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Importance of hydroxyapatite particles characteristics on cytokines production by human monocytes in vitro

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Abstract

Calcium phosphate bioceramics have been applied as bone substitutes for several decades. Aseptic loosening after total joint arthroplasty is a major problem in orthopaedic surgery. Hydroxyapatite particles from materials wear have been reported as the main cause of implant failure. For this reason, an investigation into possible wear particles from materials used in the implant may lead to longevity after arthroplasty. Monocytes are among the first cells to colonize the inflammatory site. In the present study, we have evaluated the inflammatory response after exposition to particles with different characteristics (size, sintering temperature and shape). Our data demonstrate that the most important characteristic was the shape and the size of the particles. The needle shaped particles induced the larger production of TNF-α, IL-6 and IL-10 by cells. To a less manner, the smallest particles induced an increase of the expression and production of the cytokines studied (TNF-α, IL-6 and IL-10). The sintering temperature appeared to be a less important characteristic even though it was involved in the dissolution/precipitation process.

Keywords: Hydroxyapatite particles; Cytokines; Inflammation; Physical characteristics

1. Introduction

Hydroxyapatite (HA) is widely used to coat metal parts in order to improve their biocompatibility. HA-coatings obtained under atmosphere are made of crystalline HA-particles dispersed within an amorphous calcium phosphate matrix. The early dissolution of the amorphous phase of the coating during the bone remodelling leads to the release of calcium phosphate particles having various characteristics and compositions [1–3], even with dense HA [4].

Analysis of retrieved tissues associated with failed implants, suggest that phagocytosis of HA wear debris [2] provides a potent stimulus for the release of a variety of cytokines. These products are likely responsible for the granulomatous inflammation and disturbance in the bone remodelling that lead to the local osteolytic process. Similar processes might be expected to occur with released ceramic wear debris. This could potentially lead to the induction of a local particle-induced inflammatory reaction that could adversely affect the integrity of the bone-implant interface [5].

The phagocytosis of those calcium phosphate particles has raised some concerns about their ability to activate the macrophages and to trigger the synthesis of cytokines and growth factors acting on some other cell lineages. Monocytes are among the first cells to colonize the inflammatory site [6–8]. Phagocytosis of wear particles leads to the release of several mediators that can stimulate the activity of other cells, including

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osteoclast cells [6]. Interleukin 6 (IL-6), and Tumor Necrosis Factor alpha (TNF-α) mRNA and proteins are produced by monocytes and macrophages stimulated by wear debris and are involved in the osteoclast activation [9–12]. Interleukin-10 (IL-10) was shown to inhibit IL-6 and TNF-α production following phagocytosis of polymethymetacrylate particles by monocytes/macrophages [13].

In the present study, we aimed to investigate the effect of calcium phosphate particle characteristics (size, sintering temperature and shape) on the production of inflammatory cytokines (TNF-α, IL-6) and anti-inflammatory cytokines (IL-10) by human monocytes after phagocytosis.

2. Materials and methods

2.1. Cell culture

Elutriated monocytes were used to evaluate cytokines production. Monocytes from healthy consenting donors were collected by leukapheresis and purified by counterflow elutriation as previously described [14]. Purity, after CD14 staining was, at least 95%, the rest being neutrophils. Lymphocytes and residual platelets were not counted. Cells were maintained in RPMI-1640 medium (Life Technologies, France) supplemented with 10% fetal calf serum (Life Technologies, France), 2 mM glutamine, penicillin ($5000\,U\,ml^{-1}$) and streptomycin ($25\,\mu g\,ml^{-1}$) at 37°C in a saturated 5% CO_2 95% air atmosphere. The cells were cultured in Petri dishes (Life Technologies, France). The density was 2×10^5 cells ml^{-1} in a 5-ml total volume. Cells of each donor were exposed or not (control cells) during 6 or 18 h (time chosen to have an interaction between cells and particles) to the different particles described above. A positive control was made by exposing cells to zymosan particles. Zymosan particles were chosen because of their inflammatory actions on cells [15].

The viability of cells were evaluated by trypan blue exclusion test. The viability was >95% in all of the experiments (before and after exposure to particles).

