Behavior of macrophage and osteoblast cell lines in contact with the \( \beta \)-TCP biomaterial (beta-tricalcium phosphate)

Comportement de lignées cellulaires de macrophages et d’ostéoblastes en contact avec le biomatériau \( \beta \)-TCP (phosphate bêta tricalcique)

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Available online 19 September 2017

KEYWORDS

\( \beta \)-TCP; Macrophages; Osteoblasts; Osteoconduction; Resorption

Summary Beta-tricalcium phosphate (\( \beta \)-TCP) is a synthetic ceramic used for filling bone defects. It is a good alternative to autologous grafts since it is biocompatible, resorbable and osteoconductive. Previous in vivo studies have shown that macrophages are one of the first cells coming in contact with the biomaterial followed by osteoclasts and osteoblasts that will elaborate new bone packets. Studies have focused on osteoclast morphology and very few of them have investigated the role of macrophages. The aims of this study were to characterize (i) the biomaterial surface; (ii) the in vitro behavior of macrophages (J774.2 and Raw264.7 cells) using the description of cell morphology by scanning electron microscopy (SEM) at 7 and 14 days; (iii) the behavior of osteoblasts (SaOs-2 and MC3T3-E1 cells) seeded at the surface of the biomaterial 24, 48 and 72 hours by SEM and confocal microscopy. Cell proliferation was analyzed by MTT assays. Viability and affinity of the macrophages for \( \beta \)-TCP were found significantly increased after 7 and 14d. MC3T3-E1 cells were anchored and stretched onto the \( \beta \)-TCP surface as early as 24 h with a high proliferation rate (+190%) when compared to the surface of a well plate. SaOs-2 exhibited the same morphological profile at 72 h. Proliferation became significantly higher compared to the plastic surface at only 72 h (+129%). This study emphasises the importance of choice of the cell line used in exploring the osteoconductive and osteoinductive properties of a biomaterial. Additional studies are needed to analyze differentiation of macrophages into giant multinucleated cells and how the biomaterial surface influences osteoblast differentiation.

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http://dx.doi.org/10.1016/j.morpho.2017.03.006
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Introduction

Beta-tricalcium phosphate (β-TCP) is a synthetic ceramic that belongs to the calcium orthophosphate family. Its chemical composition (β-Ca\(_3\)(PO\(_4\))\(_2\)), close to the mineral phase of bone, allows it to be used as a bone substitute for filling defects in neurosurgery, maxillofacial, reconstructive, orthopedics and spinal surgeries. TCP are known to be biocompatible since almost a century. In 1920, Albee and Morrison have reported for the first time the use calcium orthophosphate as bone graft in the rabbit radius [1]. No adverse reaction, inflammation or toxic symptoms were observed. Osteogenesis was stimulated by TCP leading to a faster bone healing. The authors concluded that this material was suitable for clinical applications and could be used in further studies on human subjects. Studies on β-TCP increased in the 70s, showing the bioreosorption ability of β-TCP with the first histologic observations in 1971: bioreorption occurred simultaneously with the apposition of new bone packets after local recruitment of osteoblasts [2]. Reliable methods of β-TCP production were subsequently proposed that led to the commercialization of the material in the 80s [3,4]. β-TCP is now recognized as osteoconductive as it provides a resorbable template for the formation of new bone [5]. Histological studies show a marked apposition of lamellar bone directly in contact with β-TCP within a period of 6 to 24 months [6,7]. In a rat model, resorption of β-TCP granules occurs 2 weeks after implantation associated with new bone formation inside β-TCP pores after 5 weeks [2]. In a rabbit model, new bone trabeculae invading the grafted biomaterials were evidenced as early as 8 days by microcomputed tomography (microCT) [8]. Bone formation occurring directly onto a biomaterial surface necessitates different types of cells: recruitment of osteoprogenitor cells from surrounding mesenchymal cells, adhesion of osteogenic cells followed by survival, proliferation and differentiation [9].

However, the degradation mechanisms of β-TCP remain unclear. Degradation of calcium/phosphate biomaterials in the body is composed of two stages: cellular resorption and the dissolution of the material [10,11]. Ca/P biomaterials can be eroded, phagocytized or degraded by pH modifications caused by osteoclasts which lead to the demineralization of the material. Besides osteoclasts, macrophages (or their derived giant cells formed by fusion) are involved at an early stage of resorption [12,13]. Some studies on β-TCP granules grafted in oral surgery suggest that resorption of the biomaterial may happen by phagocytosis with macrophages together with osteoclasts once new bone trabeculae are formed [6,14]. A double mechanism of cellular degradation of β-TCP by osteoclasts and macrophages is most probable.

