

High strength, biodegradable and osteoconductive alpha tricalcium phosphate-iron composites for temporal reduction of bone fractures

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Abstract

In this work alpha tricalcium phosphate (α -TCP)/ iron (Fe) composites were developed as a new family of biodegradable, load-bearing and osteoconductive materials. The composites with composition from pure ceramic to pure metallic samples were consolidated by pulsed electric current assisted sintering to minimise processing time and temperature while improving their mechanical performance. The mechanical strength of the composites was increased and controlled with the Fe content, passing from brittle to ductile failure. In particular, the addition of 25 vol.% of Fe produced a ceramic matrix composite with elastic modulus much closer to cortical bone than that of titanium or biodegradable magnesium alloys and specific compressive strength above that of stainless steel, chromium-cobalt alloys and pure titanium, currently used in clinic for internal fracture fixation. All the composites studied exhibited higher degradation rate than their individual components, presenting values around 200 $\mu\text{m}/\text{year}$, but also their compressive strength did not show a significant reduction in the period required for bone fracture consolidation. Composites showed preferential degradation of α -TCP areas rather than β -TCP areas, suggesting that α -TCP can produce composites with higher degradation rate. The composites were cytocompatible both in indirect and direct contact with bone cells. Osteoblast-like cells attached and spread on the surface of the composites, presenting proliferation rate similar to than on tissue culture-graded polystyrene and they showed alkaline phosphatase activity. Therefore, this new family of composites is a potential alternative to produce implants for temporal reduction of bone fractures.

Keywords: biodegradable metal; tricalcium phosphate; spark plasma sintering; mechanical properties; degradation test

1. Introduction

The functional and safe reduction of bone fractures require load-bearing biocompatible materials with elastic modulus similar to that of cortical bone [1,2]. Traditionally, biocompatible metallic materials, such as stainless steel, cobalt-chromium alloys, titanium and its alloys, are used for internal fixation of fractures because of their high yield strength and toughness [3]. Nonetheless, their stiffness greatly exceeds the stiffness of bone, leading to stress shielding, which impairs bone fracture healing [2]. Besides, their use is associated in several cases with the need for a second surgery to remove the implant when the healing process is completed. The development of biodegradable materials for osteosynthesis would be ideal to prevent long-term adverse side effects, decrease the damage caused to patient and reduce the bone fracture care costs. In particular avoid the removal of the implant has important implications during treatment of bone fractures in young patients [4]. Among resorbable materials, calcium phosphate ceramics, such as hydroxyapatite (HA) and beta tricalcium phosphate (β -TCP), are widely used for bone reconstruction in maxillofacial and orthopaedic surgeries [5,6]. They are bioactive and osteoconductive materials that promote bone regeneration. However, their low mechanical strength makes them not suitable for functional bone fracture fixation. Ceramic-metallic composites have attracted much attention due to the possibility of combining the mechanical strength and toughness of implantable metals with the osteoconductive properties of calcium phosphates. One approach involved the development of ceramic matrix composites with HA as the matrix material together with silver [7-10], titanium [11-16], stainless steel [17], metal glasses [18], carbon nanotubes [19] or even bioactive glass [20] as reinforcing particles. A second approach consisted in development of metal matrix composites, mainly based on titanium or titanium alloys together with HA particles in order to decrease the stiffness while increasing the bioactivity of the composite [11,12,21-26]. Pure iron (Fe) can be considered as a potential material to develop a new family of biodegradable composites due to its good biocompatibility, reliable mechanical performance and safe biodegradation *in vivo* [27-29]. Few works explored this idea studying Fe matrix composites produced by conventional powder metallurgy techniques [30-33]. Nonetheless, more research is needed to achieve optimal results. The objective of this work was to

develop a new family of biodegradable alpha tricalcium phosphate (α -TCP)/Fe composites for possible fabrication of temporal implants for osteosynthesis. Powders of the two components were used as starting materials and a novelty of this work was the use of pulsed electric current assisted sintering for consolidation of the composites. This is a high technology processing method that end up with materials with superior mechanical performance than common pressure-less sintering routs [18,20,34-36]. Although there is still controversy on the sintering mechanisms taking place, most of the literature agreed on the fact that fast heating rate, electric and magnetic fields and the application of a mechanical compaction load, promotes material densification at lower temperature and shorter time, thus retaining fine grain microstructure [18,20,34-37].

Within the aim of this work was study for the first time α -TCP as matrix and as reinforcement component of the composites. The hypothesis behind is that using α -TCP instead of its slightly less soluble β -TCP polymorph [38-40], can result in composites with higher degradation rate than previous Fe matrix composites incorporating calcium phosphates solely as reinforcement particles [30-33]. Besides, special interest was devoted in non-studied α -TCP matrix composites in order to achieve elastic modulus similar to cortical bone. Fe was used instead of magnesium as biodegradable metallic component because in general, the reabsorbtion of magnesium in physiological media occurs before fracture healing [41]. Conversely, based on previous work [42], commercial pure Fe powder containing carbon nanotubes was used with the aim to promote the galvanic corrosion of the metallic component of the composite.

2. Materials and methods

2.1. Synthesis of alpha tricalcium phosphate

A well-established method was used to synthetize α -TCP at 1400 °C, followed by air quenching to prevent the formation of the β -TCP polymorph [43]. The obtained block was milled (Fritsch Pulverisete

6) in isopropanol at 400 rpm, using balls (20 balls of 20 mm in diameter) and jar made of agate. Finally, the powder was dried at 37 °C. The particle size distribution of the powder was $d(10) = 1.38 \mu\text{m}$, $d(50) = 2.92 \mu\text{m}$ and $d(90) = 11.26 \mu\text{m}$. The composition of the obtained powder, determined by Rietveld refinement of the X-ray diffraction (XRD) patterns, was 97 % α -TCP with 3 % of β -TCP.

2.2. Composite fabrication by pulsed electric current sintering

A rotary cylindrical mixer was used to blend α -TCP powder with commercial Fe powder containing 3 vol.% of carbon nanotubes (Applied Carbon Nano Technologies; $d(50) = 3.68 \mu\text{m}$) for 24 h. Afterwards, the mixture was introduced into a cylindrical graphite die set and sintered under very low air pressure (8 Pa) at a constant axial compaction load of 35 MPa, using a pulsed electric current sintering device (Dr. Sinter 1050 SPS, Sumitomo Coal Mining Co., Tokyo, Japan). Samples were heated up to 1000 °C, with heating rate of 100 °C/min and kept at that temperature for 10 min. Heating was achieved by applying a direct electrical current in on-off cycles of 12 and 2 ms, respectively. Samples were cooled down inside the system until 200 °C before being removed from the die. The sintered samples were labelled according to their Fe content (0, 25, 50, 75 and 100 vol.%) as follows TCP, TCP/25Fe, TCP/50Fe, TCP/75Fe and Fe.