2.2. Particles characteristics

Twelve HA based powders were studied. They differed by their size range, shape and surface area. The variation of the surface area was obtained by sintering at different temperature. Five HA powders had a spherical shape (Table 1): their size range was between 1 and 30 μm (sintered at 600°C or 1180°C), 10–70 μm, 110–190 μm or 170 to more than 300 μm, the three last powders were sintered at 1180°C (Table 1). Five other HA powders had indifferent shapes (Table 1) with a distribution size between 1 and 30 μm (not sintered, sintered at 600°C or 1180°C), 100–250 and 150–300 μm, both were sintered at 1180°C. Two HA powders have a needle shape and a size range between 1 and 30 μm and were not sintered or sintered at 600°C (Table 1). The powders were constituted by HA particles (purity >99%) (Urodelia, Saint Lys, France) and was verified by X-ray diffraction. The distribution size was evaluated with a multisizer (Coulter Multisizer, AccuComp), the minimum detected was 1 μm and shape factors (shape factor $=(\text{Perimeter})^2/4\pi\,\text{Surface}$) were analyzed with Visilog 4.0 software (Noesis, Saclay, France) from powder optical images recorded with a CCD camera. Specific surface was evaluated with two methods: Brunauer–Emmett–Teller method and from optical images of the powders. Means of both values are

Table 1
Powder characteristics

Particle shape	Number of the powder	Size range (μm)	Mean size (μm)	Sintering temperature (°C)	Specific surface ($m^2\,g^{-1}$)	Crystal size (nm)	Shape factor
Spherical	HA 1	1–30	3	600	23.95	190	1.20±0.29
	HA 2	1–30	3	1180	5.38	350	1.20±0.26
	HA 3	10–70	44	1180	1.56	350	1.16±0.30
	HA 4	110–190	140	1180	0.63	350	1.22±0.17
	HA 5	170–300	217	1180	0.50	350	1.27±0.20
Indifferent shape	HA 6	1–30	3	20	38.06	80	1.30±0.35
	HA 7	1–30	3	600	26.72	180	1.24±0.26
	HA 8	1–30	3	1180	6.08	350	1.30±0.36
	HA 9	100–250	166	1180	0.70	420	1.30±0.36
	HA 10	150–300	196	1180	0.70	350	1.27±0.18
Needle	HA 11	1–30	2	20	18.51	200	1.92±0.87
	HA 12	1–30	2	600	13.18	300	2.10±0.93

presented in Table 1. Crystal size were determined from X-rays diffraction spectra according to the formula:

$$t = \frac{\lambda 180}{\pi \varepsilon \cos \theta}$$

where t is the crystal size, λ the wavenumber of the X-rays, ε peak full-width half-maximum, and θ the Bragg angle of the peak.

All powders characteristics are reported in Table 1. For all powders, the increase of the sintering temperature increased the crystal size and decreased the specific surface. It was impossible to obtained not sintered spherical particles, needle shape particles sintered at 1180°C and large needle particles.

In order to determine the mass powder in the cell culture, we used the work of Gonzalez et al. [16] which demonstrated an effect of surface area of polymethylmethacrylate particles on monocytes activity. Shanbhag et al. [17] calculated the surface area ratio (SAR = surface area of cell/surface area of material) and they demonstrated that cells were sensitive to the surface of material present in the cell culture. With a SAR equal to 10, cells were not activated, whereas with a SAR = 0.1, cells were very activated and the authors have obtained a maximum of cytokines production with SAR = 1. From all these data, we have chosen a surface area ratio equal to 1 for our experiments.

2.3. Analysis of cytokine steady-state mRNA levels by reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was prepared with TRIZOL reagent (Life Technologies, France). RNA extraction was performed using phenol/chloroform extraction followed by ethanol precipitation. RNA (0.4 μg) from each sample was reverse-transcripted with oligo-dT as the first-strand cDNA primer and Moloney murine leukaemia virus reverse transcriptase σM-MLV RT superscript (Life Technologies, France) as described previously by Esnault et al. [18]. Primer and probe sequences for cytokines (IL-6, IL-10, and TNF-α) and for the internal control β-actin (Life Technologies, France), as well as length of fragments obtained are described in Table 2. Reverse transcribed RNA were subjected to PCR using following cycling conditions: 95°C for 5 min; various cycles of: 94°C for 1 min; 62°C (TNF-α), 65°C (IL-6), 62°C (IL-10) or 58°C (β-actin) for 1 min; 72°C for 1 min, and 72°C for 7 min for the final extension. The number of cycles for TNF-α, IL-6, IL-10 and β-actin was 37, 29, 40 and 25, respectively. The reagents and the classical PCR conditions have been optimized [18].