Surface topography and porosity of implants and grafts can also influence bioreosorption and the behavior of cells coming in direct contact with the materials [10,15–17]. Interactions of osteoblasts and macrophages with β-TCP surface remains unclear. The aim of the study was to characterize: (i) the surface of plates made with β-TCP; (ii) the morphology of macrophages seeded onto the plates by scanning electron microscopy (SEM); (iii) the behavior of osteoblast-like cells seeded on these plates by SEM, confocal microscopy and proliferation assay.

Material And methods

Characterization and preparation of β-TCP

β-TCP samples

Plates of 3D-printed β-TCP (Sinus-Up™) were obtain from Kasios (Kasios, L’Union, France). Sinus-Up™ plates are...
prepared by rapid prototyping by using elementary β-TCP powder in hydroxypropyl-methylcellulose with water as binder, plates are then subsequently sintered at high temperature and the hydroxypropylmethylcellulose is burnt off during sintering. Sinus-Up™ are commercially available and sold for sinus floor elevation. Sinus-Up™ have a central macroporous area (which was discard in this study) and flat lateral sides which were cut in plates for experimental purposes (0.8 cm by side).

Scanning Electron Microscopy (SEM)  
Surface morphology of the β-TCP plates was analyzed by SEM on a JEOL 6301F (JEOL Paris, France). All samples were coated with a 20 nm layer of platinum by sputtering with a high vacuum coater (Leica EM ECA600, Leica, France). Images were captured in the secondary electron mode with an acceleration tension of 3 kV.

Energy-Dispersive X-ray Spectroscopy (EDS)  
An elemental analysis was performed on the β-TCP plates by energy-dispersive X-ray spectroscopy (EDS) on a Zeiss, EVO LS10 SEM. The samples were not carbon or gold coated. The working pressure was 50 Pa and the acceleration tension was 5 kV.

Raman spectroscopy  
The chemical spectrum of the β-TCP plates was analyzed by Raman spectroscopy on a Senterra microscope with OPUS 5.5 software (Bruker optic, Ettlingen). The excitation laser wavelength was 532 nm with an excitation power of 25 mW and 3–5 cm⁻¹ resolution. The final spectrum was obtained by averaging five scans of 20 sec each. A concave rubberband base line correction was applied (11 iterations, 64 points).

Cell culture reagents and preparation  
All cell culture consumables were obtained from Gibco (ThermoFisher Scientific, Illkirch, France). Four culture cell lines were used: two monocyte/macrophage cell lines J774.2 (European Collection of Authenticated Cell Culture ECACC #B5011428, Salisbury, UK) and Raw264.7 (American Type Culture Collection ATCC #TIB-71, Molsheim, France), Human SaOS-2 osteoblast-like cells (ATCC HTB-85) and pre-osteoblast cell line MC3T3-E1 subclone 4 (ATCC CRL-2593). J774.2, Raw264.7 and MC3T3-E1 cells were cultured in α-MEM (Minimum Essential Medium, alpha Modification) and SaOs-2 cells were cultured in DMEM (Dulbecco’s Modified Eagle Medium). For all cultures, the medium was supplemented with 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin and 100 μg/ml streptomycin. Medium was replaced every 2–3 days and the cultures were maintained in humidified atmosphere of 5% CO₂ at 37 °C. At 80% confluence, MC3T3-E1 and SaOs-2 cells were detached using trypsin-EDTA (trypsin/ethylene diamine tetraacetic acid) and J774.1/Raw264.7 cells were harvested by scraping.

Prior to cell seeding, β-TCP plates were sterilized in 70% ethanol during 24 hours and dried for an hour. They were subsequently immersed during a night in the media (α-MEM supplemented with 10% fetal calf serum) to remove any trace of ethanol and to allow proteins from the medium to adhere onto the β-TCP surface.

Macrophage culture and seeding on β-TCP  
J774.2 and Raw264.7 cell lines were seeded onto β-TCP plates at a density of 3.10⁴ cells/cm² and cultured during 7 and 14 days (two samples/time). β-TCP plates were immersed in 1 mL of medium in a 24-well plate and macrophages were seeded in a homogenous way in the medium above the samples. At time of seeding, the medium was supplemented with 25 ng/mL Macrophage Colony Stimulating factor (M-CSF, Biotechnne brand, R&D systems, Lille, France). Preparation of cells for SEM observation was then done (see below).

