A new biocompatible biomaterial : PEEK / β-TCP / TiO_2 composite

M.-F. HARMAND [(1) (2)] - J.-P COUGOULIC [(1)]
[(1)] LEMI – Technopole Montesquieu – 33650 MARTILLAC – FRANCE – lemi@atlantel.fr
[(2)] DETERCA – University of Bordeaux 2 – 33076 BORDEAUX - FRANCE

Introduction : Polyetheretherketone (PEEK) is an aromatic, rigid semi-crystalline thermoplastic with excellent mechanical properties and bone-like stiffness, and good biocompatibility [1-2]. Moreover for biomedical applications, particularly in the area of load-bearing orthopaedic applications [3] PEEK is able to be repeatedly sterilized, and shaped by machining or injection moulding. Devices utilising PEEK's unique combination of properties have found considerable success in spine, cardiovascular and dental applications.
PEEK composites were developed such as fiber renforced PEEK composites for bone plates [4], or PEEK-HA composites as a possible bone analogue substitute for load-bearing functions [5-6] or as scaffold for bone tissue engineering [7].
An innovant PEEK based composite has been produced consisting of a dispersion of β-tricalcium phosphate (β-TCP) (10 % w/v) and Titanium oxide (anatase) (10 % w/v) throughout a PEEK matrix. The PEEK/β-TCP/TiO_2 demonstrated excellent mechanical properties, with elastic modulus comparable to that of natural cortical bone : tensile strength of 98 MPa, flexural modulus of 4.7 GPa and a flexural strength of 16MPa. Moreover based on the recommendations of ISO 10993 "Biological evaluation of medical devices" (2004) cytotoxicity, acute systemic toxicity, irritation, sensitization, mutagenicity (Ames test, chromosome aberrations using human lymphocytes, sister chromatid exchange) were performed and demonstrated composite biocompatibility.
In this study we have investigated the cytocompatibility and bioactive nature of the material using human osteoblasts.

Materials and methods :
Test material : Discs (15.5 mm in diameter, 2 mm thick) exhibiting a smooth surface and γ-ray (25 kGy) sterilized are placed on the bottom of 24-well tissue culture plates completely covering the bottom of the culture well.
Test system : human osteoblasts (HOB) arising from trabecular bone, checked free from mycoplasma and fully characterized with regards to their phenotype expression : osteoblastic morphology, alkaline phosphatase activity, type I collagen and osteocalcin synthesis.
Protocol : HOB from the 3rd passage were seeded [8] on the test material at 5 x 10^3 cells/cm² for assessment of cell attachment at 3 h and 6 h, cell proliferation and alkaline phosphatase activity (ALP) over 27 days of culture. Polystyrene of the culture wells was the negative control. Culture medium (Iscove's Modified Dulbecco's Medium) supplemented with 5 % (v/v) heat inactivated fetal calf serum was changed every 3 days. No antibiotics were used. Plates were incubated at 37° C in a humid atmosphere containing 5 % (v/v) CO_2. Viable cells were counted with an hematocytometer using trypan blue exclusion test at 3 h and 6 h, and 1, 3, 9, 15 and 27 days. ALP activity was determined on the basis of hydrolysis of p-nitrophenylphosphate to p-nitrophenol [9] (Sigma kit : ALP-10). Morphology of the composite and colonization by HOB were recorded by scanning electron microscopy (SEM) following fixation in 2.5 % (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3 for 1 h, dehydration through an ascending alcohol series before being transferred into acetone, critical point dried, and coated with gold before examination with a scanning election microscope (Hitachi).
Statistical analysis : Data were analysed by Student's *t* test. $P < 0.05$ was considered as significant.

Results :

Cell attachment and proliferation : SEM assessment shows that HOB are able to attach, adhere and proliferate on the composite. Figure 1a shows the surface state of the composite. Figure 1b and 1c show the cell layer at day 3 and day 27 respectively : HOB are very well spread at day 3 on the test material, and constitute a multilayer at day 27. Figure 1d shows a detail of the multilayer at higher magnification.

