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Interactions of total bone marrow cells with increasing quantities of macroporous calcium phosphate ceramic granules

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Abstract The biological properties of synthetic calcium phosphate bioceramics have made them the third choice of material for bone reconstructive surgery, after autologous bone and allografts. Nevertheless, bioceramics lack the osteogenic properties that would allow them to repair large bone defects. One strategy in bone tissue engineering consists of associating a synthetic scaffold with osteogenic cells. Mesenchymal stem cells (MSC) are usually isolated from bone marrow cultured for several weeks and seeded on to a small quantity of bioceramic. We have studied the association of total bone marrow cells, harvested from femurs of rats, with increasing amounts of calcium phosphate ceramic granules (50–250 mg). A cell viability test indicated that a little quantity of bioceramics granules (50 mg) was less detrimental for culturing 1 million nucleated cells from the whole bone marrow population. Cell morphology, viability, adhesion and differentiation were studied after different culture periods. Among the heterogeneous population of bone marrow cells, only a limited amount of cells attached and differentiated on the bioceramics. To explain the influence of the amount of synthetic scaffold on cell viability, media calcium concentrations were measured. Low cell viability could be explained by calcium phosphate precipitation leading to a decrease in calcium concentrations observed with relatively large amounts of scaffold. This study showed that the chemical stability of the ceramic plays a critical role in the viability of bone marrow cells.

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Introduction

It is estimated that every year more than 1 million patients worldwide need treatment for skeletal disorders. As life expectancy increases and the population becomes more active, this number has increased steadily at a rate of about 10% per annum [1]. Bone grafting is required in the field of reconstructive surgery, spinal arthrodesis, orthopaedic surgery, cranofacial surgery and dental implantology [2].

Until now, autologous bone has remained the 'gold standard' in bone reconstruction because of its osteogenic properties [3]. However, autografts are associated with significant morbidity at the harvesting site (i.e. iliac crest), along with complications such as pain, superficial infection, haematoma or pelvic instability [4, 5]. In addition, there is a need for a second surgical site, adding to the length of the operation. The use of autologous bone is also hampered by the limited availability of bone tissue (<20 cm^3) [6].

Allografting or transplantation of devitalized femoral heads from a donor to a patient is also used clinically in bone reconstruction procedures [7]. However, allografts are less osteogenic, more immunogenic and show a greater resorption rate than autografts [8]. Despite the stringent cleaning procedures, disease transmission (e.g. HIV, hepatitis) from donor to recipient has been reported [9, 10].

Synthetic materials such as calcium phosphate bioceramics play an important role in bone reconstruction because

of their unlimited availability, excellent biocompatibilty, osteo-conductivity and even more recently reported osteo-inductivity [11, 12]. It has been shown that bioceramics with specific microstructures can induce ectopic bone formation within their macropores after implantation for few weeks in the muscles of large animals. However, the mechanism of osteoinduction by biomaterials is not fully understood and the amount of induced bone is still too limited for the reconstruction of large skeletal defects in patients [11–14].

Recent progress in both material science and biology has resulted in the possibility of bone tissue engineering with the aim of producing a bony equivalent in vitro by combining bone forming cells and a synthetic three-dimensional scaffold [15–20]. At present, in the bone tissue engineering field, three strategies make use of the patient's own bone marrow cells to engineer autologous osteogenic grafts. The first approach consists in harvesting bone marrow followed by seeding the cells on to a synthetic scaffold and immediate implantation into the bone defect. However, this strategy has not led to reproducible bone formation in animals [21, 22]. The reasons may be related to the heterogeneous nature of bone marrow, composed of haematopoietic and stromal precursors able to differentiate into a variety of lineages (blood cells, smooth muscle cells, adipocytes, chondrocytes and osteoblasts, etc). In the second approach, the harvested bone marrow cells are cultured for 1–2 weeks in order to isolate the mesenchymal stem cells (MSC). These cells are then seeded on to a suitable scaffold shortly before implantation. The hybrid constructs have shown bone-inducing ability in animal models [23–25]. The third strategy consists in harvesting bone marrow, isolating and expanding the osteoprogenitor cells for 1–2 weeks, then seeding them on to a scaffold, where they are cultured for a further 2 weeks to promote the formation of a bone-like tissue layer on the implant. The hybrid construct is finally transplanted in the same patient [26]. As such, the latter two strategies require several weeks of culturing under strict aseptic conditions making the clinical applications for bone tissue engineering extremely difficult. Nevertheless, certain clinical indications require immediate bone grafting (e.g. trauma) and large quantities of hybrid bone constructs should be available within a minimal time.