Osteoblasts culture and seeding on β-TCP  
MC3T3-E1 and SaOs-22 osteoblast cells were seeded onto β-TCP plates in 24 well plates at a density of 2 10⁴ cell/cm² and cultured during 24, 48 and 72 h. The β-TCP plates were immersed in 1 mL of culture medium and the cells were seeded in a homogenous way in the medium above the samples. Experiments for analysis of cell spreading, cell morphology and proliferation were done in duplicate at each time point.

Cell spreading analyzed by confocal microscopy  
Cells were fixed in 4% paraformaldehyde for 20 minutes at 4 °C. They were rinsed 3 times 5 min in PBS and stained with 2 μg/mL 4,6-diamidino-2-phenylindole (DAPI, Sigma, Saint Quentin-Fallavier, France) for 2 min at room temperature in the dark. After rinsing 6 times in PBS for 5 min each, cells were labeled with 6.6 μM Alexa Fluor 488-conjugated phialloidin (Thermofisher Scientific Illkirch, France) for 45 min at room temperature in the dark and rinsed in PBS (6 times, 5 min each) and distilled water (6 times 5 min). The β-TCP plates with labeled cells were mounted between glass slides with 30% glycerol. Labeled cells were observed on a Leica TCS SP8 laser-scanning confocal microscope (Leica Microsystems, Heidelberg, Germany) with a HXC PL APO 63X CS2 oil immersion objective (N.A. 1.40). Excitation and emission wavelengths were set at 405 nm for DAPI labelling and 488 nm for phialloidin labelling.

Some slides were counterstained with xylene orange 0.5 mg (Sigma) for 10 min in distilled water after the double labelling phialloidin/DAPI to label the β-TCP surface.

Cell proliferation by MTT assay  
The number of total and viable cells on the surface of β-TCP plates was measured with a colorimetric MTT assay and compared with that of cells cultured directly onto the well surface. MTT assay is based on the reduction of the yellow tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma-Aldrich) by the mitochondrial succinate dehydrogenase. At each time point, β-TCP plates were transferred in a new 24 wells plate; cells were incubated with MTT at 0.5 mg/mL for 2 hours in a humidified atmosphere of 5% CO₂ at 37 °C. MTT was reduced to purple formazan crystals which were then dissolved in 500 μL acidified isopropanol per well. Each supernatant was
transferred in 96 well plates for absorbance reading at 570 nm with a spectrophotometric plate reader (SpectraMax M2, Molecular Devices, Sunnyvale, CA) because absorbance is proportional to the number of viable cells. Number of cells was determined using a standard curve with a range of each cell type concentration between 0 and 10 cells/cm² and they were reported to the seeded surface area (well surface or β-TCP plate surface). Prior to cell culture, the β-TCP plates were imaged with a numeric radiography equipment (Faxitron X-Ray LX-60, Edimex, France) and the surface area (in cm²) was measured using the ImageJ software 1.45.

**SEM of cells seeded on β-TCP plates**

Samples were rinsed with cacodylate buffer (37°C, pH 7.4) and fixed during one night at 4°C with glutaraldehyde (2.5% in cacodylate buffer 0.2 M). Samples were subsequently postfixed with osmium tetroxide (1% in distilled water for 1 hour). They were dehydrated with a gradient of ethanol and desiccated with hexamethyldisilazane and examined as above described.

**Statistical analysis**

Statistical analysis was performed with the statistical software MedCalc version 8.2.10 (Ostend, Belgium). All data were reported as mean ± standard error of the mean (SEM). Statistical significance between groups for MTT test was determined by a non-parametric Kruskal-Wallis analysis of variance and comparison between groups was determined by post-hoc test. A difference was considered significant when \( P < 0.05 \).

**Results**

**β-TCP characterization**

**SEM**

The raw surface of the β-TCP samples appears on Fig. 1A. Although the surface of the biomaterial seemed rather flat macroscopically, the SEM analysis revealed in fact a rough surface with valleys and hills. At higher magnifications, the material surface showed a polycrystalline pavement with different polygonal crystallites separated by grain boundaries. Defect lines were also present on the surface of the material. Some crystallites showed a hexagonal pattern at the surface of the polygonal pavement (Fig. 1B). A microporosity was evidenced at the surface of the biomaterial between the sintered grains, it was also visible of fractured sections (data not shown).

