for their use as controlled drug delivery matrices is of great interest. By studying different conditions of plasma polymerization, the aim of this research is to obtain PEG-like coatings on ampicillin-loaded β–TCP materials with ability to modulate subsequent ampicillin release from the bioceramic, preserving their antibacterial properties.

2. Experimental part

2.1. Materials

Calcium hydrogen phosphate ($CaHPO_4$, *Sigma-Aldrich* C7263) and calcium carbonate ($CaCO_3$, *Sigma-Aldrich* C483*0*) were used as raw materials for the synthesis of β–Tricalcium Phosphate (β-$Ca_3(PO_4)_2$, β–TCP). Sodium phosphate dibasic (Na_2HPO_4, *Sigma-Aldrich*) was used in solution as accelerant in the synthesis of calcium deficient hydroxyapatite (CDHA) used as a precursor of β–TCP. Ampicillin sodium salt (371.39 g/mol), provided by *Sigma-Aldrich* was selected as antibiotic for loading β–TCP ceramics. Diethylene glycol dimethyl ether (Diglyme, anhydrous, 99.5%, *Sigma Aldrich*) $(CH_3OCH_2CH_2)_2O$ was used as precursor for plasma polymerization. Phosphate buffer saline (PBS), pH 7.4, was prepared from PBS tablets (*Gibco, Lifetechnologies*^{TM}, UK) and Milli-Q® deionized water. Agar bacteriological (*Scharlau S.A.*, Spain) and Brain Heart Infusion Broth (BHI Broth) (*Scharlau S.A.*, 02-599, Spain) were used to prepare the bacteriological culture media of *Staphylococcus aureus (S. aureus)* CCUG 15915 (Culture Collection University of Göteborg (CCUG), Göteborg, Sweden).

2.2. β-TCP synthesis

Microporous β–TCP discs were obtained from calcium phosphate cements prepared from α–TCP, which was obtained by solid state reaction of a 1:2 molar mixture of calcium hydrogen phosphate and calcium carbonate at 1400 ºC. A cement was produced by blending α–TCP with a solution of sodium phosphate dibasic at 2.5% (w:w) at liquid to powder ratio of 0.65. The mixture was put in a disc-shaped mold and allowed to set immersed in water for 7 days to obtain CDHA (Ginebra 2004). The former discs were sintered at 1100 ºC to obtain microporous β–TCP discs of 2 mm thickness × 12 mm ø.

2.3. Plasma polymerization

Plasma polymerization of β–TCP discs was performed using low-pressure radio-frequency plasma (13.56 MHz) (Standard Femto Plasma System, *Diener*, Germany) with a cylindrical glass chamber. Diethylene glycol dimethyl ether (Diglyme, anhydrous, 99.5%, *Sigma Aldrich*) was used as source of ethylene oxide monomers to obtain a PEG-like coating on β–TCP (Brétagnol 2006). Unloaded or ampicillin-loaded β-TCP discs were placed in the center of the reactor. To enhance the polymerization process a short surface activation step with O_2 (5.0 sccm, 40 Pa, 150 W) was performed for 60 s. Subsequent polymerization process consists in introducing Diglyme in the plasma reactor by bubbling a carrier gas (Ar) through the liquid monomer. The polymerization treatment was performed in continuous mode (15 sccm, 170 Pa, 150 W) for 10 min and 30 min. Simple (SP) and double (DP) polymerizations were performed on each side of the β–TCP materials for both plasma treatment times, and the corresponding samples were referenced as SP10, DP10, SP30 and DP30 respectively. DP corresponds to repetition of the polymerization cycle in exactly the same conditions described, with an interval of at least 15min in between cycles but without removing the sample from the reactor.

2.4. Surface topography

Topography of untreated and plasma polymerized β–TCP discs was studied by Scanning Electron Microscopy using a *Zeiss Neon 40* cross-beam workstation with *Gemini SEM* column for sample observation. Samples were C-coated before SEM observation. Observations were carried out at 5.0 kV working voltage. Coupled-Energy-Dispersive X-ray spectroscopy (EDX) equipment (INCAPentaFETx3 detector, 30 mm^2, ATW2 window) was also used for *in situ* elemental analysis of the surface of a cross-section of plasma-polymerized β–TCP to determine the depth of the effects of plasma treatment in the surface of the ceramic materials.