2.3. Chemical and microstructural analysis

Possible phase transformations or degradation of the components of the composites occurred upon sintering were evaluated by XRD (Rigaku SmartLab 3kW CF2) by scanning in Bragg-Brentano geometry, using Cu K_α radiation with the scan range in between 10 and 90 ° and scan speed of 3 °/min. Rietveld refinement of the obtained patterns was performed with High Score software to determine the percentage of crystalline phases, using the Inorganic Crystal Structure Database (ICSD), including structural models for α -TCP (ICSD No. 923), β -TCP (ICSD No. 6191), HA (ICSD No. 151414), α -iron (ICSD No. 64998) and Fe₃C (cementite; ICSD No. 16593). The microstructure of the samples was observed by scanning electron microscope (SEM; TESCAN Lyra3) using samples coated with a thin

carbon layer. In addition, a mapping of the elemental composition (Ca, P, and Fe distribution) of the samples was performed using SEM (PHILIPS XL30) equipped with an energy dispersive X-ray spectrometer (EDAX). Helium pycnometry was used to evaluate the skeletal density of the samples (Micromeritics AccuPyc II 1340).

2.4. Mechanical characterization

The diametral tensile strength (DTS) test was performed using an universal testing machine (TIRA test 2850S) at a constant cross head speed of 1 mm/min. At least three disc samples from each material were tested and their DTS was calculated according to Equation 1.

$$DTS = \frac{2F}{\pi Dh} \quad (1)$$

where F is the applied load, D is the sample diameter (12 mm) and h is the sample height (5 mm).

The DTS was considered for the brittle samples as the strength corresponding to fracture point, while for samples presenting some plastic deformation as the yield strength. The macroscopic aspect of the samples after DTS test was documented with a digital camera. In addition, a compression test was performed (TIRA test 2850S) at a constant cross head speed of 1 mm/min for the TCP/25Fe composite (samples were cylinders of 12 mm in diameter and 20 mm in height). The elastic modulus in compression was determined from the linear region of the stress-strain curve and the compressive strength was considered as the strength corresponding to fracture point of the samples. The mechanical performance of the TCP/25Fe composite was localized within an Ashby chart of specific mechanical properties including the most common implantable materials (CES Edu Pack 2013).

2.5. *In vitro* degradation test

Immersion degradation test was performed placing the samples (discs of 12 mm in diameter and 5 mm in height; apparent surface area of 414.7 mm²) in hermetic polypropylene recipients containing 40 ml of 0.9 wt.% sodium chloride (NaCl; Lach Ner) solution at 37 °C. Once a week, the NaCl solution was renewed to prevent the ionic saturation of the immersion medium. After 2, 4, 8, 12 and 16 weeks of immersion, three samples were removed from the solution, rinsed with distilled water, isopropanol and dried. While the macroscopic aspect of the corrosion products was documented with a digital camera, their surface microstructure was observed by SEM on samples coated with a thin carbon layer. The crystalline phase composition of the corrosion products was determined using XRD analysis. Mass loss and corrosion rate of the samples were determined according to Equations 2 and 3, respectively, after ultrasonic cleaning (GT Sonic) of the corrosion products in acetone for 5 minutes.

$$M_{\text{loss}} = \frac{(M_0 - M_t)}{A} \quad (2)$$

$$v_{\text{corr}} = \frac{(M_0 - M_t)}{A t \rho} \quad (3)$$

where M_0 is the initial mass of the sample and M_t is the mass of the sample at experimental time point, A is the initial area of the sample exposed to the corrosive environment, t is the immersion time and ρ is the sample density determined by helium pycnometry. Sample mass was measured using an analytical weight scale (Discovery Ohaus).

The release of calcium, phosphorous and iron ions from the samples to the immersion medium was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES; Thermo Scientific iCAP 6500 Duo) in radial mode on ionic emission lines at 317.933, 177.95 and 238.204 nm, respectively.

The concentration of phosphorus was considered as an estimation of the release of phosphate ions from the samples.

In order to evaluate influence of degradation process on the mechanical strength of the TCP/25Fe composite, samples after 4, 8 and 16 weeks of immersion were submitted to DTS test according to conditions described above.

2.6. Cell culture test

Cell culture test was performed using MG-63 human osteosarcoma cells (ECACC, UK) cultured with Dulbecco's Modified Eagle's medium (DMEM) supplemented with fetal bovine serum (FBS, 10%), penicillin/streptomycin (1%) and L-glutamine (1%), in 5 % CO₂ humidified atmosphere at 37 °C. In order to study the paracrine effect of the composites on cell proliferation, 5×10^4 cells/well were seeded on 24-well culture plates (well diameter 15.6 mm) and allowed to attach during 24 h. Afterwards, wells were washed twice with phosphate buffer solution (PBS) to remove the not attached cells and 1.5 ml of cell culture medium was added. Immediately, one sterile sample (discs of 5 mm in diameter and 2 mm in height) was placed in the centre of each well. Prior to the study, the samples were sterilized by immersion during 30 min in ethanol (70 % in water) and rinsed three times with PBS. Tissue culture-grade polystyrene (TCPS) of the culture well was used as reference material. The medium was renewed every 24 h. Cell metabolic activity was evaluated after 1 and 3 days of indirect culture using XTT cell proliferation assay kit (American Type Culture Collection) according to the instruction of the supplier. Prior to the XTT assay, the samples were removed and the cells were rinsed twice with PBS. A standard curve with decreasing number of cells was used to express the results in cell number. In addition, cell-material interactions were studied by direct culture of cells (2×10^4) on the samples using 1.5 ml of medium. After 6 h of culture samples were fixed in 2 % glutaraldehyde solution for 10 min, rinsed in PBS, dehydrated in 50, 70, 90, 96 and 100 % ethanol series and finally in hexamethyldisilazane. Cell morphology was observed by SEM in samples covered with a thin carbon layer to prevent charging. The

alkaline phosphatase (ALP) activity was assayed after 6 and 24 h of direct contact culture. The cells on the samples were lysed using 0.1 vol.% Triton X-100 in PBS for 10 min at 37 °C and ALP activity was evaluated using a ALP colorimetric assay kit (Bio vision) following the instruction of the supplier. Absorbance was measured at 405 nm and the ALP activity was calculated as the p-nitrophenol (pNP) generated by samples during the assay per unit of sample volume per minute of reaction. The experiments were run in triplicate for statistical analysis.