**Raman spectroscopy**

The Raman spectrum of the β-TCP plates appears on Fig. 2 for a wavenumber ranging from 50 to 1550 cm⁻¹. The labeled peaks are characteristic of the internal vibration of the PO₄²⁻ tetrahedric groups of the β-TCP molecule. The symmetric stretching (\( \nu_1 \)) of P–O bonds of the tetrahedron corresponds to the peaks with the highest intensity at around 950 cm⁻¹ and 970 cm⁻¹. The asymmetric stretching (\( \nu_3 \)) has a lower intensity and is located in the 1015–1090 cm⁻¹ range. The other vibrational modes (\( \nu_2 \) and \( \nu_4 \)) correspond to O–P–O bending deformations of the tetrahedron. They are respectively located at 407 and 548 cm⁻¹.

**Monocyte/macrophage cells morphology by SEM**

After 7 and 14 days, RAW 264.7 and J774.2 cells have survived on β-TCP surface. At 7 days of culture (Fig. 3A), the majority of Raw264.7 cells had a round shape. After 14 days, some RAW cells appeared flattened when some others had maintained a round shape (Fig. 3B). These cells have
emitted long filopodia that anchor them onto the β-TCP surface (Fig. 3D). The number of filopodia increased between 7 days (Fig. 3C) to 14 days; even if they cannot be counted on such a rough material, this corresponds to a firmer anchor capacity of the cells to the biomaterial.

At 7 and 14 days, the osteoblastic J774 cells seeded on the β-TCP had a round shape (Fig. 4). They appeared anchored to the surface with less filopodia than RAW 264.7 cells in the same conditions; cytoplasmic veil-like structures were observed on their surface (Fig. 4C-D). No difference in morphology between the two times of culture was observed.

**Morphological aspect of SaOs-2 and MC3T3-E1 cells on β-TCP**

**SEM**

SEM analysis of SaOs-2 and MC3T3-E1 cells in contact with β-TCP at 24, 48 and 72 h appears on Fig. 5. At 24 h, SaOs-2 cells had a round shape; at 48 h, they flattened and stretched on the β-TCP pavement (Fig. 5A). Cells exhibited pseudopodia allowing a direct anchorage to the biomaterial surface. At 72 h, SaOs-2 cells appeared flat and more stretched than at 48 h. Cells exhibited long cytoplasmic extensions (pseudopodia with some filopodia) that allowed them to be anchored on the rough surface of the β-TCP (Fig. 5B). At 72 h, SaOs-2 cells showed a morphological adaptation to the relief made of valleys and hills.

At 24 h, MC3T3-E1 cells were flat and affixed onto the β-TCP surface (Fig. 5C). Cells were anchored by both filopodia and larger pseudopodia. At 48 h, cells had proliferated and formed a dense layer. At 72 h, they were flat, with an enlarged surface and had established numerous contact between each other; thus a monolayer of MC3T3-E1 cells covered almost all the surface of the biomaterial (Fig. 5D).

**Confocal microscopy**

Fig. 6 shows confocal images obtained after a double labelling DAPI/phalloidin of SaOs-2 and MC3T3 cells. At 24 h after cells seeding, phalloidin labelling showed that SaOs-2 cells were attached on the β-TCP surface but appeared less spread than MC3T3-E1 cells (Fig. 6A-C). The actin network appeared clearly much more developed in the cytoplasm of MC3T3-E1 cells compared to SaOs-2 cells. As early as 24 h, MC3T3-E1 cells appeared in contact and overlapped; confocal images evidenced cytoplasmic extensions that interact with neighboring cells (Fig. 6C). At 48 h, the actin cytoskeleton of SaOs-2 cells remained poorly developed and these cells were not well spread. On the contrary, MC3T3-E1 cells appeared well spread and cytoplasmic extensions were observed. Confocal observation at a smaller magnification, clearly showed a dense layer of MC3T3-cells on the β-TCP surface (that appeared in red after xylene orange counterstaining) (Fig. 7).