2.5. Wetting properties

Determination of the wettability of the β–TCP surfaces, to compare the untreated with the PEG-like coated ceramics by plasma polymerization was done by static contact angle measurements. A Contact Angle System OCA15 (*Dataphysics*, Germany) was used with the SCA20 Software (*Dataphysics*, Germany) to analyze the images acquired with a CCD. 10 µL water droplets were deposited on the β–TCP surface. Measurements were carried out on the plasma-polymerized side of the samples. In this study, a minimum of 4 replicates of each kind of treatment were carried out.

2.6. X-Ray Photoelectron Spectroscopy (XPS)

To determine the chemical composition of the surface of bare and treated β–TCP samples and assess the influence of plasma polymerization, X-Ray Photoelectron spectroscopy (XPS) was acquired in ultrahigh vacuum (5.0×10^{-7} Pa) with an XR50 Mg anode source operating at 150 W and a Phoibos 150 MCD-9 detector. Spectra were recorded at pass energy of 25 eV with a step size of 1.0 eV for survey spectra and 0.1 eV for high resolution spectra, on an area of the sample of 3.5 x 2 mm. The recorded core levels were C_{1s}, O_{1s}, Ca_{2p} and P_{2p}. C_{1s} peak was used as a reference. CasaXPS software (*Casa Software Ltd.*, UK) was used for the determination of atomic elemental composition applying the manufacturer set of relative sensitivity factors. The relative error associated to the survey spectra XPS measurements is of 0.5%.

2.7. Ampicillin loading of β–TCP

Loading of ampicillin was done, previous to plasma polymerization, by soaking the β–TCP discs in 1.0 mL of 4.0% ampicillin aqueous solution at 50 r.p.m. and 20 °C during 30 min, by complete immersion of the sample. Samples were dried at 37 °C for 24 h.

2.8. Drug release experiments

Ampicillin release experiments were performed using untreated and plasma-polymerized β – TCP discs previously loaded with the 4.0% ampicillin solution. For the drug release study, an USP equipment (TDT-08L Dissolution Tester (USP), *Pharma AllianceGroup*, U.S.A.) with 8 thermo-jacketed opaque cells of 300 mL was used, each one filled with 150 mL of PBS at pH 7.4 as receptor media. Temperature and rotation were maintained constant at 37 °C and 100 r.p.m. respectively. 1 mL samples were withdrawn from the receptor liquid media for latter spectroscopy analysis to determine the ampicillin released from the untreated and plasma-polymerized β–TCP discs and plot their corresponding release kinetics. After each sample withdrawn, the same volume of PBS was added to the receptor media. Release experiments were performed with four replicates of each plasma polymerization condition.

For the quantification of the ampicillin release, an UV-visible-NIR spectrophotometer *UV-3600 Shimadzu* was used at λ = 204 nm, corresponding to the wavelength of maximum absorbance of ampicillin in PBS solution. The concentration of ampicillin was below 10% saturation concentration (SINK conditions) in the receptor solution during the experiment. Stability of ampicillin after plasma polymerization on the ampicillin-loaded β–TCP was also checked by UV-spectroscopy after release of ampicillin in PBS through comparison of the general spectra.

2.9. Antibacterial assays

The antibacterial activity of the ampicillin-loaded plasma polymerized β-TCP discs was tested in suspension against *Staphylococcus aureus* (*S. aureus*) in BHI Broth at [BHI] = 37.0 $g.L^{-1}$. After incubation for 24 h at 37 °C, 1 mL of the inoculate media was put in each well of a 48-well Falcon™ culture well-plates, previously prepared by connecting two adjacent wells. The β–TCP materials were placed in one of the connected wells, while the second was employed to measure absorbance and monitoring the growth of *S. aureus* by means of a *Synergy HTX* Multimode Reader (*BioTek Instruments, Inc.*). The antibacterial activity was monitored during 72 hours by measuring absorbance at λ=600 nm (Deng 2015). Measurements were recorded using *Gen. 5 software* (*BioTek Instruments, Inc.*) and results are normalized and presented in growth % with respect to the positive control.

3. Results and Discussion

3.1. Influence of plasma polymerization on wettability, surface chemistry and topography

Untreated β–TCP displays hydrophilic properties and due to its microporous nature it absorbed water instantaneously (Table 1); however, the observation of water droplet persistence on the surface of plasma-polymerized β–TCP indicated modified wettability, so contact angles were measured (θ_s) to compare the influence of the different plasma treatments on the wettability of

β–TCP ceramics. While a single polymerization treatment for 10 min (SP10) only led to a slight delay in the water absorption (around 2 s), no water absorption was observed for a double polymerization treatment of 10 min (DP10), neither for longer treatments of 30 s either in single polymerization (SP30) or double polymerization (DP30). These three samples displayed contact angles between 122.6° and 128.45, as shown in Table 1 and water absorption times longer than 10 min, and above 1h for the longest plasma treatments (DP30).