2.7 Statistical analysis

Results are reported as average values together with their standard deviation of at least 3 samples. A Student's t-test was performed to analyse the statistical significance of the results, assuming equal variances and using 0.05 as the significance level.

3. Results

3.1. Microstructure and phase composition of the composites

Figure 1 shows representative XRD patterns and backscattered electron SEM images of the sintered composites. Rietveld analysis of the XRD patterns (Table 1) shows that pure TCP sample was mainly composed by both α -TCP and β -TCP polymorphs and small amount of HA. Nonetheless, the presence of other crystalline phases such CaO, was not detected. For the composites, the XRD patterns and Rietveld analysis revealed the presence of Fe with body-centred cubic (BCC) crystal lattice (ferrite), alpha and beta TCP, with no signals of HA or other phosphates, carbides or oxides of calcium or iron. The intensity of the diffraction peaks of the two polymorphs of TCP decreased with the increment of Fe, but the α -TCP to β -TCP phase ratio did not follow any trend as regards the content of Fe. The XRD pattern of pure Fe sample was indexed as alpha Fe with traces of cementite (Fe_3C). In backscattered electron SEM images the Fe component presented a brighter aspect due to higher average in atomic number with respect to TCP component, appeared as dark phase. For the composites with 25 and 50 vol.% of Fe the images show an

interpenetrated TCP matrix reinforced with homogeneously dispersed Fe particles or aggregates with broad size distribution. In contrast, for the composite with 75 vol.% of Fe, the interpenetrated matrix was Fe with small reinforcing TCP particles as well as few bigger TCP aggregates. The sum of the α -TCP and β -TCP polymorphs at the sample surface, or equivalently surface content of Fe, determined by Rietveld analysis was within the nominal composition of the composites (Table 1). Furthermore, the high densification of the samples was corroborated by the experimental densities, which were above 95 % of their correspondent theoretical density (Table 1). This is in agreement with the absence of porosity in the microstructure of the samples as observed by SEM (Figure 1).

Table 1. Percentage of crystalline phases in the surface of the samples determined by Rietveld refinement of the XRD patterns shown in Figure 1, together with the theoretical and experimental densities of the samples.

Sample	Phase composition (wt.%)						α -TCP/ β -TCP ratio	Density [g/cm ³]	
	α -TCP	β -TCP	α -TCP + β -TCP	HA	Fe	Fe ₃ C		Theoretical	Experimental
TCP	29.2	64.5	93.7	6.3	N.D.	N.D.	0.45	2.86 [38]	2.79 ± 0.01
TCP/25Fe	16.8	63.0	79.8	N.D.	20.2	N.D.	0.27	3.97*	3.90 ± 0.01
TCP/50Fe	26.9	30.4	57.3	N.D.	42.7	N.D.	0.88	5.08*	5.13 ± 0.02
TCP/75Fe	3.2	19.8	23.0	N.D.	77.0	N.D.	0.16	6.19*	6.10 ± 0.01
Fe	N.D.	N.D.	-	N.D.	95.8	4.2	-	7.87 [42]	7.46 ± 0.01

*Calculated using the rule of mixtures for composite materials; N.D. Not detected.

The representative elemental mapping of iron, calcium, and phosphorus on the surface of the TCP/50Fe composite is presented in Figure 1f. The chemical element distribution confirmed the separate presence of the two components of the composite as the locations of calcium and phosphorus were clearly apart from the locations of iron. Besides, the atomic calcium to phosphorus ratio measured by EDAX analysis in the TCP component (Ca/P = 1.44) perfectly fits the theoretical ratio for this phase (1.5 [38]). A more detailed analysis of the interface between Fe and TCP showed a sharp transition of their chemical elemental composition, in other words, there was no evidence of either reaction or diffusion between TCP

and Fe during sintering. Nonetheless, direct interfacial contact between the ceramic and the metallic components was observed.

3.2. Mechanical characterization

Figure 2 shows the DTS values and representative images of the type of fracture of the sintered composites after DTS test. The DTS of pure TCP significantly increased with the incorporation of Fe ($p < 0.05$), showing a maximum at 75 vol.% of Fe. The Fe sample presented slightly lower but not statistically significant difference ($p = 0.3317$) in DTS with respect to TCP/75Fe composite. The DTS of pure Fe was also statistically equivalent ($p = 0.2086$) to the DTS of the TCP/50Fe composite. The images in Figure 2b show that the crack growth resistance of the samples increases with the Fe content, going from completely brittle fracture behaviour of pure TCP to plastic deformation of pure Fe. Nonetheless, although the TCP/75Fe composite showed some plastic deformation, the presence of ceramic particles induced a more brittle behaviour with respect to the pure Fe sample. Also, notice that the fracture occurred along the diameter of the samples and between the points of the application of the load. This corroborates the presence of a tensile stress perpendicular to the direction of loading.

The compressive strength and the elastic modulus in compression for the TCP/25Fe composite were 629.2 ± 17.9 MPa and 26.9 ± 3.3 GPa, respectively. The specific compressive strength of this composite calculated with the corresponding experimental density determined by helium pycnometry (Table 1) was 161.5 ± 4.6 MPa/(g/cm³). These experimental values were used to localize the expected performance of the TCP/25Fe composite within the Ashby chart of mechanical properties presented in Figure 3. The chart also shows the performance of bone and nacre along with that of metals, ceramics and polymers currently used for the fabrication of orthopaedic implants. As can be observed in Figure 3, the TCP/25Fe composite showed a lower elastic modulus as compared to most used metallic and ceramic biomaterials. In particular, the composite showed lower elastic modulus than titanium, titanium alloys and magnesium alloys which have the most similar modulus with respect to that of cortical bone in the longitudinal

direction. Besides, the specific strength of the TCP/25Fe composite was larger than the specific strength of load-bearing implantable materials, such as polymethyl methacrylate, stainless steel, cobalt-chromium alloys, alumina, zirconia, commercially pure titanium and cast magnesium alloys. The specific strength of the composite was within the range of titanium alloys and wrought magnesium alloys.