PCR products (10 μl) were incubated with denaturation buffer (10 N NaOH, 250 mM EDTA) for 10 min at 20°C. The denaturation was stopped by addition of neutralizing buffer (1 M Tris–HCl pH 8). Then denatured PCR products were vacuum dot blotted onto Hybond-N^+ membrane (Amersham, Les Ulis, France). Specific probes were 3′-end labelled with fluorescein-11-dUTP using the enhanced chemiluminescence (ECL) 3′-oligolabelling reagents (RPN 2130, Amersham, Les Ulis, France) according to the manufacturer's instructions. The membrane was placed in presence of 1–2 μl/ml of hydridization buffer (ECL 3′-oligolabelling reagents, Amersham, Les Ulis, France) in a total volume of buffer of 20 ml for 2 h at 55°C under gently and rotary agitation. Following hybridization, membrane was washed 3 times with washing buffer (SSC5X, 0.1% SDS) for 5 min under gently agitation and incubated in stringent buffer (SSC1X, 0.1% SDS) pre-heating at 45°C for 15 min under gently agitation. Then the membrane was incubated with anti-fluorescein-horseradish peroxidase for 30 min at 20°C under gently agitation. The detection was performed using hydrogen

Table 2
Oligonucleotide primers and PCR conditions

Gene	Primer	Sequence	Length of amplified fragments (bp)
TNF-α	5′	5′-ATGAGCACTGAAAGCATGATC	702
	3′	5′-TCACAGGGCAATGATCCCAAAGTAGACCTGCCC	
	Probe	5′-CAGGCAGTCAGATCATCTTCT	
IL-6	5′	5′-ATGAACTCCTTCTCCACAAGC	639
	3′	5′-CTACATTTGCCGAAGAGCCCTCAGGCTGGACTG	
	Probe	5′-AAGCTGCAGGCACAGAAC	
IL-10	5′	5′-ATCAAGGCGCATGTGAACTC	296
	3′	5′-AGAGCCCCAGATCCGATTTT	
	Probe	5′-CATCGATTTCTTCCCTGTGA	
β-actin	5′	5′-TGCTATCCAGGCTGTGCTA	443
	3′	5′-ATGGAGTTGAAGGTAGTTT	
	Probe	5′-TTCCAGCCTTCCTTCCTGG	

peroxide and luminol (RPN 2105, Amersham, Les Ulis, France). The luminescence was detected on blue light-sensitive autoradiography film (Hyperfilm-ECL, RPN 3103, Amersham, Les Ulis, France). The quantification of each spot was determined by densitometry analysis (Visilog 4, Noesis, Saclay, France). All cytokine PCR products were analyzed comparatively to the amount of β-actin detected in the same mRNA sample. Results are presented in arbitrary unit.

2.4. Quantitation of cytokines by ELISA

After 6 and 18 h, TNF-α, IL-6 and IL-10 determinations in cell culture supernatants were performed using commercially available ELISA kits (Quantikine, R&D Systems, Inc., Minneapolis, MN) according to the manufacturer's instructions. The sensitivity of each kit was 4.4 pg ml^{-1} for TNF-α, 0.70 pg ml^{-1} for IL-6 and 3.9 pg ml^{-1} for IL-10. Results are presented in pg ml^{-1} for each cytokine studied.

3. Results

3.1. Effect of HA particles on TNF-α expression and production

In order to investigate how HA particles modulate TNF-α synthesis, human monocytes were incubated with different HA particles for 6 and 18 h. In control cells, non-exposed to HA particles, the level of TNF-α mRNA expression was low (Table 3). When cells were exposed to zymosan particles (positive control), high level of TNF-α mRNA expression was shown at 6 h, the expression was four times that of the control and decreased at 18 h. Addition of HA particles modified the expression of TNF-α by monocytes. At 6 h, when monocytes were exposed to spherical particles HA1, HA2, HA3, HA4, HA5 and indifferent shape particles HA9 and HA10, the expression was lower than the control one, the expression was comparable to the control with indifferent shape particles HA6, HA7 and HA8. It was higher than the

Table 3
mRNA and protein production of TNF-α by the monocytes exposed to particles during 6 and 18 h

	Control	HA1	HA2	HA3	HA4	HA5	HA6	HA7	HA8	HA9	HA10	HA11	HA12	Zymosan
6 hours mRNA														
6 hours β-actin														
6 hours SQ	100	10	55	43	3	11	80	107	98	71	52	249	333	453
6 hours Protein (pg.ml^{-1})	12	9	15	6	6	6	9	9	9	12	11	78	66	2587
18 hours mRNA														
18 hours β-actin														
18 hours SQ	100	123	161	37	25	44	30	46	36	58	54	95	140	151
18 hours Protein (pg.ml^{-1})	7	14	24	4	4	4	13	7	13	8	9	91	116	5260

