In-vitro comparison of hydroxyapatite coatings obtained by cold spray and conventional thermal spray technologies

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Abstract

Hydroxyapatite (HA) coatings onto Ti6Al4V alloy substrates were obtained by several thermal spray technologies: atmospheric plasma spray (APS) and high velocity oxy fuel (HVOF), together with the cold spray (CS) technique. A characterization study has been performed by means of surface and microstructure analyses, as well as biological performance. In-vitro tests were performed with primary human osteoblasts at 1, 7 and 14 days of cell culture on substrates. Cell viability was tested by MTS and LIVE/DEAD assays, cell differentiation by alkaline phosphatase (ALP) quantification, and cell morphology was analyzed by scanning electron microscopy.

The HA coatings showed an increase of HA crystallinity from 62,4% to 89%, but also an increase of hydrophilicity from ~32° to 0°, with the decrease of the operating temperature of the thermal spray techniques (APS > HVOF > CS). Additionally, APS HA coatings showed more surface micro-features than HVOF and CS HA coatings; cells onto APS HA coatings showed faster attachment by acquiring osteoblastic morphology in comparison with the rounded cell morphology observed onto CS HA coatings at 1 day of cell culture. HVOF HA coatings also showed proper cell adherence but did not show extended filopodia as cells onto APS HA coatings. However, at 14 days of cell culture, higher cell proliferation and differentiation was detected on HA coatings with higher crystallinity (HVOF and CS techniques). Cell attachment is suggested to be favoured by surface micro-features but also moderate surface wettability whereas cell proliferation and differentiation is suggested to be highly influenced by HA crystallinity and crystal size.

Keywords: Atmospheric plasma spray; High velocity oxy fuel; Cold spray; Hydroxyapatite coatings; In-vitro tests; Joint prosthesis

1 Introduction

Hydroxyapatite (HA) has been recognized for its biocompatibility and usefulness in promoting biointegration for implants in osseous and soft tissue due to its similar composition with the bone. Atmospheric Plasma Spraying (APS) is a well-established process in commercial prosthesis for the production of HA coatings and it has been validated by FDA regulations [1,2]. Even so, the use of high operating temperatures and high cooling rates produces changes in the phase composition, thus leading to more likely implant failure [3]. The weakest part tends to be the coating-substrate interface, where HA decomposes into secondary phases that have higher dissolution rates in body fluids, ending up with the delamination of the coating [1].

Some alternatives are the performance of post-heat treatments of such HA coatings in order to transform amorphous HA into crystalline phases. Another strategy is the use of metallic porous-rough surfaces to enhance mechanical properties by the interlocking of bone ingrowth into the pores [4].

The dissolution behavior of the coating is a critical factor to achieve long-term stability coatings. The two main factors that control the dissolution rates of the coatings are (i) the inherent material properties, such as composition and crystallinity, and (ii) environmental factors, such as media composition and pH. It is clearly known that the dissolution behavior of HA coatings is very sensitive to HA crystallinity; secondary HA phases such as tricalcium phosphate (α/β form), calcium oxide (CaO), tetracalcium phosphate (TTCP) as well as amorphous calcium phosphate (ACP) increase dissolution rates [5,6]. Also, it was shown that nanoscale particles provided better stabilization, bone ingrowth and osteointegration than of uncoated and microscale HA-coated coatings [7]. Some attempts were found in the literature by the use of High Velocity Oxy Fuel (HVOF) due to its low temperatures in comparison with APS. Although higher crystalline HA coatings were obtained by HVOF in comparison with APS, it was still observed the formation of ACP phase in the interface coating/substrate [8,9], which is detrimental for the bond strength of the coating under in-vitro performance. As an alternative, crystalline HA coatings by HVOF were obtained after a heat treatment at 700 °C for 60 min, leading to higher bond strength after immersion in simulated body fluids (SBF) than without treatment [9]. However, osteoblastic differentiation was higher in the presence of ACP. Thus, HA coatings with graded crystallinity seems to be optimal in order to achieve a balance between the biological properties of the as-sprayed coatings and the adhesive strength of the crystalline coatings [10].