This low wettability was surprising, considering that PEG is well-known for its hydrophilic properties. Depending on the polymerization process, static contact angles <60 ° can be expected for PEG surfaces (Li 2008). Our previous observations on PEG-like plasma coatings obtained from Tetraglyme deposited on different substrates (ie. polypropylene meshes (Labay 2015), or on titanium (Buxadera 2015)) displayed much lower contact angles (23 º). In this case, the hydrophobic behaviour of the plasma-polymerized β-TCP ceramics should be attributed to the substantial surface roughness of the sample (Figure 1a), that after plasma polymerization could mainly be due to trapping of air on the surface, increasing the measured contact angles and in some cases progressive clogging of the pores of β–TCP, limiting water absorption. In a recent work, very low wettability was also observed on calcium phosphate scaffolds plasma polymerized with hydrophilic PEG-co-PCL polymers (Canal 2016).

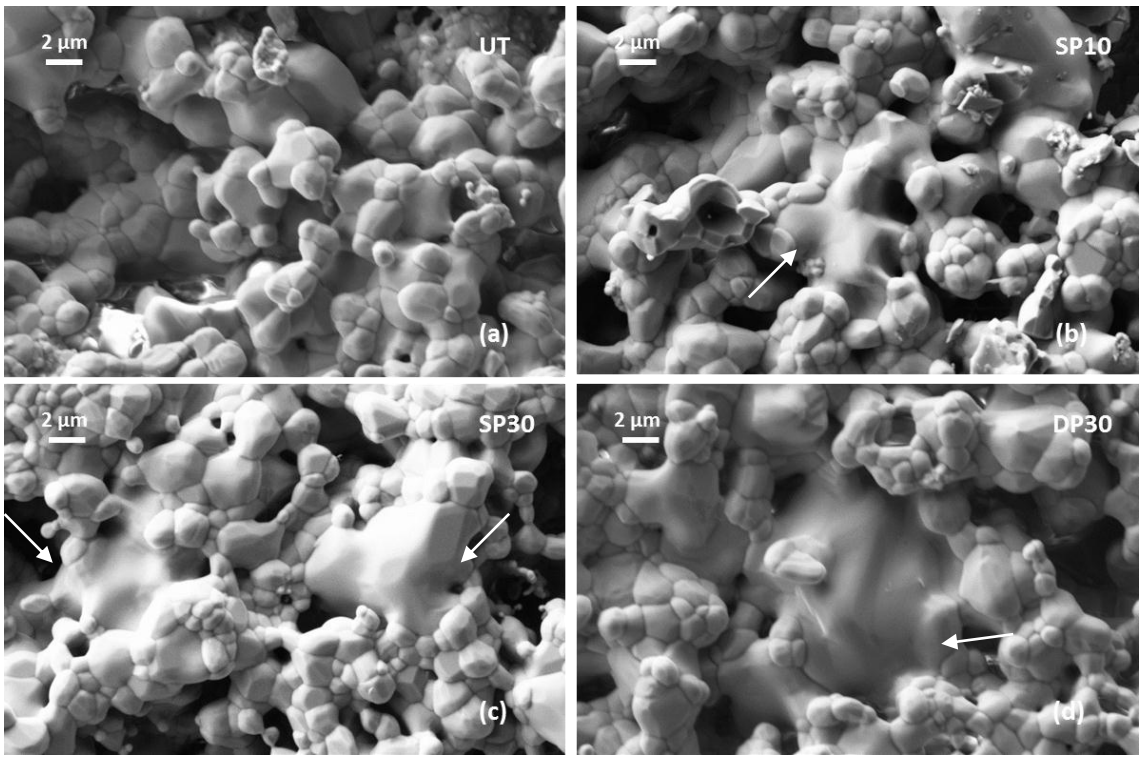

Figure 1. Scanning Electron Microscopy of untreated (a), SP10 (b), SP30 (c) and DP30 (d) β-TCP surfaces. Arrows indicate areas with polymer coatings.

This evolution of the wetting properties of β-TPC indicates a change in its surface chemistry due to plasma polymerization. Therefore, the elemental relative composition of untreated and plasma-polymerized β–TCP surfaces was been by XPS and is reported in Table 1.