Total RNA was isolated and the cytokine RT-PCR products were dot-blotted, hybridized with fluorescein-labelled specific probes, and detected by an autoradiography of the chemiluminescence on a sensitive film. The β-actin is the RT-PCR control. Results are shown as autoradiographs and as quantification of signals with Visilog 4. Results are representative of three independent experiments in triplicate. The first line is the name of the experiment (control, cells and HA particles or cells and zymosan particles). The second line represents the dot of the mRNA of TNF-α. The third line represents the dot of the mRNA of β-actin. The fourth line represents the semi-quantification (SQ) of the signal (mRNA of TNF-α/mRNA of β-actin) in arbitrary unit. The fifth line represents the quantity of TNF-α protein determined by ELISA. The next four lines represent the same things after 18 h of culture.

control one with needle shape particles HA11 and HA12. After 18 h of cell exposure to HA particles, the expression of TNF-α was lower than the control one when cells were exposed to spherical particles (HA3, HA4, and HA5) and indifferent shape particles (HA6, HA7, HA8, HA9, and HA10). With spherical particles (HA1, and HA2) and needle shape particles (HA11 and HA12), the expression of TNF-α was close to the control, at 18 h.

After 6 and 18 h of treatment cell culture supernatants were collected for the determination of the TNF-α secretion levels. The production of TNF-α by the control was low at 6 and 18 h (Table 3). Addition of zymozan particles increased the production of this cytokine. When monocytes were exposed to spherical particles (HA1, HA3, HA4, and HA5) or to indifferent shape particles (HA6, HA7, HA8, HA9, and HA10), the production of TNF-α was close to the control one. Addition of spherical HA2 particles induced an increase of TNF-α production (twice the control one) and addition of needle shape particles (HA11 and HA12) provokes an important production of this cytokine.

In summary, the smallest particles, the particles sintered at the highest temperature and the needle particles induced an increased of the TNF-α production.

3.2. *Effect of HA particles on IL-6 expression and production*

We next studied how HA particles modulate another inflammatory cytokine, IL-6, human monocytes were incubated with different HA particles for 6 and 18 h. In control cells, non-exposed to HA particles, the level of IL-6 mRNA expression was low (Table 4).

Addition of zymosan particles increased two fold the expression of IL-6 at 6 h and 10 times this expression compared to the control one at 18 h. At 6 h, when monocytes were exposed to spherical particles (HA2), indifferent shape particles (HA7), or needle shape particles (HA11 and HA12) the expression of IL-6 was close to the control one. When monocytes were exposed to spherical particles (HA1, HA3, HA4, and HA5), or indifferent shape particles (HA6, HA7, HA8, HA9, and HA10), the expression of IL-6 decreased. At 18 h, the expression of IL-6 was almost non-existent in the case of

Table 4
mRNA and protein production of IL-6 by the monocytes exposed to particles during 6 and 18 h

	Control	HA1	HA2	HA3	HA4	HA5	HA6	HA7	HA8	HA9	HA10	HA11	HA12	Zymosan
6 hours mRNA														
6 hours β-actin														
6 hours SQ	100	58	87	47	51	44	41	95	73	38	17	112	112	216
6 hours Protein ($pg.ml^{-1}$)	35	13	31	12	13	7	30	35	31	13	13	57	49	2876
18 hours mRNA														
18 hours β-actin														
18 hours SQ	100	14	80	0	0	5	0	0	0	0	0	350	785	1260
18 hours Protein ($pg.ml^{-1}$)	45	36	66	19	21	12	54	23	35	28	37	117	221	18007

Total RNA was isolated and the cytokine RT-PCR products were dot-blotted, hybridized with fluorescein-labelled specific probes, and detected by an autoradiography of the chemiluminescence on a sensitive film. The β-actin is the RT-PCR control. Results are shown as autoradiographs and as quantification of signals with Visilog 4. Results are representative of three independent experiments in triplicate. The first line is the name of the experiment (control, cells and HA particles or cells and zymosan particles). The second line represents the dot of the mRNA of IL-6. The third line represents the dot of the mRNA of β-actin. The fourth line represents the semi-quantification (SQ) of the signal (mRNA of IL-6/mRNA of β-actin) in arbitrary unit. The fifth line represents the quantity of IL-6 protein determined by ELISA. The next four lines represent the same things after 18 h of culture.

spherical particles (HA1, HA2, HA3, HA4, and HA5), or indifferent shape particles (HA6, HA7, HA8, HA9, and HA10). The expression was high in cells exposed to needle shape particles (HA11 and HA12).