In order to control HA composition, low-temperature coating deposition processes were purposed such as Aerosol Deposition (AD) [11,12] and Nano Particle Deposition System (NPDS) [13]. However, the use of sub-micron sized particles as feedstock powder led to very thin (nanometric) layers. Dense nanostructured HA coatings were obtained by AD with a 98.5% density and a tensile adhesion of 30.05 ± 1.2 MPa. However, further heat treatment up to 400 °C needed to be applied in order to avoid amorphous phases. Heat treatments up to 400 °C increased HA crystal size from 16.2 to 29.3 nm, as well as biological properties. In contrast, heat treatments above 400 °C induced an increase of HA crystal size up to 99.7 nm, but a decrease of biological properties [11]. Those results agreed with Webster et al. [14] which reported that osteoblast cells are sensible to HA grain size under 100 nm. Both AD HA coatings showed good biocompatibility promoting osseointegration, however, it was suggested a successful outcome without post-heating treatment showing a high bone implant contact length [12]. Moreover, different HA surface roughnesses were achieved by manipulating the particle size distribution of the feedstock powder. An increase of roughness was perceived from 0.65 to 1.03 μm together with a thickness increase up to 10 μm. It was found that the optimal biological performance was for 5 μm thick HA coating with an intermediate surface roughness of 0.82 μm [15].

As an alternative, Cold Sprayed (CS) was purposed for the obtaining of different HA coatings. CS is a solid-state coating process in which powder particles are accelerated up to 1200 m/s over the supersonic velocities through a de Laval nozzle onto a substrate and, unlike the conventional processes, powder particles here do not reach their melting points. Therefore, CS is a suitable technique to spray oxygen and temperature sensitive materials, obtaining deposits with the same composition as in the feedstock. In the case of metals, particles undergo plastic deformation during the impact and adhere to the substrate. Spraying ceramics is more challenging but recent studies have dealt with their deposition mechanisms, which is also very dependent on the feedstock characteristics [16–19]. Thus, CS allows the option to spray customized coatings with the desired microstructure and composition, which can be especially beneficial in biomedical field [20].

The present research is focused on the in-vitro comparison of APS, HVOF and CS HA coatings. Surface parameters such as topography, phase composition, crystallinity, wettability and microstructure were evaluated to analyze cell response.

2 Materials and experimental method

2.1 Feedstock materials

HA coatings have been previously optimized by APS and HVOF [10] technologies, using a sintered crystalline HA (C-HA) powder from Plasma-Biotal Ltd (Captal 30, Derbyshire, UK). On the other hand, an agglomerated nanocrystalline HA (NC-HA) powder from Medicoat (France) was used to produce and optimize the HA coatings by CS [17,21], since C-HA powder did not show a proper build-up of a coating. Both HA powders were sprayed onto 7x7x2 mm Ti6Al4 alloy substrates for the performance of the in-vitro tests.

2.2 Deposition and characterization of coatings

The set of spraying parameters for APS and HVOF HA coatings were selected with the aim to reach a high amount of crystallinity [10]. Optimized APS and HVOF HA coatings were deposited onto grid blasted Ti6Al4V alloy substrates (Table 1). An APS A-3000 system with an F4 plasma torch (Sulzer Metco, Germany) and DJH 2600 HVOF system (Sulzer Metco, Inc., Westbury, NY) were used for that purposed. On the other hand, CS HA spraying conditions were optimized onto highly rough commercial pure titanium bondcoat for proper deposition [4,21]. The CS equipment used was a CGS KINETICS® 4000 (Cold Gas Technology, Ampfing, Germany) with a maximum operating pressure of 40 bar, temperature of 800 °C and it operated with nitrogen as the propellant gas.

Table 1 Optimal APS and HVOF spraying conditions [10][a].

alt-text: Table 1

APS		HVOF	
Primary gas (Ar) [l min-1]	50	Oxygen [l min-1]	193
Secondary gas (H2) [l min-1]	1	Hydrogen [l min-1]	635
Carrier gas (Ar) [l min-1]	3.6	Air [l min-1]	275
Intensity [A]	500	Feeding rate [g s-1]	33
Stand off-distance [mm]	80	Stand off-distance	225
Torch speed [mms-1]	600	Torch speed [mms-1]	1000
Gun passes [n°]	5	Gun passes [n°]	5

[a] Spraying conditions of HA coatings by CS are summarized in the following intellectual property [21].