Table 1. Surface elemental composition, atomic ratios and contact angles of the untreated, activated β -TCP and plasma-polymerized β–TCP.

	C_{1s}	O_{1s}	Ca_{2p}	P_{2p}	C/O	Ca/C	θ_s (°)
Untreated	14.98	52.12	20.21	12.70	0.29	1.35	*
Act. β-TCP	9.06	54.23	21.86	14.85	0.17	2.41	*
SP10	80.45	14.48	2.67	2.41	5.56	0.033	*
DP10	72.10	24.48	1.73	1.70	2.95	0.024	122.60 ±2.92†
SP30	79.29	17.61	1.64	1.46	4.50	0.021	125.63 ±1.27†
DP30	78.38	19.54	1.20	0.88	4.01	0.015	128.45 ±2.41‡

* Quick water absorption did not allow static contact angle measurement. Different symbols († and ‡) indicate statistically significant differences with $p<0.05$.

While the untreated sample shows a relative proportion between O, Ca and P atoms fairly concordant with the β–TCP formula (theoretical Ca/P ratio = 1.5), the presence of carbon atoms can be highlighted. Since β–TCP ($Ca_3(PO_4)_2$) does not include carbon atoms in its formula, the C_{1s} proportion (14.98%) found in the elemental composition of the untreated β–TCP surface corresponds to the adsorption of ambient contamination or the presence of surface carbonates. The main effect obtained with the activation treatment with Oxygen plasma is related to decrease in C, so a cleaning effect is observed mainly attributable to etching processes.

Plasma polymerization of β–TCP using Diglyme ($(CH_3OCH_2CH_2)_2O$) as monomer leads to a huge increase of carbon on the surface of β–TCP with C_{1s} atomic percentages between 72.10% and 80.45% for the plasma polymerized samples. This increase of carbon ratio alongside the decrease of combined Ca and P (i.e. from 5.08% for DP10 to 2.08% for DP30) confirms a screening of the β–TCP surface by an increasingly thicker polymer coating of the β–TCP (Figure 2). However, the fact that some Ca and P can still be detected in the surface atomic composition of plasma treated samples indicates that the average thickness of the PEG-like coating obtained by plasma polymerization is of nanometric order and/or that the surface is not still fully coated, as the aggregates observed by SEM at shorter polymerization times.

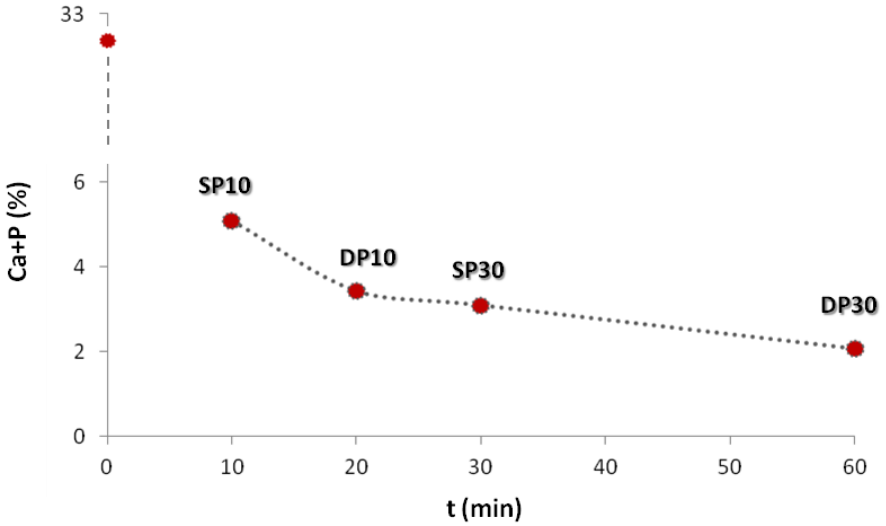

Figure 2. Evolution of (Ca+P) percentage detected by XPS surface analysis on the plasma-polymerized β-TCP ceramics as function of the plasma polymerization time.

Decomposition of the C_{1s} peak (Table 2) revealed significant differences between simple and double polymerization processes. In the deconvolution of C_{1s} peak, four different types of carbon bindings corresponding to C-C (284.93 eV), C-O (286.33 eV), C=O (287.91 eV), COOH (289.42 eV) functional groups can be found on the PEG-like coated β-TCP surface. The ether peak present in the plasma polymerized samples is indicative of the PEG character of the coating, and can be related to the fragmentation process during the plasma polymerization (Michel 2005, Zhang 1998, Michelmore 2013, Labay 2015).