3.3. *In vitro* degradation behaviour

Figure 4a shows the mass loss of the samples as a function of degradation time until a maximum period of 16 weeks (4 months). All samples presented a similar trend, *i.e.* an increment of mass loss with the immersion time. TCP sample showed statistically inferior mass loss with respect to the other studied samples followed by Fe. No statistical significant differences in mass loss were observed between composites during all the experiment. The degradation rate of the samples is presented in Figure 4b. The degradation rate during the experiment was constant for the TCP, TCP/25Fe, TCP/50Fe and Fe samples, while for the TCP/75Fe sample the degradation rate increases over time, reaching after 12 weeks a value similar to the other composites. Although no statistically significant difference existed between composites, the average degradation rate decreased according to the following trend TCP/25Fe (206 $\mu\text{m}/\text{year}$), TCP/50Fe (180 $\mu\text{m}/\text{year}$) and TCP/75Fe (123 $\mu\text{m}/\text{year}$). Pure Fe and TCP samples presented statistically significant lower ($p < 0.001$) average degradation rates than the composites, 69 and 44 $\mu\text{m}/\text{year}$, respectively.

Consistent with the mass loss (Figure 4a), all the samples released calcium, phosphorous and/or iron ions to the immersion medium (Figure 4c). The weekly increment of calcium concentration in the medium was similar for TCP, TCP/25Fe and TCP/50Fe samples and negligible in the medium in contact with the pure Fe sample. The TCP/75Fe sample produced a half increment in calcium concentration, which was statistically significant different ($p < 0.0001$) with respect the other samples. The weekly increment of phosphorous concentration was maximum for the medium in contact with the pure TCP sample and progressively decreased with the increment of Fe content in the samples, until becoming negligible for

pure Fe sample. As expected, the release of iron was negligible for the pure TCP sample. The weekly increment of iron concentration reached its maximum for TCP/50Fe sample followed by TCP/25Fe sample, without statistically significant differences between them ($p = 0.1465$) due to wide dispersion in the results. The weekly increment in iron concentration decreased for TCP/75Fe and Fe samples, without statistically significant differences between them ($p = 0.8391$). In agreement with the mass loss (Figure 4a) and ion release (Figure 4c), the DTS of the TCP/25Fe composite slightly decreases with degradation time (Figure 4d), presenting a statistically significant difference ($p = 0.0112$) with respect to the initial condition only after 16 weeks of immersion.

As it is shown in Figure 5, the macroscopic aspect of the TCP samples did not present apparent evidences of degradation after the immersion test. Nonetheless, the sample surface observed by SEM revealed severe pitting, characteristic of grain dissolution without signals of precipitates. XRD results show that the crystalline composition of the TCP sample after degradation corresponded to β -TCP (Figure 6), suggesting a preferential dissolution of the slightly more soluble α -TCP areas of the sample. After ultrasonic cleaning, the morphology of the surface was maintained. In contrast, macroscopically the composites and the pure Fe sample presented the formation of orange-reddish and black coloured corrosion product on their surfaces (Figure 5). This suggests that the corrosion products contained hydrated Fe (III) oxides and/or Fe (III) oxide-hydroxides. Nonetheless, the XRD patterns of the corrosion products (Figure 6) were indexed as Fe (II,III) oxide (Fe_3O_4 ; magnetite). In addition to magnetite, the XRD patterns of the degraded composites and degraded Fe sample showed the presence of cementite, being more evident in the pure Fe sample (Figure 6). Last but not least, while the presence of Fe was hidden below the diffractions of the corrosion products, the presence of β -TCP was evident in all the composites without signals of α -TCP (Figure 6). The microstructure of the corrosion products had the morphology of compact aggregates, displaying needle-like or plate-like features (Figure 5). After ultrasonic cleaning, the surface morphology of the TCP/25Fe and TCP/50Fe composites was similar to

the TCP sample, *i.e.* pits with signals of surface etching. In contrast, the cleaned surface of the TCP/75Fe and Fe samples displayed pores and parallel plates typical for perlite microstructure.

3.4. Indirect cell proliferation assay

Figure 7 shows the proliferation of MG-63 osteoblast-like cells in indirect contact with the samples and TCPS. The trend observed after one day of culture was the slight decrease in number of cells with the increment of Fe content in the composites. Nonetheless, in any case, the differences with respect to the TCPS were statistically significant ($p > 0.05$). After three days of culture, the number of cells in all the samples increased comparing to the day one, reaching similar cell number without statistically significant differences between samples.

3.5. Cell morphology and ALP activity in direct contact

MG-63 osteoblast-like cells were able to attach and spread on the surface of the composites, showing different cell morphology depending on the Fe content (Figure 8). Cells on the pure TCP sample were flat, spread, smooth, presented several lamellipodia and filopodia and covered almost all the sample surface. Cells on the TCP/25Fe and TCP/50Fe composites were polarized and presented rather elongated morphology instead of spread one. Cells also presented lamellipodia but considerably more filopodia than on TCP. Cells on the TCP/75Fe composite were again spread and presented a flat morphology. Nonetheless, their size was smaller and the cell membrane was rougher with respect to the cells on TCP. Figure 9 shows that ALP activity was similar in all samples after 6 h of direct contact culture. ALP activity increased after 24 h of culture, being statistically significant different with respect to 6 h of culture. The increment of ALP activity was higher for cells in contact with TCP and TCP/25Fe samples ($p < 0.0002$) than for cells in contact with TCP/50Fe, TCP/75Fe and Fe samples ($p < 0.0376$), without statistically significant differences within these two groups ($p > 0.2147$).

4. Discussion

One of the main purposes for the development of TCP/Fe composites is to improve the mechanical strength of TCP, while decreasing the stiffness of Fe to values comparable with cortical bone. The search of load-bearing materials with low elastic modulus is driven to avoid the stress shielding effect, which may impair bone healing during bone fracture reduction [1,2]. TCP/25Fe composite attained an elastic modulus much closer to that of cortical bone than currently clinically available titanium or in particular magnesium alloys, which are considered as the most biocompatible materials with bone in terms of stiffness. Moreover, the addition of Fe particles considerably reinforced the TCP matrix, increasing its tensile strength and toughness. A proof of the later is the less brittle mode of fracture of the composites (Figure 2b). In fact, the composite with 75 vol.% of Fe can be considered as a metal matrix composite instead of ceramic matrix composite, showing higher yield strength than Fe, although with less plastic deformation before fracture. The improvement of the mechanical performance of TCP through the inclusion of ductile Fe particles was probably accomplished by microstructural toughening mechanisms, such as deflection of crack tips, formation of Fe bridges across crack faces, absorption of energy during pull-out of Fe particles and redistribution of stresses in regions adjacent to crack tips [13,16,19,44]. The extent of interfacial contact and mechanical anchorage between the two components are of paramount relevance to achieve the optimal transference of strength and stiffness between the reinforcing material and the TCP matrix. As can be observed in Figure 1, the pulsed electric current sintering while applying pressure, allowed a close interfacial contact between TCP and Fe components, without porosity and secondary phases between them, therefore, allowing excellent stress transference. This highlights the advantages of pulsed electric current assisted sintering against conventional pressure-less sintering methods.