Field Emission Scanning Electron Microscopy (FESEM) JEOL JSM 7100F equipment was used to evaluate the top surface of the HA coatings. Thickness and porosity values were measured according to ASTM F1854 with Optical Microscopy (Leica DMI5000 M), while Confocal Microscopy (Leica DCM3D) was used to measure coating roughnesses. The X-Ray Diffraction (XRD) measurements were carried out on a Bragg–Brentano θ/2θ Siemens D-500 diffractometer with Cu Kα radiation. The phase identification was analyzed with X'Pert PRO MPD diffractometer (PANalytical). A Rietveld analysis, using the FullProf software [22], was carried out to determine the percentage of the crystalline and amorphous phases [23]. A home-made water contact angle goniometer with ImageJ software program has been used for the measurement of the contact angles, performing static measurements after 10 s in sessile drop mode with 2 ml volume Milli-Q H_2O droplet.

2.3 Cell culture

Human osteoblastic cells (HOBs) have been obtained from knee trabecular bone of postmenopausal women undergoing knee replacement due to osteoarthritis, following the protocol described by Nacher et al. [24]. The entire study has been approved by the Parc de Salut Mar Ethics Committee. Briefly, trabecular bone was dissected into small pieces, thoroughly washed in phosphate-buffered solution (PBS) and placed into a 15 cm diameter Petri dish containing 15 ml Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, penicillin (100 UI/ml), streptomycin (100 UI/mL), ascorbic acid (100 µg/ml) (Invitrogen) and fungisone (0.4%) (Gibco). The explants were incubated at 37 °C in a humidified atmosphere of 5% CO_2, changing the medium once a week until cell confluence. Finally, cells were subcultured into new 75 cm_2 flasks until the needed cell number has been reached. A maximum of a third subculture has been used in the experiments. For materials testing, samples were overnight sterilized in ethanol 70º, washed in PBS and placed on a 48-well polystyrene culture plate (Nunc A/S). Each material was seeded with 100.000 cells and cultured with DMEM supplemented with 10% FBS and ascorbic acid. Samples were tested at 1, 7 and 14 days of cell culture. Tests were carried out three times in order to ensure reproducibility. Each test contained two replicas of each sample and was tested together positive and negative controls. Results were normalized by the APS HA coating samples within each experiment and each time in order to avoid the inter-experiment variability.

2.4 Cell viability assays

Cell viability on materials was tested using MTS assay CellTiter 96® AQueous One Solution Cell Proliferation assay (Promega) according to manufacturer's protocol. 50 µl of MTS were added in each sample cultured with 250 µl of medium, incubating for 3 h and then recording the absorbance at 490 nm.

LIVE/DEAD Viability/Cytotoxicity assay Kit for Mammalian Cells (Invitrogen) discriminates live from dead cells by simultaneously staining with green-fluorescent calcein-AM (life cells) and red-fluorescent ethidium homodimer-1 (dead cells). Live/Dead assay was performed by adding 300 µl of 4 µM EthD-1 and 2 µM of calcein AM in PBS per sample and incubated for 30–45 min at room temperature. Then, surfaces were observed with a Confocal TCS SP5 Upright from Leica Microsystems.

2.5 Alkaline phosphatase assay

Osteoblastic differentiation was evaluated through alkaline phosphatase (ALP) activity, with Abcam's Alkaline Phosphatase Assay Kit. This assay uses p-nitrophenyl phosphate (pNPP) as a phosphatase substrate which turns yellow when dephosphorylated (it turns to p-nitrophenol) by ALP. The resulting absorbance was measured at 405 nm using a scanning multi-well spectrophotometer.

2.6 Cell morphology

Cell morphology was observed in a FESEM JEOL JSM 7100F after 1 and 7 days of cell culture. For that, cells were fixed by immersing the samples in 3.7% formaldehyde (Probus) solution in PBS (1 h at room temperature)

followed by extensively wash with PBS. Then, cells were deshydratated by immersing the samples (x1 or x3 repetitions) in different percentage solutions of ethanol with distilled water: at 50% (x1), 70% (x1), 80% (x1), 90% (x1), 96% (x3) and 100% (x3) of ethanol.