At 72 h, SaOs-2 cells formed a dense layer on the β-TCP surface and their morphological aspect clearly showed improvement by exhibiting round shaped nuclei, a developed actin network and cytoplasmic extensions (Fig. 6B). MC3T3-E1 cells covered almost the β-TCP surface and these
Figure 4  J774 cells on β-TCP after (A) 7 days and (B) 14 days of culture. Higher magnification of J774 cells on β-TCP surface after (C) 7 days and (D) 14 days of culture.

Figure 5  SEM observations of SaOs-2 cells behavior in contact with β-TCP plates at 48 h (A) and 72 h (B); MC3T3-E1 cells behavior in contact with β-TCP plates at 24 h (C) and 72 h (D).
Figure 6 Confocal microscopy observations of SaOs-2 cells adhesion (A-B) and MC3T3-E1 cells adhesion (C-D) on β-TCP surface at 24 and 72 h. The actin filaments are stained in green with phalloidin and the nuclei are stained in blue with DAPI.

Proliferation of osteoblast-like cells on β-TCP

Proliferation kinetics of SaOs-2 and MC3T3-E1 cells on the β-TCP surface at 24, 48 and 72 hours appears on Fig. 8. The number of SaOs-2 cells significantly increased at 48 h \( (P<0.05 \text{ vs } 24\text{h}) \) and at 72 h \( (P<0.05 \text{ vs } 48\text{h}) \). At 24 and 48 h SaOs-2 proliferation was not significantly higher than on the plastic surface. At 72 h proliferation of SaOs-2 cells on the biomaterial had increased by 129% compared to control conditions on the plastic surface \( (P<0.05) \). The number of MC3T3-E1 cells on β-TCP increased from 24 to 72 h but it became statistically significant only at 72 h vs 48 h \( (P<0.05) \). Proliferation on the β-TCP surface was significantly higher when compared to controls on the plastic surface at each time point at 24, 48 and 72 h \( \text{resp. } +190\%, +177\%, +181\% \).

Discussion

The surface of the β-TCP plates observed by SEM presented three main characteristics: micropores, a polygonal pavement of polycrystalline tessels limited by grain joints and defect lines. The defect lines observed on our SEM images, represent shear bands, a characteristic surface defect on ceramics. During sintering at high temperature, the motion of atoms allows the material to form crystallites. When forming, these crystallites are submitted to high shear stress that leads to the formation of structural defects called dislocations; they are 2D linear plastic deformations of the crystal. Under the shear stress, they have the ability to move over the crystallite leading to the formation of defect lines, also called shear bands. Some shear bands show an hexagonal pattern characteristic from the hexagonal lattice of the β-TCP structure [18]. These specificities of the surface topography is of the upmost importance and may influence adhesion of macrophages and expression of different cytokines [19]. It has been shown that the chemical
Morphology of macrophages and osteoblasts on β-TCP

The two mouse monocyte/macrophage cell lines used in this study were cultured with M-CSF. This cytokine is known to regulate and control the survival, proliferation and differentiation of phagocytic macrophages from undifferentiated precursors [22,23]. Adding M-CSF in the cultures allows cells to differentiate into fully mature macrophages. RAW 264.7 and J774 cells survived between 7 and 14 days. They had cytoplasmic veil-like expansions on their surface characteristic of healthy macrophages. The cells presented pseudopodia and numerous filopodia that anchored them at the surface of the β-TCP. Previous work on rabbit bone biopsies showed that cellular resorption of β-TCP occurred in two steps [24]. Giant nucleated TRAcP-negative cells first colonized the surface of the biomaterial from 7 to 14 days. These cells contained a great amount of mineral crystals from inside their vacuoles suggesting degradation by phagocytosis. As new bone is formed, multinucleated TRAcP positive cells with a ruffled border (characteristic of osteoclasts) are evident on the surface of Ca/P ceramics [24]. The number of osteoclasts increases upon time. So, a double population of multinucleated cell is responsible for the cellular resorption of ceramics: giant TRAcP-negative cells that erode the biomaterial and osteoclasts that resorb the biomaterial and remodel the newly formed bone [24,25].

Besides osteoclasts, macrophages could also be involved at an early stage of biomaterial resorption. In a series of 14 patients that had sinus lift augmentation in oral surgery with β-TCP granules, TRAcP-positive multinucleated cells were observed in contact with granules [6]. However, slightly TRAcP-positive cells (characteristic of macrophage activation) were also observed with β-TCP grains inside their cytoplasm after phagocytosis. Similar findings were also reported by others [8,14,26,27]. This suggests that resorption happens by phagocytosis due to macrophages together with osteoclasts. The early vascularization around the grafted β-TCP particles allows in situ migration of precursor cells, macrophages and osteoprogenitors [28]. In our study, no giant multinucleated cells were observed meaning that macrophages did not fuse into giant cells in vitro. In the future, it could be interesting to analyze the expression of TRAcP by macrophages in presence of β-TCP and how it varies over time.