In this study, we attempt to determine the optimal quantity of scaffold for culturing bone marrow cells in order to produce an osteogenic graft within a minimal time. Bone marrow cells were harvested from rat femurs. Instead of selecting the MSC sub-population and expanding them in vitro, whole bone marrow cells were directly seeded on to granules of macroporous biphasic calcium phosphate (MBCP) ceramic. Total bone marrow cells were cultured on increasing quantities of MBCP granules for periods ranging from a few hours to days in culture media containing osteogenic factors such as dexamethasone, beta-glycerophosphate and ascorbic acid. Cell attachment, morphology, viability and differentiation on the MBCP scaffold were assayed following these culturing conditions.

Materials and methods

Materials

Cell culture plastic ware was purchased from Falcon (Becton-Dickinson, Franklin Lakes, NJ, USA). Alpha-Minimal Essential medium (α-MEM), L-Glutamine (L-Glu), penicillin/streptomycin antibiotics (AB), trypsin/EDTA and phosphate buffered saline (PBS) were purchased from Invitrogen Corporation (Paisley, UK). Fetal calf serum (FCS) was supplied by Dominique Dutcher (Brumath, France). Surfactant Nonidet P-40 was obtained from Amersham Biosciences (Orsay, France). Dexamethasone (Dex), sodium L-ascorbate (AA), glycerol 2-phosphate disodium salt hydrate (β-GP), para-nitrophenylphosphate (p-NPP), 2-amino-2-methyl-1-propanol and $MgCl_2$ were purchased from Sigma (St. Quentin Fallavier, France). Promega (Charbonnière les Bains, France) supplied the 3-(4,5-Dimethylthiazol-2-gl)-5-(3-carboxymethoxylphenyl)-2-(4-sulphophenyl-2H) tetrazolium inner salt (MTS). All other chemicals were from standard laboratory suppliers and were of the highest purity available.

Preparation and characterisation of scaffolds

Scaffolds were made of MBCP ceramic (MBCP^{TM}, Biomatlante, France). MBCP granules measuring 1–2 mm in diameter were used. The MBCP ceramic consisted of 60% hydroxyapatite (HA) and 40% beta-tricalcium phosphate (βTCP). The chemical purity of the MBCP was analysed using X-ray diffraction (XRD, Philips PW 1830, CuKα source) and Fourier transform infrared spectroscopy (FTIR, Nicolet, Magna-IR 550). Finally, traces of CaO in the MBCP were checked by using a phenolphthalein test. The structure of the MBCP was observed using a scanning electron microscope (SEM, Leo 1450VP, Zeiss, Germany). Prior to the SEM observations, the samples were coated with gold-palladium at 20 mA for 4 min (EM Scope, UK). As microparticles were found, the granules were washed in demineralised water and ethanol for 10 min using an ultrasonic bath and finally air-dried. Prior to the cell culture experiments, the MBCP granules were sterilized in an autoclave at 121 °C for 30 min.

Harvesting bone marrow cells

Bone marrow cells were obtained from rat femurs using the method developed by Maniatopoulos et al. [26]. Our study