Additional advantages of pulsed electric current assisted sintering are (1) fast heating rate, (2) significant reduction of the sintering time and temperature, (3) high material densification and (4) superior mechanical strength. In the present study a heating rate of 100 °C/min was used, however, it can be easily

increased up to 500 °C/min without gradients of temperature in the sample and keeping the accuracy in the control of the process [35,37]. These heating rates are considerably superior to those available in conventional furnaces (approximately 30 °C/min) where the main heat transference mechanism is convection. The fast heating allows to reduce both the sintering time and temperature. Commonly pressure-less sintering of TCP is performed for 1 to 12 h and between 1100 and 1300 °C [45], while in the present study, sintering was carried out only for 10 min at 1000 °C. Although that, in all cases, the samples attained more than 95 % of their theoretical density (see Table 1) with a consequent superior mechanical strength. For example, previous works on metal matrix composites reported compressive strength around 700 MPa for Fe/5% β -TCP composite fabricated by uniaxial pressing and conventional sintering [30], 500 MPa for Fe/30% β -TCP composite fabricated by powder injection moulding and conventional sintering [31] and around 760 MPa for Ti/30% β -TCP composite fabricated by spark plasma sintering [46]. In the present study, the TCP matrix composite with only 25 vol.% of Fe achieved a similar compressive strength of around 630 MPa. It is expected that TCP/Fe composites can attain even higher compressive strength with the increment of Fe content. Although differences in stress conditions should be considered, TCP/75Fe and Fe samples developed in this work also presented superior DTS (above 100 MPa, see Figure 2) than pure Fe and Fe-matrix/HA composites fabricated by conventional powder metallurgy method (yield strengths in tension of 96 and 82 MPa, respectively) [33].

The stability during sintering is one of the most important advantages of the TCP/Fe composites in comparison with the most studied HA/metal composites. Even the slightest deviation from the stoichiometry can lead to HA decomposition during sintering [14,45], producing secondary phases that may impair the mechanical performance of the composite [15,23,25]. This thermal instability of HA is amplified when it is sintered in contact with high reactive metals such as titanium, which reacts with OH groups of HA, making its decomposition during sintering faster [12-15,24,25]. The reactivity of titanium is so high that it also partially decomposes TCP during sintering [46]. In contrast, XRD patterns and elemental maps (Figure 1) confirmed that TCP and Fe powder mixtures are stable, allowing together with

pulsed electric current assisted sintering the retention of pure components without interfacial reaction between them. Nonetheless, TCP presented partial allotropic alpha to beta phase transformation. Similarly to HA, TCP is considered as bioactive and resorbable material, appropriate for bone regeneration and it is successfully used in clinic as synthetic bone filler material [5,6]. A second advantage of TCP is its higher solubility with respect to HA, that makes it more resorbable, *i.e.* it can be resorbed *in vivo* in shorter time [39]. Therefore, in combination with a proper biodegradable reinforcing metal, such as Fe, TCP allows the possibility to fabricate biodegradable, load-bearing and low elastic modulus composite materials. Furthermore, although Fe is a heavy metal, it produces composites with low density in combination with 75 vol.% of TCP (Table 1), and more important, with high specific compressive strength (Figure 3). This is a paramount advantage of this composite for the fabrication of lighter and probably thinner and less invasive implants that potentially could be resorbed in a shorter time, producing less amount of degradation products, but as well mechanically reliable. Nonetheless, attention should be pay to the magnetic properties of the composite in concern with its compatibility with common clinical imaging techniques.

The ideal biodegradable material for bone fracture fixation should impart mechanical stability during fracture consolidation, which depending on the bone and type of fracture could be around 3 to 12 weeks [29]. The present study shows that the DTS of TCP/25Fe composite did not change significantly during the first 8 weeks of degradation and presented only a 20 % reduction after 16 weeks (Figure 4d). Therefore, this composite, or in general the other composites tested, can provide reliable mechanical integrity during fracture healing since they present similar degradation rate. Besides, the thickness of the implant can be designed to match specific mechanical and reabsorbtion requirements. After bone fracture healing, the degradation rate of the biodegradable implant should increase, but within the natural buffer capacity of the body to eliminate the degradation products without adverse effects. Only some recent works studied the degradation of Fe-matrix/ β -TCP composites [30-32]. However, α -TCP that is slightly more soluble than β -TCP has never been studied to increase the degradation rate. Therefore, a novelty of

this work was use of α -TCP in a wider compositional range. However, the sintering temperature below the stability temperature of the α -TCP phase (1275 °C [38]), together with the mass transport mechanisms promoted by the high temperature and the electric current field, the process resulted in the TCP component composed of both α -TCP and β -TCP polymorphs (Figure 1). In spite of that, XRD results from the degradation test (Figure 6) prove that the α -TCP crystalline phase dissolved faster than the β -TCP, concluding that α -TCP can produce more resorbable composites. Besides, the incorporation of Fe considerably increased the mass loss and degradation rate of the composites with respect to the pure components (Figure 4). Nonetheless, the experimental degradation rates attained are still too low for proper degradation of temporal implants for osteosynthesis. For example, considering a standard bone plate with 2 mm of thickness, the implant will be resorbed in around 10 years. Therefore, although pure α -TCP is technically more difficult to retain, it may form composites with more suitable degradation rates. Alternatively, other calcium phosphates with solubility greater than α -TCP, such as dicalcium phosphate, could be used as matrix of the composite to increase its degradation rate.