2.7 Statistical analysis

Statistical analyses were performed by Mann-Whitney U test for group comparisons in the SPSS v.12.0 for Windows. All analyses were two-tailed, and p-values<0.05 were considered significant.

3 Results

3.1 Surface characterization of HA coatings

Fig. 1 shows the as-sprayed top surface area of HA coatings. APS and HVOF HA coatings (Fig. 1a,b) show irregular micro-featured morphologies due to the combination melted and un-melted particles On the other hand, CS HA coating (Fig. 1c) shows a more regular surface as a result of the compaction of the particles [19].

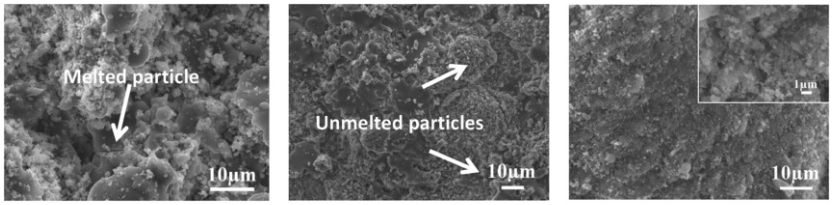

Fig. 1 FESEM micrographs of the top surface areas of a) APS, b) HVOF and c) CS HA coatings.

alt-text: Fig. 1

Table 2 shows the coatings characteristics such as thickness, roughness, porosity and crystallinity. Regarding coating thickness, some authors concluded that an optimum coating of 50 µm would avoid fatigue failure, which commonly occurred in coatings thicker than 100 µm. There is not stablished range of thickness for coating implants, but a thickness of 50–75 µm has been followed by most manufacturers for commercially used orthopedic implants [1]; thus, all coating's thicknesses were optimized to a similar values between 50 and 100 µm. Regarding surface microroughness, both APS and HVOF HA coatings, exhibit similar values. However, CS HA coatings show higher surface roughness due to the rough CS CP-Ti bondcoat [4]. Moreover, an increase of HA crystallinity (APS < HVOF < CS) can be observed with the decrease of the operating temperature of the technique, since higher temperatures, as well as fast cooling rates promotes HA decomposition. Regarding the 89% crystallinity of CS HA coatings, it should be pointed that comes from the feedstock powder, not from CS technique indeed; such coating showed a content of around 7.56% of amorphous phase [17]. Regarding porosity, an amount in the ranges of 21–23% and 11–15%, respectively for APS and HVOF coatings, was found. The resolution given by the ASTM F1854 procedure with Optical Microscopy is however too low to evaluate the porosity of the CS HA coatings; a more detailed study on its microstructure can be found in the previous study [16].

Table 2 Surface characterization of HA coatings obtained by APS and CS *(*Nanoporosity)*.

alt-text: Table 2

PROPERTIES	APS	HVOF	CS
Thickness (µm)	84.5 ± 6.1	68.6 ± 6.0	45 ± 20
Microroughness Ra (µm)	5.8 ± 0.4	4.2 ± 0.4	12 ± 1
Crystallinity (%)	62.4	82	89
Porosity (%)	21–23	11–15	*

Fig. 2 shows the wettability measurements by the calculation of the two contact angles of Milli-Q H_2O droplet onto HA coatings. A higher contact angle was measured onto APS HA coatings with values of $\alpha_1 = 32.4 \pm 6.9$° and $\alpha_2 = 29.8 \pm 5.3$°, followed by HVOF HA and CS HA coatings, with values of $\alpha_1 = 10.8 \pm 2.5$° and $\alpha_2 = 13.8 \pm 2.6$°, and 0° (both angles) respectively. During the test, it was observed that superhydrophilic behaviour of CS HA coatings;

this might be explained by its nanoporosity, as the Milli-Q H_2O water was absorbed by the coatings.