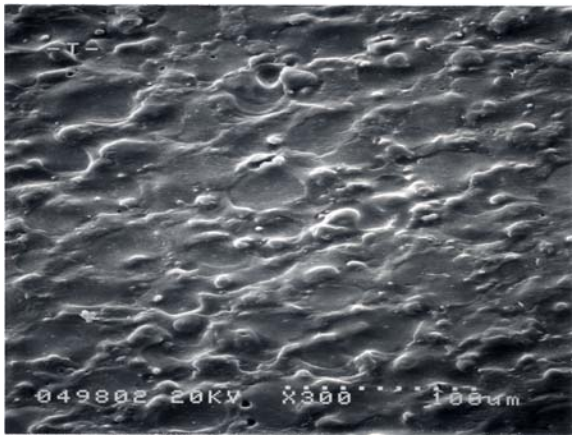

Figure 1a

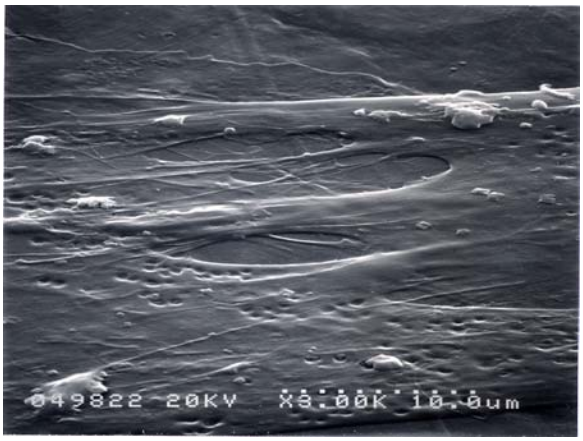

Figure 1b

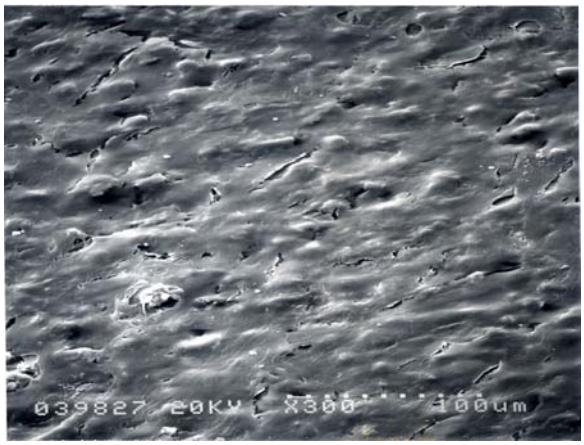

Figure 1c

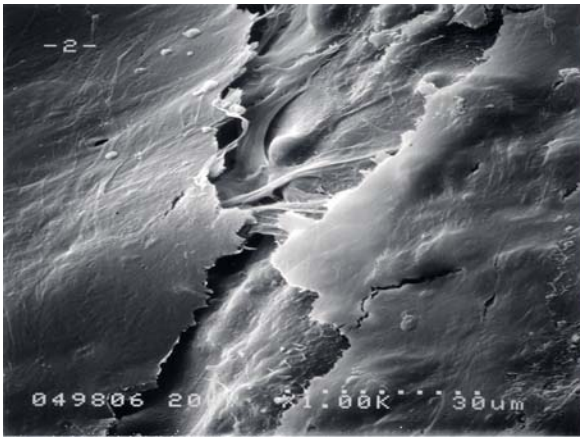

Figure 1d

Figure 1 : Scanning electron micrographs of the test material surface state (Figure 1a), HOB on the test material at day 3 (magnification x 3000) (Figure 1b) and at day 27 (magnification x 300 and x 1000 in Figure 1c and 1d respectively).

A slight increase in cell attachment kinetics (Figure 2) is observed for the composite with regard to negative control : + 12 %, $P < 0.05$ at 3 h. HOB proliferated better on the compound than on the negative control. At 27 days cell density was 17 % ($P < 0.02$) higher than on the negative control. This is the consequence of a lower HOB doubling time : for the control T_1^{112} (1st exponential phase of growth) is 5.5 days and T_2^{112} (2nd exponential phase of growth) is 23.5 days, whereas for the test material T_1^{112} and T_2^{112} are 5 days and 18.5 days respectively.

ALP activity : Figure 3 shows the amount of ALP activity (nMPi/min/10^6 cells) after 3, 15 and 27 days of culture. HOB exhibited an increase in ALP activity over time on both negative control and test material. ALP activity, an early marker of HOB differentiation, was significantly higher on the test material as soon as day 3. At day 27, ALP activity is increased by 21 % ($P < 0.01$) on the PEEK composite.