was approved by the local committee for animal care and ethics. Inbred rats (Lewis 1A, male, 7 weeks old, 250 g) were purchased from a professional breeder (Elevage Janvier, Le Genest St Isle, France). The rats were anaesthetised by inhalation of isoflurane (Laboratoires Belamont, Boulogne, France) at a flow rate of 3 mL/min for 5 min. The rats were then euthanised by intra-cardiac injection of 1 mL of sodium pentobarbital (Doléthal, Vétoquinol, Lure, France). Both femurs were dissected and immediately placed in α-MEM containing 1% of AB. Under a laminar flow cabinet, the surrounding muscles were detached from the femurs. Both femoral epiphyses were cut and the medulla was carefully flushed with 3 mL of α-MEM using a syringe with an 18-gauge needle. The bone marrow was then filtrated through a nylon sieve (70 μm, BD Falcon, Becton Dickinson Bioscience, Erembodegem, Belgium) in order to remove bone debris and blood clots. Nucleated cells were counted on Malassez haemocytometer slides. Cell viability was greater than 90% as determined by the trypan-blue dye exclusion test. The number of nucleated cells was around 25×10^6 per mL of medium. A cytological myelographic analysis (cytospin) was performed in order to assess the quality of the harvested rat bone marrow. The different populations of cells were expressed as relative percentages.

Bone marrow cell culture

In this study, total bone marrow was used without isolating MSC. After harvesting from the femurs of rats and counting of nucleated cells, the total bone marrow cells were directly seeded on tissue culture plastic (control) and on increasing amounts of MBCP granules. A cell density of 1×10^6 nucleated cells per well in 1 mL of culture medium was used in 24-well tissue culture dishes. The culture medium was α-MEM supplemented with 10% FCS, 1% AB and 1% L-glut with or without adding Osteogenic Factors (OF) consisting of 10^{-8} M Dex, 20 mM AA and 1 mM β-GP. Bone marrow cell behaviour was studied on 50, 100, 150, 200 and 250 mg of MBCP granules using culture medium supplemented with OF at the same cell density of 1×10^6 nucleated cells/mL. The cultures were maintained in a humidified atmosphere of 95% air with 5% CO_2 at 37 °C. The culture medium was renewed every 2 days.

Cell viability assay

Different quantities of MBCP granules were tested in 24-well plates with 1 mL of culture medium: 50, 100, 150, 200 and 250 mg. Prior to cell seeding, the MBCP granules were incubated for 2–3 days in α-MEM with 1% AB. During this pre incubation period, the culture medium was renewed twice a day. Freshly harvested total bone marrow cells were then seeded on to the MBCP granules at a cell density of 1×10^6 cells per well. Subcultures were performed in 1 mL of α-MEM supplemented with 10% FCS, 1% AB and 1% L-Glu with or without adding OF (Dex, 10^{-8} M; AA, 20 mM and β-GP 1 mM). The subcultures were maintained in a humidified atmosphere of 95% air with 5% CO_2 at 37 °C. The culture medium was renewed every 2 days. After culturing for 8 days bone marrow cells with increasing amounts of MBCP granules, the cell viability was measured using the MTS assay. Briefly, mitochondrial NADH/NADPH-dependent dehydrogenase activity, resulting in the cellular conversion of the tetrazolium salt into a soluble formazan dye [27, 28], was assayed by using a MTS CellTiter 96 Aqueous non-radioactive cell proliferation assay (Promega). Rat bone marrow cells were cultured on to culture dishes (plastic) and on to MBCP granules in 24-well plates at a cell density of 1×10^6 cells per mL. After 8 days, the culture media were removed and 100 μL of MTS solution was added to each well for 2–3 h according to the manufacturer's instructions. Finally, colorimetric measurement of formazan dye was performed on a spectrophotometer with an optical density reading at 490 nm. The results were expressed as relative MTS activity as compared to control conditions (cells cultured in the absence of the materials on plastic).

Cell morphology by scanning electron microscopy

Freshly harvested bone marrow cells were directly seeded on the MBCP granules at a density of 1×10^6 nucleated cells per mL. Cells were maintained in culture for 30 min, 4, 8 and 48 h and 8 days in a humidified atmosphere of 95% air with 5% CO_2 at 37 °C. After these culture periods, the MBCP granules were removed from the wells and washed three times with PBS. The cells were then fixed using a 4% glutaraldehyde solution for 1 h, washed with PBS and then dehydrated through series of ethanol graded from 50% to 100%. The samples were then dried with ethanol/trichlorotrifluoroethane solutions using the following ratios: 75/25, 50/50, 25/75, 0/100 for 15 min and 0/100 until evaporation of the solution. Prior to the SEM observations, the granules were coated with gold-palladium at 20 mA for 4 min (EM Scope, UK). SEM and BSEM observations were made using secondary and back-scattered electrons respectively, at 15 kV.