Table 2. Determination of carbon and oxygen functional groups respectively from decomposition of high-resolution C_{1s} and O_{1s} XPS peaks of activated β-TCP and plasma-polymerized SP10, DP10, SP30 and DP30 β–TCP.

	C_{1s}				
	C carbide	C (C-C, C-H)	C (C-O)	C(C=O)	C (COO^-)
Act. β-TCP	6.53	69.83	11.66	11.98	-
SP10	-	43.29	49.73	6.96	-
DP10	-	59.19	35.14	4.64	1.03
SP30	-	75.18	15.21	6.98	1.60
DP30	-	80.75	14.85	2.20	2.20

Simple polymerization (SP10 and SP30) on β-TCP leads to combined i) etching of β-TCP surface by removing the contaminant moieties and ii) to introduction of C-C and C-O functional groups, possibly indicating some degree of cross-linking of the plasma polymer. In double polymerization processes (DP10 and DP30) or in long processes (SP30), COO^- groups appear,

indicating that other mechanisms must possibly be taken into account beside deposition of polymer coating on β–TCP: in fact, while simple polymerization proceeds through coating of a ceramic β-TCP surface, the second-step of the double polymerization or in long treatments it proceeds through a polymer surface, as the surface of β–TCP is already coated by the first plasma polymerization cycle. This can lead to further oxidation of the C=O groups. In this sense, it could be speculated that different mechanisms are involved in simple and double polymerizations. Possibly the ether (C-O) groups present in the SP coated β-TCP surfaces, can be further oxidized during the plasma treatment due to different reactions, as proposed in Figure 3, followed by subsequent reaction with air after the treatment.

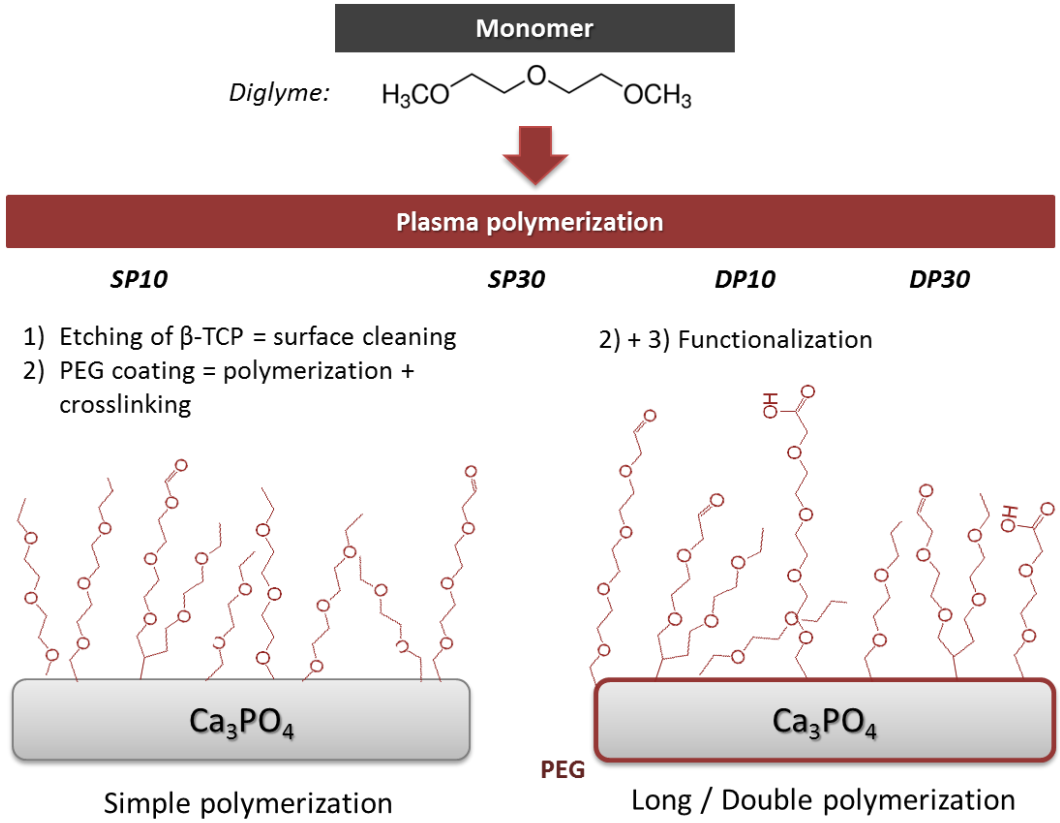

Figure 3. Plasma polymerization of PEG-like coating from Diglyme precursor. Proposed processes involved in simple (SP) and long/double (DP) polymerization processes.