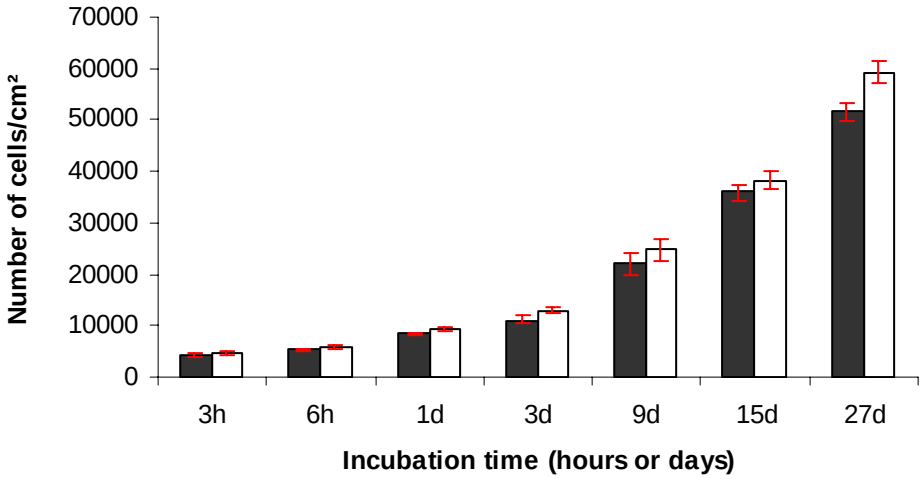

Figure 2 : Histogram showing HOB attachment and proliferation on negative control ■ and test material □. Each point is the mean of four samples.

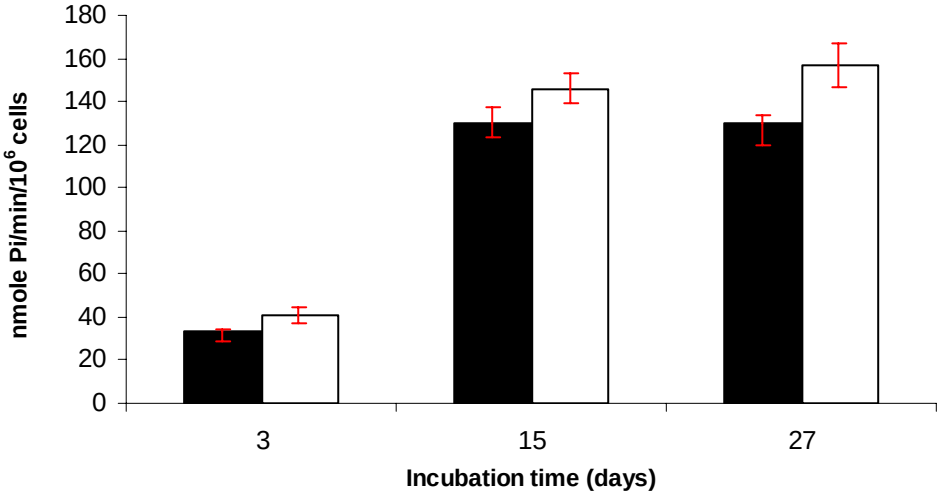

Figure 3 : Histogram showing ALP activity after 3, 15 and 27 days of HOB cultured on the negative control ■ and on the test material □.

Discussion: HOB growth and phenotype expression (ALP) are slightly enhanced when cultured in direct contact with the test material PEEK/β-TCP/TiO_2 composite : this could be the consequence of a better cell adhesion process, and/or related to the release of Ca^{2+} ions from the material surface.
A biodegradation study performed in simulated body fluid (SBF) over 42 days has shown that calcium release, arising from the surface of the PEEK composite, was about 0.5 µg/cm²/day (unpublished results). One can assume that this Ca^{2+} release could stimulate HOB proliferation and differentiation. Osteoblasts express the calcium sensing receptor (CaR) ; accumulating evidence indicates the involvement of this receptor to the changing of extracellular ionic environment [10]. Local release of Ca^{2+} was shown [11] to stimulate osteoblastic cells (MC3T3-E1) proliferation, migration and mineralisation. Ca^{2+} could be an extracellular first messenger acting via the CaR of rat calvarial osteoblasts increasing proliferation [12], and expression of the osteoblast differentiation markers, osteocalcin, collagen I mRNAs, mineralization [13].
TiO_2 powder was added in the PEEK matrix in order to give some radio-opacity to the composite. However it is possible that the powder grains of TiO_2 located at the surface of the samples could have created micro- or nanodomains more favourable to cell adhesion than the PEEK/β-TCP

mixture alone. One of the main regulators of cell growth and proliferation in anchorage dependent cells in shape. Cells in rounded configuration divide at a lower rate than those flattened and well spread on a substratum. SEM observations (Figure 1b) were well correlated with an increase in cell attachment and proliferation.