The degradation mechanisms of the composites *in vitro* involve the passive dissolution of both TCP polymorphs and the electrochemical corrosion of Fe, resulting in the release of calcium, phosphate and iron ions. The next stage is the precipitation of magnetite as solid corrosion product. However, the presence of hydrated ferric oxides and hydroxides as well as calcium and phosphate substituted amorphous phases cannot be discarded. In comparison with as-sintered samples, the degraded samples that contain Fe also exhibited the presence of cementite. Cementite or rather perlite microstructure was formed within Fe particles during sintering due to the dissolution of carbon nanotubes in Fe matrix. During degradation, the anodic dissolution of ferrite at the surface left behind the typical parallel plates of cementite (Figure 5). Over time, the degradation of the matrix of the composite will produce the detachment of the reinforcement particles as observed in the case of Ti/ β -TCP composites. In this case, the released β -TCP particles were reabsorbed *in vivo* while the titanium matrix was osteointegrated [46]. It is expected that for the new TCP/Fe composites, both, the particles and the matrix, will be reabsorbed

regardless of whether they are TCP or Fe. Besides, the release of particles from the osteosynthesis device should not have the same adverse effect such as that taking place in total joint arthroplasties, where particles debris catalyses the degradation of the articular surface inducing severe osteolysis [47].

Osteoblasts in indirect contact with the composites were viable and proliferated in a similar rate as in the contact with TCPS (Figure 7), showing that release of calcium, phosphate and iron ions is not detrimental for osteoblast metabolism. Ullum *et.al.* reported similar cytocompatible results using muscle cells in indirect contact with Fe/ β -TCP composites [30]. Therefore, it can be expected that ions released during the degradation of the composite will not impair fracture healing or near muscles function *in vivo*. Besides, it is expected that the natural circulation of body fluids *in vivo* together with the body buffer capacity dilute the concentrations of ions, minimising possible adverse effects. For example, the vascular implantation of Fe-stents does not produce local or systemic toxicity and only moderate signals of inflammatory reactions are reported [28]. Furthermore, the concentration of calcium, iron and phosphorus in the blood of sheep did not change after being implanted with Fe/HA composites [32]. A further proof of the cytocompatibility of the TCP/Fe composites is that osteoblasts are able to attach, spread and produce active ALP on their surface (Figure 8 and Figure 9). In fact, there was an increment of ALP activity between 6 and 24 h of direct contact culture, which was more pronounced on TCP and TCP/25Fe samples, probably due to a bioactive effect of the more abundant TCP matrix. Despite the fact that no cytotoxicity neither biocompatibility of the degradation products were studied in the present work, there are studies reporting that they are well tolerated *in vivo*. Magnetite nanoparticles are commonly used for cancer ablation [48] and good osteointegration has been observed in biodegradable pure Fe, Fe alloys and Fe composites, presenting similar degradation products [27,32,46]. The exact composition of the degradation products *in vivo* may differ from that observed in the *in vitro* degradation test, independently of the composition of the immersion medium used. Therefore, the conclusions draw from the present study must be taken as a first approximation, but not as an exhaustive explanation of what can actually

happen during *in vivo* degradation. Further experimental work is required to proof the degradation mechanism and biocompatibility of the new TCP/Fe composites *in vivo*.

5. Conclusions

The incorporation of Fe particles allowed the control of the mechanical behaviour of TCP composites, passing from brittle fracture to some degree of plastic deformation before failure. Toughening of TCP was achieved due to the advanced processing method used for powder consolidation, which combines high heating rate, high temperature, direct electrical current pulses and mechanical compaction pressure. All together results in high material densification and direct contact between the matrix and the reinforcements, condition required for proper load transmission. In particular, the incorporation of only 25 vol.% of Fe resulted in the development of a light-weight composite with stiffness and specific strength that fulfil the requirements for the fabrication of implants for bone fracture fixation. *In vitro* study showed that TCP/Fe composites have higher degradation rate than their components, but are mechanically reliable during the time required for bone fracture healing. Furthermore, osteoblast-like cells attached and spread on the surface of the composites, presenting similar proliferation rate as on TCPS and showed ALP activity. Therefore, the developed composites successfully combine the strength and toughness of Fe with the osteoconductivity of TCP. Further improvement of the sintering conditions is still required to retain the pure α -TCP matrix, in order to obtain implants that potentially could be resorbed in a shorter time.

6. Acknowledgments

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie and it is co-financed by the South Moravian Region under grant agreement no. 665860. Authors also acknowledge the project CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Program II. Part of the work was carried out with the support of core facilities of

research infrastructure CEITEC Nano of CEITEC-BUT. MCL acknowledges to Brno Ph.D. Talent scholarship founded by the Brno City Municipality. SDT acknowledges to CONACYT-SNI and SIP-IPN. Special thanks to Dr. A. Danhel from the Institute of Biophysics, Academy of Science of the Czech Republic for the support with ICP-OES, Dr. M. Rampichová from Czech Technical University in Prague for supplying the cells for this work, Eng. G. Diéguez-Trejo and A. Patiño-Pineda from CIITEC-IPN for their technical assistance during SPS, Dr. L. Klakurková from CEITEC-BUT for her technical assistance during sample characterization and Dr. Z. Pavlouskova from CEITEC-BUT for her assistance in administrative tasks.

7. Disclosures

Authors have no competing interests to declare.

8. References

- [1] V.K. Ganesh, K. Ramakrishna, D.N. Ghista, Biomechanics of bone-fracture fixation by stiffness-graded plates in comparison with stainless-steel plates, *Biomed. Eng. Online*. 4 (2005) 46.
- [2] J. Kärrholm, R. Razaznejad, Fixation and bone remodeling around a low stiffness stem in revision surgery, *Clinical Orthopaedics and Related Research* 466 (2008) 380–388.
- [3] M. Navarro, A. Michiardi, O. Castaño, J.A. Planell, Biomaterials in orthopaedics, *J. R. Soc. Interface* 5 (2008) 1137–1158.
- [4] P.P. Schmittenebecher, Implant removal in children, *Eur. J. Trauma Emerg. Surg.* 39 (2013) 345–352.
- [5] R.Z. LeGeros, J.P. LeGeros, Calcium phosphate bioceramics: past, present and future, *Key Eng. Mater.* 240-242 (2003) 3-10.
- [6] M. Espanol, R.A. Perez, E.B. Montufar, M.P. Ginebra, Ceramics, in: M. Santin, G.J. Phillips (Eds.), *Biomimetic, Bioresponsive, and Bioactive Materials: An Introduction to Integrating Materials with Tissues*, John Wiley & Sons, 2012, pp 162-190.