From the Scanning Electron Micrographs of untreated and plasma-polymerized β-TCP surfaces presented in Figure 1, the untreated β–TCP surface revealed a highly microporous material, with crystalline grains bound together through sintering necks (Figure 1 a). In general, the plasma polymerization coating was visible only in some regions, where accumulation of the polymer-coating led to areas of smooth appearance which clogged some of the surface pores of β–TCP. These were present already in SP10 (Figure 1 b), although longer treatment times led to bigger areas visibly covered with the thick plasma polymer (Figure 1 c and d).

EDX of a DP30 cross-section indicated that depth of penetration of the plasma polymer was of the order of 7-9 µm within the porous structure of β-TCP ceramics, with higher concentration of carbon and thereby higher concentration of polymer on the bioceramic surface than 7-9 µm in the bulk.

SEM was also employed to confirm that previous loading of ampicillin in β-TCP did not affect the topography of the plasma polymer coating with respect to the unloaded samples.

3.2. Influence of plasma polymerization on ampicillin release & antibacterial activity

The influence of plasma polymerization on the ampicillin release from β-TCP in physiological conditions (Figure 4) was monitored along 24 hours for untreated, DP10, SP30 and DP30 β-TCP ceramics. Due to the lack of uniformity of the SP10 coating of β-TCP as inferred from the wettability experiments, this sample was not considered for the drug release assays. The amount of ampicillin loaded in the β–TCP ceramics prior to the plasma polymerization was of 5.01±0.59 mg.

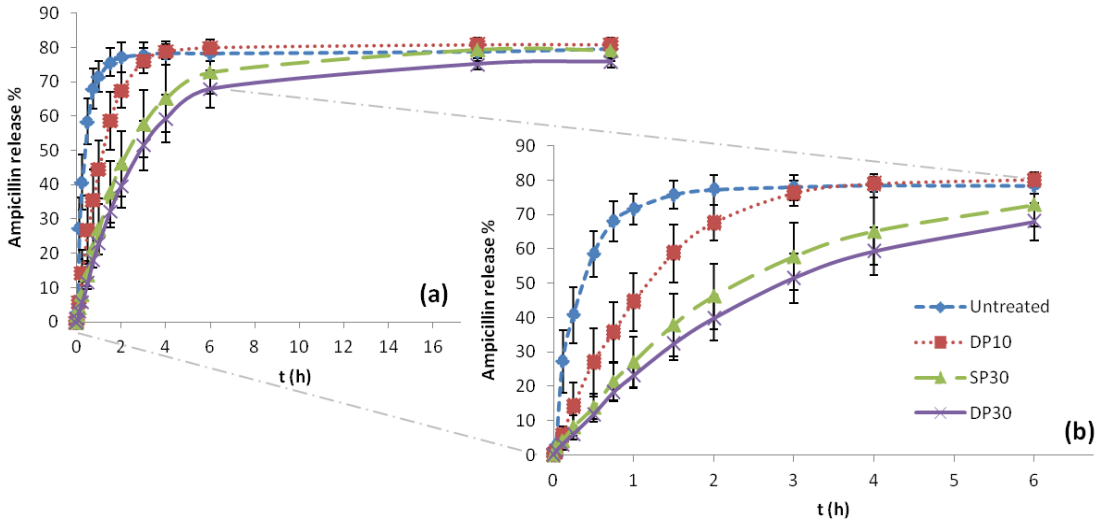

Figure 4. Ampicillin release kinetics from the untreated, DP10, SP30 and DP30 β-TCP ceramics (a), with enlargement of the first 6 hours of ampicillin release (b).