Conclusion: In conclusion, the results obtained in this study justify further investigation, specially at the level of osteoblast adhesion into the use of this new composite PEEK/β-TCP/TiO_2 in dental and orthopaedic applications : the composite demonstrates excellent mechanical properties, can be considered biocompatible in the framework of ISO 10993 "Biological evaluation of Medical devices". It supports human osteoblasts adhesion, proliferation and differentiation suggesting bioactivity and osteointegration potential through osteoconduction. This was confirmed by clinical results. CE mark was obtained for a dental implant made of PEEK/ β-TCP/ TiO_2 : 4000 cylindric implants have been implanted over a 10 year period, 50 % as immediate implants after extraction and 50 % after socket bone healing, with 96 % success.

References :

[1] Wenz LM., Merritt K., Brown SA., Moet A., Steffee AD. In vitro biocompatibility of polyetheretherketone and polysulfone composites. J Biomed Mater Res. 1990 ; 24(2) : 207-215. [2] Katzer A., Marquardt H., Westendorf J., Wening JV., von Foerster G. Polyetheretherketone – cytotoxicity and mutagenicity in vitro. Biomaterials. 2002 ; 23(8) : 1749-1759.
[3] Evans SL., Gregson PJ. Composite technology in load-bearing orthopaedic implants. Biomaterials. 1998 ; 19(15) : 1329-1342.
[4] Fujihara K., Huang ZM., Ramakrishna S., Satknanantham K., Hamada H. Feasibility of knitted carbon/PEEK composites for orthopaedic bone plates. Biomaterials. 2004 ; 25(17) : 3877-3885.
[5] Meenan BJ., McClorey C., Akay M. Thermal analysis studies of poly(etheretherketone)/hydroxyapatite biocomposite mixtures. J Mater Sci Mater Med. 2000 : 11(8) : 481-489.
[6] Abu Bakar MS., Cheng MH., Tang SM., Yu SC., Liao K., Tan CT., Khor KA., Cheang P. Tensile properties, tension-tension fatigue and biological response of polyetheretherketone-hydroxyapatite composites for load-bearing orthopaedic implants. Biomaterials. 2003 ; 24(13) : 2245-2250.
[7] Tan KH., Chua CK., Leong KF., Cheah CM., Cheang P., Abu Bakar MS., Cha SW. Scaffold development using selective laser sintering of polyetheretherketone-hydroxyapatite biocomposite blends. Biomaterials. 2003 ; 24(18) : 3115-3123.
[8] Naji A., Harmand MF. Cytocompatibility of two coating materials, amorphous alumina and silicon carbide, using human differentiated cell cultures. Biomaterials. 1991 ; 12(7) : 690-694.
[9] Luben RA., Wong GL., Cohn DV. Biochemicals characterization with parathormone and calcitonin of isolated bone cells : provisional identification of osteoclasts and osteoblasts. Endocrinology. 1976 ; 99(2) : 526-534.
[10] Dvorak MM., Riccardi D. Ca^{2+} as an extracellular signal in bone. Cell Calcium. 2004 ; 35(3) : 249-255.
[11] Yamauchi M., Yamaguchi T., Kaji H. Sugimoto T., Chihara K. Involvement of calcium-sensing receptor (CaR) in osteoblastic differentiation of mouse MC3T3-E1 cells. Am J Physiol Endocrinol Metab. 2004 Nov 16. [Epub ahead of print].
[12] Chattopadhyay N., Yano S., Tfelt-Hansen J., Rooney P., Kanuparthi D., Bandyopadhyay S., Ren X., Terwilliger E., Brown EM. Mitogenic action of calcium-sensing receptor on rat calvarial osteoblasts. Endocrinology. 2004 ; 145(7) : 3451-3462.
[13] Dvorak MM., Siddiqua A., Ward DT., Carter DH., Dallas SL., Nemeth EF., Riccardi D. Physiological changes in extracellular calcium concentration directly control osteoblast function in the absence of calciotropic hormones. Proc Natl Acad Sci U S A. 2004 ; 101(14) : 5140-5145.