- [7] X. Zhang, G.H.M. Gubbels, R.A. Terpstra, R. Metselaar, Toughening of calcium hydroxyapatite with silver particles. *J. of Mater. Sci.* 32 (1997) 235-243.
- [8] B.T. Lee, N.Y. Shin, J.K. Han, Y.H. Song, Microstructures and fracture characteristics of spark plasma-sintered HAp–5 vol.% Ag composites, *Mater. Sci. and Eng. A* 429 (2006) 348–352.
- [9] S. Nath, S. Kalmodia, S. Basu, Densification, phase stability and in vitro biocompatibility property of hydroxyapatite-10 wt% silver composites. *J. Mater. Sci. Mater. Med.* 21 (2010) 1273–1287.
- [10] A. Rajendran, R.C. Barik, D. Natarajan, M.S. Kiran, D.K. Pattanayak, Synthesis, phase stability of hydroxyapatite–silver composite with antimicrobial activity and cytocompatibility, *Ceram. Int.* 40 (2014) 10831–10838.
- [11] C. Chenglin, Z. Jingchuan, Y. Zhongda, W. Shidong, Hydroxyapatite–Ti functionally graded biomaterial fabricated by powder metallurgy, *Mater. Sci. and Eng. A* 271 (1999) 95–100.
- [12] C.Q. Ning, Y. Zhou, In vitro bioactivity of a biocomposite fabricated from HA and Ti powders by powder metallurgy method, *Biomaterials* 23 (2002) 2909–2915.
- [13] C. Chenglin, X. Xiaoyan, Z. Jingchuan, Y. Zhongda, Mechanical and biological properties of hydroxyapatite reinforced with 40 vol. % titanium particles for use as hard tissue replacement, *J. Mater. Sci. Mater. Med.* 15 (2004) 665-670.
- [14] Y. Yang, K.H. Kim, C.M. Agrawal, J.L. Ong, Interaction of hydroxyapatite–titanium at elevated temperature in vacuum environment, *Biomaterials* 25 (2004) 2927–2932.
- [15] S. Salman, O. Gunduz, S. Yilmaz, M.L. Ovecoglu, Robert L. Snyder, S. Agathopoulos, F.N. Oktar, Sintering effect on mechanical properties of composites of natural hydroxyapatites and titanium, *Ceram. Int.* 35 (2009) 2965–2971.
- [16] A. Kumar, K. Biswas, B. Basu, On the toughness enhancement in hydroxyapatite-based composites, *Acta Mat.* 61 (2013) 5198–5215.
- [17] M.I. Ramli, A.B. Sulong, N. Muhamad, A. Muchtar, A. Arifin, Stainless steel 316L–hydroxyapatite composite via powder injection moulding: rheological and mechanical properties characterisation, *Mater. Res. Innovations* 18 (2014) S6-100-S6-104.

- [18] S. Zhu, X. Wang, M. Yoshimura, A. Inoue, Synthesis of Ti-based glassy alloy/hydroxyapatite composite by spark plasma sintering, *Mater. Trans.* 49 (2008) 502–505.
- [19] A.A. White, S.M. Best, Hydroxyapatite–carbon nanotube composites for biomedical applications: A review, *Int. J. Appl. Ceram. Technol.* 4 (2007) 1–13.
- [20] D. Bellucci, L. Desogus, S. Montinaro, R. Orrù, G. Cao, V. Cannillo, Innovative hydroxyapatite/bioactive glass composites processed by spark plasma sintering for bone tissue repair. *J. Eur. Ceram. Soc.* 37 (2017) 1723–1733.
- [21] C. Chenglin, X. Xiaoyan, Z. Jingchuan, Y. Zhongda, Fabrication and characterization of titanium-matrix composite with 20 vol% hydroxyapatite for use as heavy load-bearing hard tissue replacement, *J. Mater. Sci. Mater. Med.* 17 (2006) 245–251.
- [22] C. Ning, Y. Zhou, Correlations between the in vitro and in vivo bioactivity of the Ti/HA composites fabricated by a powder metallurgy method, *Acta Biomaterialia* 4 (2008) 1944–1952.
- [23] E.S. Thian, N.H. Loh, K.A. Khor, S.B. Tor, In vitro behavior of sintered powder injection molded Ti-6Al-4V/HA, *J. Biomed. Mater. Res. Part B* 63 (2002) 79–87.
- [24] H. Ye, X.Y. Liu, H. Hong. Characterization of sintered titanium/hydroxyapatite biocomposite using FTIR spectroscopy, *J. Mater. Sci. Mater. Med.* 20 (2009) 843–850.
- [25] P. Balbinotia, E. Gemelli, G. Buerger, S.A. de Lima, J. de Jesus, N.H. Almeida Camargo, V.A. Rodrigues Henriques, G.D. de Almeida Soares, Microstructure Development on sintered Ti/HA biocomposites produced by powder metallurgy, *Mater. Res.* 14 (2011) 384–393.
- [26] Anawati, H. Tanigawa, H. Asoh, T. Ohno, M. Kubota, S. Ono, Electrochemical corrosion and bioactivity of titanium–hydroxyapatite composites prepared by spark plasma sintering, *Corr. Sci.* 70 (2013) 212–220.
- [27] T. Kraus, F. Moszner, S. Fischerauer, M. Fiedler, E. Martinelli, J. Eichler, F. Witte, E. Willbold, M. Schinhammer, M. Meischel, P.J. Uggowitzer, J.F. Löffler, A. Weinberg, Biodegradable Fe-based alloys for use in osteosynthesis: Outcome of an in vivo study after 52 weeks, *Acta Biomaterialia* 10 (2014) 3346–3353.