Untreated β-TCP presents a release percentage of 79.59±2.67% after 24 h, with a burst release profile in which 75% of ampicillin was released after only 90 min and the stationary stage was soon reached. For any bone graft, delivering the drug in a controlled manner, following a more extended release profile should be a target, and from Figure 4 two main issues may be highlighted: Firstly, the final ampicillin release percentage (at 24 h) did not show significant differences between the untreated and the plasma-polymerized β-TCP, with final release values of 80.84±1.95%, 79.27±3.16% and 75.87±1.83% for DP10, SP30 and DP30, respectively. Secondly, and more interestingly, progressive modifications in release kinetics were observed with plasma polymerization of β-TCP: the antibiotic release rate progressively slowed down with the treatment time and thus the coating thickness. As can be more clearly observed in the zoom of the first 6 hours of the drug delivery assay (Figure 4b), the initial release slope was

progressively reduced following UT>DP10>SP30>DP30 (from 2.174 for untreated β-TCP to 0.284 in DP30 sample in the first 60% of release), corresponding to the observed slowdown of the release kinetics. Therein, to reach 75% of ampicillin release, β-TCP coated by double polymerization for 10 min (DP10) took 3 hours, doubling the time of untreated β-TCP. This is was extended to more than 6 hours for SP30 and DP30, where no statistically significant differences were observed in the release kinetics of SP30 and DP30.

To describe the drug release mechanism involved, Korsmeyer-Peppas semi-empirical equation (Equation 1) was applied to the first 60% of the ampicillin release curves (Peppas 1984, Peppas 1985, Korsmeyer 1986a, Korsmeyer 1986b, Kosmidis 2003)

$$\frac{M_t}{M_\infty} = k.t^n \quad [1]$$

Where M_t and M_∞ are the absolute cumulative amount of drug released at time t and infinite time, respectively; k is a constant incorporating structural and geometric characteristics of the system, and n is the release exponent, which might be indicative of the mechanism of drug release, being n the exponent which is used to describe the kinetics behind the release (Ritger, 1987). Table 3 shows that plasma polymerization on the β-TCP modified the drug release mechanism since untreated β-TCP presents Fickian-diffusion release of ampicillin (n = 0.50) while plasma-polymerized β-TCP ceramics show non-Fickian diffusion of the antibiotic (0.5<n<1.0) (Ritger 1987).

Table 3. Evaluation of the nature of ampicillin release mechanism from PEG-like coated β-TCP materials at the different plasma polymerization conditions studied from the Korsmeyer-Peppas model for thin films.

	n	**R^2**	**Drug release mechanism**
Untreated	0.50	0.896	Fickian diffusion
DP10	0.74	0.987	Non-Fickian diffusion
SP30	0.79	0.984	Non-fickian diffusion
DP30	0.82	0.989	Non-fickian diffusion

Modification of the release mechanism following plasma polymerization was also observed by Bhatt et al. on a glass surface using a dye as model drug. They studied plasma polymerized multilayer PCL-co-PEG coatings (poly (ε-caprolactone)-poly (ethylene glycol) copolymer) prepared at different deposition times, using ε-CL/DEGME mixture of monomers. They observed that short plasma polymerization times (up to 10 min) of the drug-loaded substrate presented a zero order release as the uncoated sample, while longer plasma treatment times (between 20 and 50 min in their case) led to a modification of the drug release mechanism with an anomalous non-Fickian diffusion of the model drug (Bhatt 2013).

The results obtained suggest that longer plasma treatment times led to higher thickness of polymer coating (Figure 2), which consequently contributed to improve the slow-down in the

release kinetics of ampicillin from β-TCP ceramics (Figure 4). These promising results need still to be improved in further works as for bone applications, as an extended release over three or four weeks preceded by a burst release would be highly desirable.

It is important to ascertain the conservation of the therapeutic activity of the antibiotic after the plasma coating processes and after its release from the material. The influence of plasma polymerization on the antibacterial activity of ampicillin against *S. aureus* was studied by antibacterial assays in BHI suspension, and results of the bacterial growth after 1.5, 6 and 72 hours are presented in Figure 6. All β-TCP ceramics loaded with ampicillin and subsequently coated by plasma polymerization displayed antibacterial activity: therefore, the drug was not degraded and antibacterial properties were preserved after plasma polymerization. Moreover, within the timeframe evaluated none of the coated ceramics presented any significantly statistical differences between them, indicating that the Minimum Inhibitory Concentration (MIC) was reached in all cases. This is related with the amount of drug released, ie. after 6 h and 72 h, since it has been shown that all samples release nearly the same amount (about 3.7 mg) of ampicillin after 6 hours-release experiment.