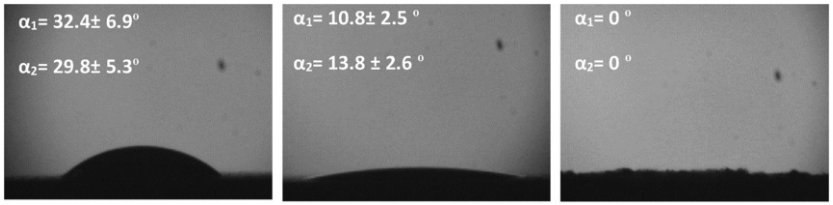

Fig. 2 Contact angle of a Milli-Q H_2O droplet a) APS, b) HVOF and c) CS HA coatings.

alt-text: Fig. 2

3.2 Osteoblast viability and proliferation

Osteoblast cell viability and proliferation were qualitatively analyzed by Live/Dead (Fig. 3) after 1, 7 and 14 days cell of culture. Live/Dead images of APS and HVOF HA coatings showed a continuous and faster cell proliferation. On contrary, CS HA coating showed a slower cell proliferation up to 7 days cell of culture, then cell proliferation increases faster than APS and HVOF coatings overpassing their values at 14 days of cell culture. In addition, for all the conditions, no significant number of dead cells was found along days of cell culture. Fig. 4 shows the MTS assay test, which corroborates quantitatively the results obtained with Live/Dead assay.

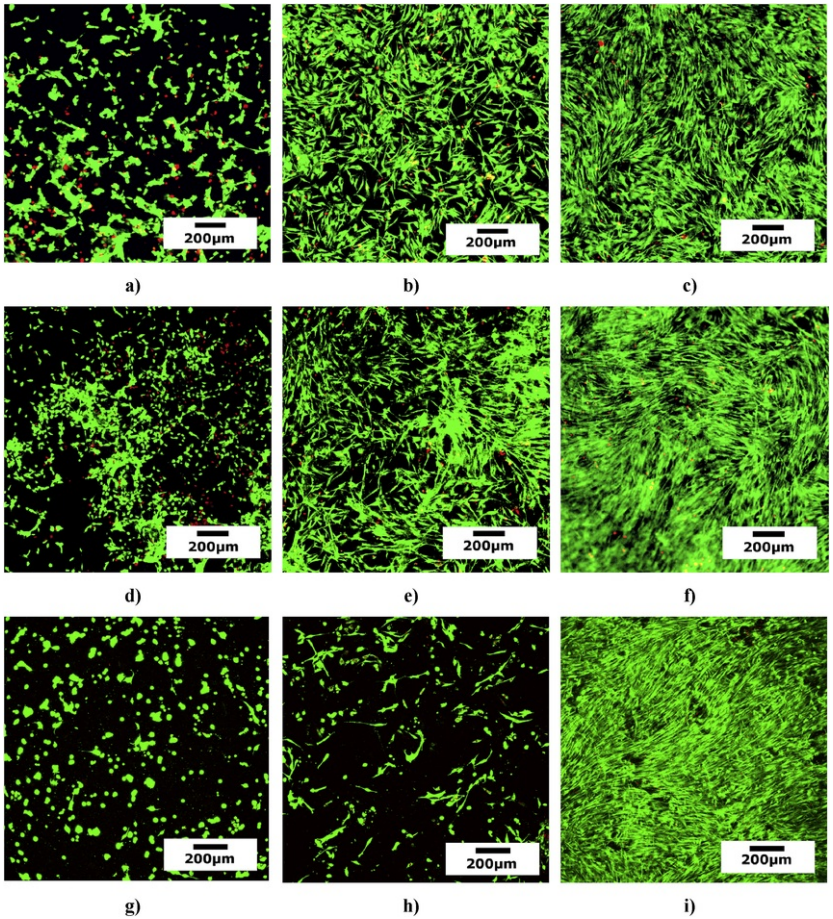

Fig. 3 Live/Dead assay at 1, 7 and 14 days (from left to right) of cell culture onto a,b,c) APS, d,e,f) HVOF and g,h,i) CS HA coatings.

alt-text: Fig. 3

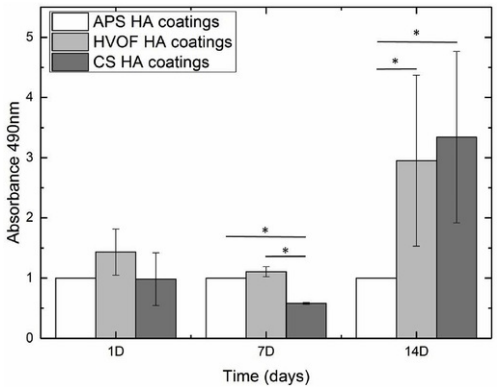

Fig. 4 MTS assay at 1, 7 and 14 days of cell culture onto APS, HVOF and CS HA coatings (n = 3; *p-values<0.05).

alt-text: Fig. 4

3.3 Osteoblast differentiation

The ALP is an early marker of cell differentiation that allows us to monitor the osteoblast status (Fig. 5). The ALP activity of HA surfaces is maintained constant along 1 and 7 days of cell culture. However, ALP activity of HVOF and CS HA coatings showed significantly higher values in comparison with APS HA coatings, especially HVOF HA coating.