To determine whether the modulation of IL-6 mRNA following exposure to HA particles is associated with a concomitant modulation of protein secretion, conditioned medium of cells were analyzed by ELISA (Table 4). The production of IL-6 by the control was the same at 6 and 18 h. When cells were exposed to zymosan, the production was highly increased compared to the control one (82 times at 6 h and 400 times at 18 h). At 6 and 18 h, addition of spherical particles (HA1, and HA2), or indifferent shape particles (HA6, HA7, and HA8) did not change the production of IL-6 whereas spherical particles (HA3, HA4, and HA5), and indifferent shape particles (HA9 and HA10) induced a decrease of the expression of this cytokine. Only needle shape particles (HA11 and HA12) induced an increase of the production of IL-6. Such differences between the level of protein and mRNA have already been observed with titanium particles [19] and explained in yeast [20].

In summary, only needle particles induced an increase of the IL-6 production. The size and the sintering temperature do not seem to modify the production of IL-6.

3.3. Effect of HA particles on IL-10 expression and production

Then we finally studied how HA particles modulate the anti-inflammatory cytokine, IL-10, human monocytes were incubated with different HA particles for 6 and 18 h. The expression of IL-10 by the control was low and almost the same at 6 and 18 h (Table 5). The expression is highly increased by addition of zymosan compared to the control one (5 times at 6 h and 7 times at 18 h). At 6 h, the expression of IL-10 was decreased when monocytes were exposed to spherical particles (HA4, and HA5) and indifferent shape particles HA10. It was the same in the case of HA3 (spherical particles) and HA9 (indifferent shape particles) and the expression was increased with addition of spherical particles (HA1 and HA2), indifferent shape particles (HA6, HA7, and HA8), and needle shape particles (HA11 and HA12). At 18 h, addition of HA5 (spherical particles), HA6, HA7, HA9, and HA10 (indifferent shape particles), decreased the expression of IL-10 by cells. With indifferent shape

Table 5
mRNA production of IL-10 by the monocytes exposed to particles during 6 and 18 h

	Control	HA1	HA2	HA3	HA4	HA5	HA6	HA7	HA8	HA9	HA10	HA11	HA12	Zymosan
6 hours mRNA														
6 hours β-actin														
6 hours SQ	100	227	204	108	42	77	164	181	194	142	64	267	358	578
6 hours Protein ($pg.ml^{-1}$)	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	78
18 hours mRNA														
18 hours β-actin														
18 hours SQ	100	142	93	120	96	0	61	50	113	0	13	165	251	768
18 hours Protein ($pg.ml^{-1}$)	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	116

Total RNA was isolated and the cytokine RT-PCR products were dot-blotted, hybridized with fluorescein-labelled specific probes, and detected by an autoradiography of the chemiluminescence on a sensitive film. The β-actin is the RT-PCR control. Results are shown as autoradiographs and as quantification of signals with Visilog 4. Results are representative of three independent experiments in triplicate. The first line is the name of the experiment (control, cells and HA particles or cells and zymosan particles). The second line represents the dot of the mRNA of IL-10. The third line represents the dot of the mRNA of β-actin. The fourth line represents the semi-quantification (SQ) of the signal (mRNA of IL-10/mRNA of β-actin) in arbitrary unit. The fifth line represents the quantity of IL-10 protein determined by ELISA. The next four lines represent the same things after 18 h of culture.

particles HA8, the expression was close to the control one and with spherical particles (HA1, HA2, and HA3), and needle shape particles (HA11 and HA12), the expression was higher than the control one.

After 6 and 18 h of incubation cell culture supernatants were collected for the determination of the IL-10 secretion levels. Although level of IL-10 mRNA expression was detected, in the cell-free culture medium the quantity of IL-10 protein was under the kit detection limit using HA particles. Addition of zymosan induced a high production of IL-10 by monocytes: 78 and 116 pg ml^{-1} were obtained after 6 and 18 h. Surprisingly, when cells were exposed to HA particles, whatever their characteristics, no IL-10 protein was obtained in the culture medium even using particles that induced the most inflammatory cytokines (TNF-α and IL-6) production such as HA11 and HA12 (needle shape particles).