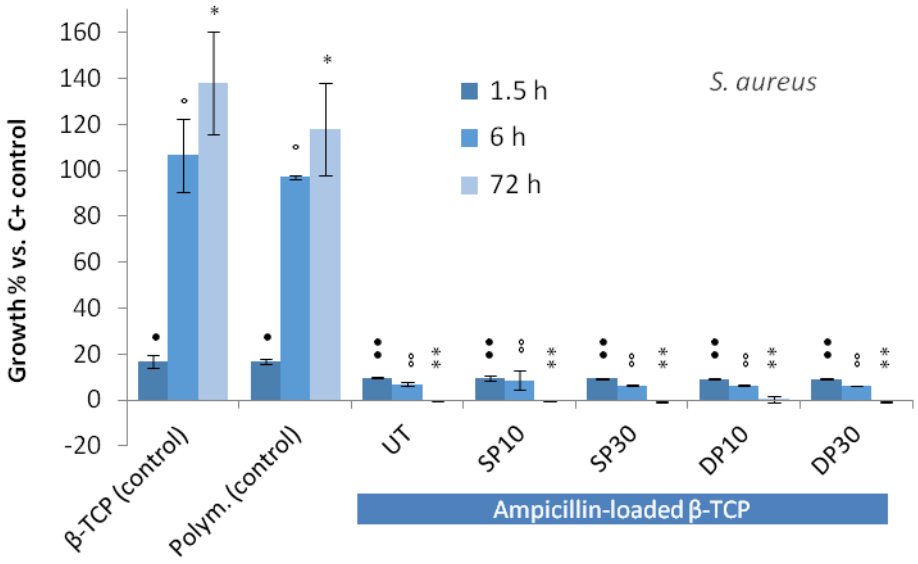

Figure 5. Antibacterial properties of ampicillin-loaded untreated and plasma polymerized β-TCP ceramics in contact with S. aureus at 1.5, 6 and 72 hours. •, °, * indicate statistically significant differences among samples for the same time of assay: the series with the same number of symbols show no statistically significant differences.

The conservation of antibacterial activity of plasma-coated polymer meshes was also shown for meshes for hernia repair coated by plasma polymerization from another monomer (Tetraglyme) (Labay, 2015), being a good indicator that the low pressure plasma process is not altering the molecule loaded on the materials. Moreover, in that and other works of the group (Buxadera-Palomero 2015) very suitable biological behavior was found on PEG-like plasma coated materials (Polypropylene or titanium). Given the important changes produced on the surface chemistry of β-TCP, future works will be needed to evaluate the cell response to the novel surfaces.

4. Conclusions

Microporous β-Tricalcium Phosphate (β-TCP) bioceramics with intricate surfaces have been successfully coated with polyethyleneglycol plasma polymer, thus with significant modifications on surface chemistry. Different plasma polymerization conditions were evaluated, in particular single or double coatings at different times. Progressive screening of the Ca and P atoms from β-TCP by C-containing moieties confirmed the deposition of a nanometric coating. Despite the hydrophilic characteristics of the polymer, the coated materials were hydrophobic possibly due to air entrapment in the rough β-TCP surface. Moreover, it has been shown that different physical mechanisms take place in simple and double polymerizations, mostly since in the second-step of a double plasma polymerization process the surface is no longer a ceramic as it has a prior PEG-like coating. In addition to the modification of surface chemistry, topographical changes could be visualized even for short plasma polymerization times (SP10), with accumulation of polymer in some areas. SEM observation combined with contact angle measurements revealed that long polymerization times and double coating processes improved the coating of the ceramics.

As consequence of the physical and chemical changes in β-TCP surface induced by plasma polymerization, the antibiotic release from the ceramic materials was successfully modified, significantly slowing down the ampicillin release kinetics. It has been demonstrated that longer plasma polymerization times either in single or double coatings were the most effective in controlling the drug release, which has been related with higher thickness of the plasma polymer coating. While untreated β-TCP reached its final ampicillin release percentage after only 90 min, DP30 needed more than 6 hours, with a change in the drug release mechanism from Fickian-release to non-Fickian diffusion. Moreover, the antibacterial activity vs. *Staphilococcus aureus* of all plasma polymerized samples was maintained.

Plasma polymerization of the β-TCP in the conditions tested not only avoided burst release of the antibiotic but also led to a controlled release of the loaded ampicillin, and everything suggests that plasma conditions could be optimized with longer and/or multiple plasma sequences to further slow down the release kinetics, opening great perspectives for drug delivery from bone bioceramics which require longer and sustained release. In this sense, plasma polymerization can be considered as relevant for the design of implantable β-TCP matrices for controlled release applications, opening new routes for prophylaxis or treatment in post-operatory infections in bone repair surgery.

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