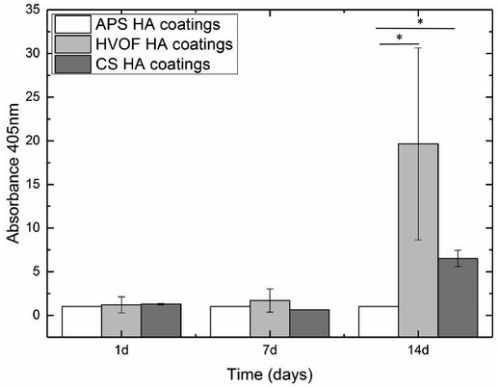

Fig. 5 ALP assay at 1, 7 and 14 days of cell culture onto APS, HVOF and CS HA coatings (n = 3; *p-values<0.05).

alt-text: Fig. 5

3.4 Morphological aspects of osteoblast

Cells were observed by FESEM after 1 and 7 days of cell culture (Fig. 6). At 1 day of cell culture, cells onto APS and HVOF HA coatings exhibited a typical adherent osteoblast morphology (Fig. 6 a,b). Cells showed a flattened cytoplams with long filopodia attached onto the surface, while at 7 days of culture cells started connecting their filopodia between them (Fig. 6 d,e). On the other hand, cells onto CS HA coating showed spherical cell morphology at 1 day of cell culture (Fig. 6c) showing a slower cell attachment on the HA surface. After 7 days of cell culture, cells on CS HA coating adquired flattened adherent morphology with extended filopodia showing good cell attachment (Fig. 6f).

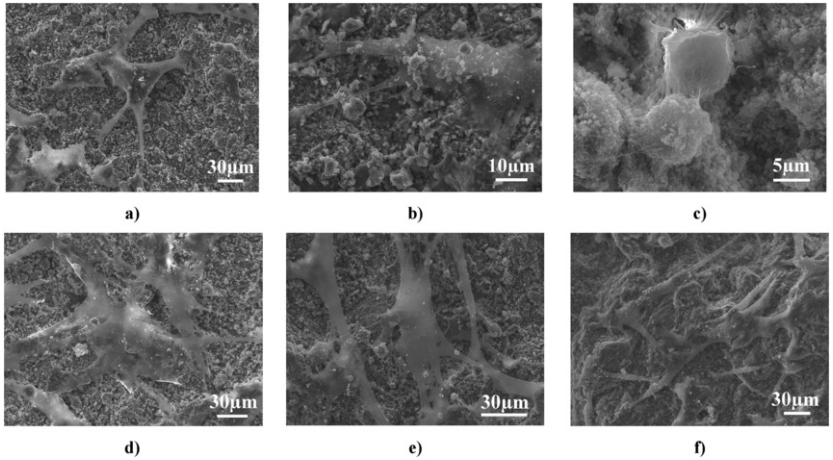

Fig. 6 FESEM micrographs of cells at a,b,c) 1 and d,e,f) 7 days of cell culture onto (a,d) APS, (b,e) HVOF and (c,f) CS HA coatings (n = 3).