- [28] A. Francis, Y. Yang, S. Virtanen, A.R. Boccaccini, Iron and iron-based alloys for temporary cardiovascular applications, *J. Mater. Sci. Mater. Med.* 26 (2015) 138.
- [29] Y.F. Zheng, X.N. Gu, F. Witte, Biodegradable metals, *Mater. Sci. and Eng. R.* 77 (2014) 1–34.
- [30] M.F. Ulum, A. Arafat, D. Noviana, A.H. Yusop, A.K. Nasution, M.R. Abdul Kadir, H. Hermawan, In vitro and in vivo degradation evaluation of novel iron-bioceramic composites for bone implant applications, *Mater. Sci. and Eng. C* 36 (2014) 336–344.
- [31] A. Reindl, R. Borowsky, S.B. Hein, J. Geis-Gerstorfer, P. Imgrund, F. Petzoldt, Degradation behavior of novel Fe/ β -TCP composites produced by powder injection molding for cortical bone replacement, *J. Mater. Sci.* 49 (2014) 8234–8243.
- [32] M.F., Ulum, A.K. Nasution, A.H. Yusop, A. Arafat, M. Rafiq, A. Kadir, V. Juniantito, D. Noviana, H. Hermawan, Evidences of in vivo bioactivity of Fe-bioceramic composites for temporary bone implants, *J. Biomed. Mater. Res. Part B* 103 (2015) 1354–1365.
- [33] M. Dehestani, E. Adolfsson, L.A. Stanciu. Mechanical properties and corrosion behavior of powder metalurgy iron-hydroxyapatite composites for biodegradable implant applications, *Mater.s and Des.* 109 (2016) 556–569.
- [34] M. Tokita, Trends in advanced SPS spark plasma sintering systems and technology. *Joztrital of the Society of Powder Techtrology Jayat*, 30 (1993) 790-804.
- [35] R. Orrú , R. Licheri, A.M. Locci, A. Cincotti, G. Cao, Consolidation/synthesis of materials by electric current activated/assisted sintering. *Mater. Sci. Eng. R.* 63 (2009) 127-287.
- [36] O. Guillon¹, J. Gonzalez-Julian, B. Dargatz, T. Kessel, G. Schierning, J. Räthel, M. Herrmann. Field-Assisted Sintering Technology/Spark Plasma Sintering: mechanisms, materials, and technology developments, *Adv. Eng. Mater.* 16 (2014) 830–849.
- [37] Y. Aman, V. Garnier, E. Djurado, Pressure-less spark plasma sintering effect on non-conventional necking process during the initial stage of sintering of copper and alumina, *J. Mater. Sci.* 47 (2012) 5766–5773.

- [38] R.G. Carrodeguas, S. de Aza, α -Tricalcium phosphate: synthesis, properties and biomedical applications, *Acta Biomaterialia* 7 (2011) 3536–3546.
- [39] H. Rojbani, M. Nyan, K. Ohya, S. Kasugai, Evaluation of the osteoconductivity of α -tricalcium phosphate, β -tricalcium phosphate, and hydroxyapatite combined with or without simvastatin in rat calvarial defect, *J. of Biomed. Mater. Res. Part A* 98 (2011) 488–98.
- [40] M. Yamada, M. Shiota, Y. Yamashita, S. Kasugai, Histological and histomorphometrical comparative study of the degradation and osteoconductive characteristics of α - and β -tricalcium phosphate in block grafts, *J. of Biomed. Mater. Res. Part B* 82 (2007) 139–148.
- [41] F. Witte, F. Feyerabend, P. Maier, J. Fischer, M. Stormer, C. Blawert, W. Dietzel, N. Hort, Biodegradable magnesium–hydroxyapatite metal matrix composites, *Biomaterials* 28 (2007) 2163–2174.
- [42] J. Cheng, Y.F. Zheng, In vitro study on newly designed biodegradable Fe-X composites (X = W, CNT) prepared by spark plasma sintering, *J. Biomed. Mater. Res. Part B* 101 (2013) 485–497.
- [43] E.B. Montufar, Y. Maazouz, M.P. Ginebra, Relevance of the setting reaction to the injectability of tricalcium phosphate pastes, *Acta Biomaterialia* 9 (2013) 6188–6198.
- [44] A. Kumar, K. Biswas, A.B. Basu, Hydroxyapatite-titanium bulk composites for bone tissue engineering applications, *J. of Biomed. Mater. Res. Part A* 103 (2014) 791–806.
- [45] E. Champion, Sintering of calcium phosphate bioceramics, *Acta Biomaterialia* 9 (2013) 5855–5875.
- [46] D. Mondal, L. Nguyen, I.H. Oh, B.T. Lee, Microstructure and biocompatibility of composite biomaterials fabricated from titanium and tricalcium phosphate by spark plasma sintering, *J. Biomed. Mater. Res. Part A* 101 (2013) 1489–1501.
- [47] R. Dattani, Femoral osteolysis following total hip replacement, *Postgraduate Medical Journal*. 83 (2007) 312–316.
- [48] R.A. Revia, M. Zhang, Magnetite nanoparticles for cancer diagnosis, treatment, and treatment monitoring: recent advances. *Mater. Today* 19 (2016) 157–168.

Figure captions

Figure 1. XRD patterns and backscattered electron SEM images of the composites with different volume contents of Fe a) 0 %, b) 25 %, c) 50 %, d) 75 % and e) 100 %. In backscattered electron SEM images TCP appears as dark phase, while Fe is brighter. f) Shows a representative backscattered electron SEM image together with the corresponding elemental mapping of iron, calcium and phosphorous for the TCP/50Fe sample.

Figure 2. Reinforcement effect of Fe on TCP a) DTS of the TCP-Fe composites as function of the Fe content and b) representative images of the fractured samples after DTS test.

Figure 3. Elastic modulus (E) plotted against specific strength (σ_y/ρ) for mineralized biological materials (bone and nacre), commonly implantable materials (divided in metals, ceramics and polymers) and the new TCP/25Fe composite. Plotted strength corresponds to tensile yield strength for metals and polymers and compressive strength for ceramics and composites. The chart was built using the software CES Edu Pack 2013.

Figure 4. Results of the *in vitro* degradation test. a) Mass loss and b) degradation rate as a function of the immersion time in 0.9 wt.% NaCl solution at 37 °C. Legends for graphs (a) and (b) are shown in (a). c) Percentage increase of concentration of calcium (Ca), phosphorus (P) and iron (Fe) ions in the NaCl solution. Values represent the average increment every week during the 16 weeks of experiment. d) Changes in DTS for the TCP/25Fe composite as function of the immersion time in 0.9 wt.% NaCl solution at 37 °C.

Figure 5. Macroscopic aspect (left) and microstructure (centre) of the corrosion products formed on the surface of the TCP-Fe samples after 16 weeks of degradation in NaCl solution at 37 °C. Right images

show the microstructure of the sample surfaces after removal of the corrosion products. Scale bar of the SEM images corresponds to 10 μm .

Figure 6. XRD patterns showing the surface crystalline composition of the TCP-Fe composites after 16 weeks of degradation in NaCl solution at 37 °C.

Figure 7. Indirect contact effect of the TCP-Fe composites on the proliferation of MG-63 cells. Tissue culture-grade polystyrene (TCPS) was used as reference material.

Figure 8. Representative SEM images showing the direct contact effect of the TCP-Fe composites on the morphology of MG-63 cells cultured during 6 h.

Figure 9. Direct contact effect of the TCP-Fe composites on ALP activity of MG-63 cells cultured during 6 and 24 h.