In summary, the smallest particles, the particles sintered at the highest temperature and the needle particles induced an increased of the IL-10 production.

4. Discussion

Data presented here show that HA particles can trigger cytokine production by human monocytes depending on their physical characteristics. By studying TNF-α, IL-6 and IL-10 production, we addressed two major questions: (1) what is the effect of HA particles on the inflammatory reaction and (2) does there exist a down-regulation by cells of the inflammatory response by IL-10?

This work demonstrates that cytokine production by human monocytes can be influenced by the physical characteristics of HA particles (size, sintering temperature and shape).

Indeed exposition of monocytes to HA particles with different sizes allow us to demonstrate that spherical particles that are phagocytable induced an increase of the expression and production of both inflammatory cytokines (TNF-α and IL-6) studied whereas non-phagocytable did not induce such increase whatever their size. The production was almost the same when cells were in contact with all non-phagocytable particles. In the presence of irregular shape particles, there was no difference of expression or production of inflammatory and anti-inflammatory cytokines between phagocytable and non-phagocytable. Using phagocytable spherical particles, the production of IL-10 was important suggesting a down-regulation of the inflammatory response by monocytes. It is known that the inflammatory response is resolved by a release of endogenous anti-inflammatory mediators, such as IL-10 [21]. This shows that particles have to be phagocytosed to provoke the production of cytokines and this production seems also depend of the particles shape.

There are conflicting results in the literature data on the effect of the particles size which can be explained by two facts: the first one is that authors did not take care of the shape of the studied particles and the second one is that authors did not use the SAR procedure for their cells culture. Harada et al. [22] obtained results close to ours with milled and unmilled HA particles (which are assumed to have indifferent shape) regarding the inflammatory cytokines production by monocytes/macrophages and in vivo, Malard et al. [23] obtained a decreased of the inflammatory reaction with the decrease of the irregular shape HA particles sizes (the shape was evaluated according to their scanning electron microscopy micrographs). On the other hand, without any indications of the HA particles used, Sun et al. [24] did not observe differences in the TNF-α production by osteoclasts cells. The presence of large particles also altered fibroblastic cells proliferation compared to fine particles [25]. In addition, a decrease in HA particle sizes have been demonstrated to increase metalloproteinase production [26,27].

Concerning the effect of the sintering temperature, in the case of spherical and needle shape particles, the production and expression of the cytokines studied (TNF-α, IL-6 and IL-10) was more important when cells were exposed to particles sintered at the highest temperature. Using irregularly shaped particles, the expression of the three cytokines was quite the same whatever the sintering temperature. All these data demonstrated that there was an effect of the surface area (or crystal size) of the particles on the interactions with cells as demonstrated Prudhommeaux et al. [28] and Nagase et al. [29]. Harada et al. [22] also demonstrated an effect of the sintering temperature of HA biomaterials on cytokines productions but they obtained results opposite to ours. That may be due to the different quantity of powder used. On the other hand, Fukuchi et al. [30] demonstrated that HA sintered were most suitable in term of biocompatibility.

All these results could be explained by the dissolution/reprecipitation process that occurs at the interface between cells and HA particles which depends on the sintering temperature of HA [31].

The study of the effect of the particles shape demonstrated that the needle particles always induced the most important expression and production of inflammatory cytokines. There were only slight differences between the effects of particles sintered at 600°C and 1180°C. Therefore, we can conclude that the needle shape particles induced the most inflammatory reaction. To our knowledge, this work is the first study, that points out a shape effect of the HA particles on inflammatory response. Only studies with inhaled mineral or glass particles have shown the toxicity of needle shape particles [32,33]. In this case, Guthrie has supposed that fiber particles might affect particle–cell

interaction by causing mechanical stresses on the cell surface. Whatever their sintering temperature, needle particles induced the largest expression of IL-10. This may suggest that cells try to down-regulate the inflammatory reaction.

In conclusion, HA-particles can be more or less toxic according to their characteristics. They must be phagocytosed to elicit a cellular effect. This fact must be taken into account when calcium–phosphate ceramics are made. The particles, which will be released during the material degradation and bone remodelling, can have an influence on the biocompatibility of the material. In the present study, we have demonstrated that the smallest particles and especially needle-like particles induced the most important inflammatory reaction that coincided with the most expression of anti-inflammatory cytokines. So this kind of particles released should be avoided by manufacturer.

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