alt-text: Fig. 6

4 Discussion

4.1 HA spraying features by thermal spray techniques

The crystallinity of HA coatings by APS and HVOF is strongly influenced by the higher operating temperatures and high cooling rates. In the case of APS, operating temperature varies between 6000 and 15000 °C in comparison with HVOF, which reaches 2500 to 3100 °C; the much higher particle velocities achieved during HVOF also involves much lower in-flame times of particles. Thus, it is easier to produce HA coatings with higher crystallinity by HVOF. With the present spraying conditions, APS HA coatings were successfully obtained with up to 62.4% of crystalline phases. However, this value can be varied according to the process variables, mainly gun power and nozzle-sample distance [25–27]. HA HVOF HA studies found the preferred optimized settings to achieve high crystallinity and purity of percentages of up to 95%. This research found crystallinity and purity values of 93.8 and 99.8%, respectively [28]. Although coatings obtained by HVOF have higher crystallinity than APS, the operating temperatures are still too high to have full control of the phase composition at the same time of mechanical properties. The use of CS permits the maintenance of the feedstock crystallinity to the coatings due to its low operating gas temperatures (below 1100 °C), as well as the size of those HA crystallites [17]. Similar results have been reported with other ceramic materials such as TiO_2, by retaining the crystalline pure anatase phase [29].

Additionally, the thermal history of the particles within the flame or gas stream can influence surface morphology of the ceramic HA coatings, thus modifying cell response. A similar surface morphology comparison can be performed by other coatings obtained by APS, HVOF and CS [29–31]. APS coatings mainly show well flattened splats due to the higher temperatures, showing microcracks caused by the rapid quenching of the process which induces large residual tensile stresses and, due to the inherent ceramic brittleness as well [30]. On the other hand, the lower temperatures of CS technique led to ceramic coating surface morphologies similar to the original agglomerate feedstock powder [29], in the present HA study of nanometer size. In the case of HVOF ceramic coatings, in which temperatures are between APS and CS techniques, the surface morphology is composed by a mixture of melted and non-melted particles [30,31].

Although the two feedstocks are different, the comparison for the discussion of cell behavior is presented based on the final surface properties, mainly considering roughness, wettability and crystallinity. Other surface features such as the influence of presence of hydroxyl groups might be also considered for their importance [32] although zeta potential for the measurement of surface charge is not here provided. The presence of hydroxyl groups in HA coatings has been reported to promote the calcium and phosphate precipitation and improve the interactions with osteoblastic cells [33]. In our previous studies, the presence of hydroxyl group in CS HA coatings was observed [17]. However, results on APS and HVOF HA coatings showed different results. The higher temperatures of APS technique led to the disappearance of vibration band at 633 cm^{-1}, as well as hydroxyl stretch band at 3572 cm^{-1} [10]. On the other hand, HVOF HA coatings showed lower intensity hydroxyl stretch band at 3572 cm^{-1} in comparison with the original feedstock powder, but it did not show the presence of the liberation mode at 630 cm^{-1} of the hydroxyl vibration band [34].

Many works within the literature can be then found concerning the influence of the composition, crystallinity, surface topography and wettability on the in-vitro performance. It is hard to stablish a compendium among the parameters, as all of them are related and involved on cell response. The discussion will be addressed trying to evaluate the characteristics that play a more predominant role based on surface features.

4.2 Cell attachment and proliferation

A synergic effect on composition, crystallinity, surface roughness and wettability when considering prosthetic implant surface plays a key role. Two different osteoblast morphologies were found at 1 day of culture: (i) flattened cell morphology on APS and HVOF HA coatings, and spherical cell morphology on CS HA coating. Annaz et al. [35] studied the role of macro- and micro-porosities in synthetic pure HA phase. The cellular attachment was described by the following phases: rounded cellular morphology, protrusion of filopodia oriented towards micropores, extensions of broad lamellipodia followed by flattening of the cells with organized cytoskeletal arrangement. It was concluded that cells have affinity to micropores through filopodia extensions, at initial stage of attachment. The microporosity provided by APS and HVOF HA coatings could suggest a faster initial cell attachment in comparison with the CS HA coatings. At 1 day of cell culture, cells on APS HA coatings show good adherence and osteoblastic morphology in comparison with cells on HVOF HA coatings, which has less extended filopodia and CS HA coatings, which have round cellular morphology. At the time, there is no compendium, limits, or ideal surface roughness for cell attachment. High roughness leads to large surface areas, beneficial for higher cell attachment and deposition. However, cells seem to be much sensitive to “smaller surface features” that facilitate their anchoring [36]. Deligianni et al. [37] studied in-vitro and separately the influence of surface roughness (Ra = 0.73, 2.85 and 4.68 μm), keeping constant the rest of surface parameters. The study concluded that the increase of cell attachment enhances cell adhesion and proliferation. Also,a delayed increase of cell differentiation on rougher surfaces was reported.