It is admitted that the resorption in case of a biodegradable material occurs simultaneously with apposition of new bone packets after recruitments of osteoblasts. Numerous studies have focused on the osteoconductive characteristics of a biomaterial in culture using an osteoblast cell line and/or bone marrow stroma cells (BMSC) [20,29,30]. The choice of a cell type in an in vitro study is of the utmost importance. BMSCs are interesting because they can differentiate into osteoblasts. Indeed, such an osteogenic differentiation in contact with a biomaterial can reflect its osteoinductive potential [30]. In the present study, SaOs-2 are mature osteoblast derived from a human osteosarcoma as they express alkaline phosphatase [31]. In contrast, MC3T3-E1 cells are pre-osteoblasts as they do not expressing alkaline phosphatase in the absence of ascorbic acid and β-glycerophosphate [31]. MC3T3-E1 have been shown to be the most appropriate model in biomaterial studies [31]. Culture of MC3T3-E1 on calcium phosphate ceramics induces alkaline phosphatase gene expression after 14 days.

Figure 7 Confocal microscopy observation of MC3T3-E1 cells cultures 48 h on β-TCP surface stained in red with xylene orange. The actin filaments are stained in green with phalloidin and the nuclei are stained in blue with DAPI.

Figure 8 Proliferation of SaOs-2 and MC3T3-E1 cell cultured directly on 24 well plates and on β-TCP plates, expressed in number of cells per cm² at 24, 48 and 72 hours. *P < 0.05 vs plastic surface; †P < 0.05 vs 24h on β-TCP surface; ‡P < 0.05 vs 48h on β-TCP surface.

and roughness surface of β-TCP favored the adhesion process osteoblast cells in vitro [20].

The present study focused on cells morphology which have developed in vitro on β-TCP. Several studies have been done using macrophages cultured on β-TCP [10,16,17]. In most of them, macrophages were cultured in presence of receptor activator of nuclear factor kappa-B ligand (RANK-L) to form osteoclast-like cells but none of them have focused on the macrophage morphology. In a previous study, we have found by a time-laps in vitro study that macrophages were able to resorb β-TCP granules and that osteoblast-like cells could climb at the surface of the biomaterial [21].
of culture without any medium supplementation [30]. In our study MC3T3-E1 cells adhered and spread out on the β-TCP surface more rapidly than SaOs-2 cells. At 72 h, the two cell lines occupied most of the surface and exhibited a developed cytoskeleton with a marked actin network [32]. Interaction between cells and the biomaterial surface is crucial to induce proliferation, followed by differentiation. Our results showed an influence of the β-TCP surface on cell proliferation as early as 24 h which could be correlated with a rapid adhesion process at 24 h. Interaction with an extracellular matrix or a biomaterial involves is mediated by integrins that interacted with the matrix. Another study has shown that spreading of SaOs-2 osteoblastic cells occurred within 1 day on β-TCP (as also found here) and that focal adhesion are observed at 4 days [20]. In our study, cytoplasmic extensions were observed after 48 hours allowing a firm anchorage of the cells onto the biomaterial surface.

In conclusion, the present study emphasises the importance of the choice of a cell line in exploring the osteoconductive and osteoinductive properties of a biomaterial. Additional studies are needed to better understand the resorption process involving differentiation of macrophage into giant multinucleated cells. The topographical, chemical and physicochemical characteristics of β-TCP may account for its excellent capacity of inducing a regenerative bone formation associated with progressive resorption of the biomaterial.

Disclosure of interest

B.A. received a PhD scholarship from Kasios SAS.

Acknowledgments

This work was made possible by grants, from ANR, program LabCom “NextBone”- SEM and confocal analysis were performed at Service Commun d’Imagerie et d’Analyses Microscopiques (SCIAM), Université d’Angers, thanks to R. Perrot and R. Mallet. Many thanks for Kasios SAS, 18, chemin de la Violette 31240 L’UNION—France for providing the Sinus-Lift™ devices.

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