On the other hand, it is usually reported that biomaterial surfaces with moderate hydrophilicity provides better cell growth and higher biocompatibility [38]. Surface wettability is considered to increase production of osteoblastic factors and bone formation as well as roughness [39]. In addition, a considerable number of studies have indicated that cells tend to attach better to hydrophilic surfaces than to hydrophobic ones [40,41]. However, some other works argued that cells adhere better on intermediate hydrophobic surfaces with contact angles of around 70° [42,43]. In the present study, different contact angles ~31°, 12° and 0° were obtained for APS, HVOF and CGS HA coatings, respectively. This also suggests that different cell morphologies could be observed according to its degree of wettability, by acquiring faster cell attachment on those surfaces that showed moderated wettability (APS and

HVOF HA coatings) and slower cell attachment on superhydrophilic surfaces (CS HA coating). At 7 days of cell culture, cells onto APS and HVOF HA coatings showed significant increase of cell proliferation due to the faster cell attachment, in comparison with rounded cells of CS HA coatings up to 7 days of cell culture.

Cells on CS HA coatings showed slower cell attachment up to 7 days of cell culture, in which acquire an osteoblastic morphology. Results showed a significant increase of cell proliferation on samples with higher HA crystallinity at 14 days of cell culture. APS HA coatings showed the lowest values followed by HVOF and CS HA coatings. In-vivo results proved that HA coatings with higher crystallinity led to low dissolution rates, thus obtaining high shear strength between the bond and the implant [44]. Crystalline HA phase seems to stimulate cell proliferation. However, the difference in the increase of cell proliferation between HVOF and CS HA coatings could be due to the small crystal size [14].

4.3 Cell differentiation

Crystalline HA is well known to enhance cell differentiation, especially with small crystal size [45], but also in-vitro and in-vivo that the dissolution of the amorphous phase inhibited the osteogenic differentiation and bone formation [46]. Contrary, other in-vitro studies show that osteogenic differentiation is higher in amorphous calcium phosphates substrates compared to crystalline substrates [47]. During the 1 and 7 days cell of culture, cell differentiation values of HA coatings are quite similar with no significant differences. However, at 14 days of cell culture, there is an increase of the number of differentiated cells on the coatings with higher crystallinity (HVOF and CS HA coatings). Difference between HVOF and CS HA coatings could be due to the slower cell attachment of cells on CS HA coatings, thus delaying cell differentiation, or the higher density of cells at 7 days of cell culture on HVOF HA coatings. Thus, long period tests should be performed.

5 Conclusions

- HA coatings were produced by three thermal spray technologies: APS, HVOF and CS. An in-vitro characterization has been performed according to the analyses of surface properties, such as topography, phase composition, crystallinity and microstructure. To sum up:All HA coatings showed different cell morphology at 1 day of cell culture, suggesting different cell attachment. Cells onto APS HA coatings show good attaching by acquiring osteoblastic morphology in comparison with the rounded cell morphology of CS HA coatings. HVOF HA coatings also show proper cell adherence but did not show as extended filopodia as cells onto APS HA coatings. APS HA coatings show surface micro-features which suggest higher stimulation of cell anchoring. Therefore, moderate wettability is suggested to favour it.
- Cells start proliferating and differentiating after a proper cell attachment. However, the increasing of these values is favoured by the amount of crystallinity of HVOF and CS HA coatings. Thus, the higher increase of cell proliferation at 14 days of cell culture between both specimens is suggested to be influenced by HA crystal size on CS HA coatings.

Finally, it is important to clarify that the current results give a trend just considering the specific coating conditions used here; this should not be taken categorically since the variation of spraying conditions, as well as feedstock powder and substrate can change considerably the final properties of the coatings (surface roughness, wettability composition...), thus different cell response.

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Highlights

- In-vitro comparison of optimized HA coatings obtained by APS, HVOF and CS is evaluated.
- Osteoblastic cells showed a faster cell attachment onto APS and HVOF HA coatings than CS HA coatings.
- Cells start proliferating and differentiating after a proper cell attachment.
- Cell response has been discussed according to HA microstructure and surface parameters.

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