Alkaline phosphatase activity

ALP activity was evaluated as previously described [29]. Rat bone marrow cells were cultured on to culture dishes (plastic) and on to MBCP granules or in the absence of materials in 24-well plates (1×10^4 cells per cm^2). After the indicated times, the cells were washed twice with ice-

cold PBS and scraped into a 0.2% aqueous solution of Nonidet P-40. The cell suspension was sonicated on ice for 30 s and centrifugated for 5 min at 4 °C. Aliquots of supernatants were subjected to both protein assay with the Pierce Coomassie Plus assay reagent (Pierce, Rockford, USA) and ALP activity measurement. ALP activity was assessed at pH 10.3 in 0.1 M 2-amino-2-methyl-1-propanol containing 1 mM $MgCl_2$. P-NPP (10 mM) was used as the chromogenic substrate for an optical density reading at 405 nm. The results were expressed as relative ALP activity compared with control conditions (cells cultured in the absence of material on plastic).

Calcium measurements in culture media

Increasing amounts of MBCP granules, 0, 50, 100, 150, 200 and 250 mg were incubated in 1 mL of α-MEM medium using 24-well plates without bone marrow cells. After incubation for 7 and 24 h, conditioned media were collected and the calcium quantities were measured via atomic absorption spectrometric analyses. About 1 mL of each sample was diluted in 2 mL of a 1% $LaCl_3$ solution. Analyses were performed at 422.7 nm, under an air/acetylen flame with an Na-lamp using a Unicam 989 spectrometer. Measurements were performed in triplicate and data were averaged.

Statistical analysis

Each experiment was repeated at least twice with similar results. The results are expressed as mean ± SEM of triplicate determinations. Comparative studies of means were performed using one-way ANOVA followed by a post hoc test (Fisher projected least significant difference) with statistical significance at $p < 0.05$.

Results

The structure of the MBCP granules is shown in Fig. 1. SEM micrographs show granules ranging in size from 1 mm to 2 mm. Macropores with a diameter ranging from 100 μm to 600 μm were visible but these pores were not always interconnected (Fig. 1a). At higher magnification, a microporous surface was observed. These micropores ranged in diameter from 0.1 μm to 0.5 μm (Fig. 1b).

Total bone marrow cells were seeded and cultured on these MBCP granules. About 25×10^6 of nucleated cells per mL of media were harvested from the rat femurs. The cytological myelographic counting revealed a heterogeneous cell population with haematopoietic and stromal lineages. The major cell sub-populations were erythroblasts (36%), lymphocytes (26%), polynuclear neutrophils (11%) and monocytes (5%). The bone marrow had a normal cell population repartition.

Following pre incubation of the MBCP granules in culture media, the whole population of rat bone marrow cells was directly seeded on to increasing quantities of MBCP granules at a density of 1×10^6 nucleated cells/mL. The quantity of MBCP granules varied from 0 (plastic), 50, 100, 150, 200 to 250 mg. The corresponding volumes of MBCP granules were 0.1, 0.2, 0.3, 0.4 and 0.5 cm^3, respectively. For 200 and 250 mg of ceramic, the surface of the well was completely covered by the MBCP granules. After culturing for 8 days, the cell viability was measured using the MTS activity assay for increasing MBCP amounts. As shown in Fig. 2, a gradual decrease in the viability of the bone marrow cells was found with increased quantities of MBCP. Cell viability decreased by 20% with the addition of 50 mg of granules while it fell by 60% with 100 mg. High quantities of granules led to a similar effect on cell viability. The MTS activity was only 10% with 250 mg of granules as compared to plastic conditions.

For studying cell morphology, the freshly harvested rat bone marrow cells were seeded and cultured on 50 mg of MBCP granules. As evidenced by the MTS assay, this quantity of MBCP granules was less detrimental for cell viability. The morphology of the cells on the scaffold was then observed by SEM (Fig. 3). After culturing for 30 min, numerous cells had colonized the surface of the MBCP. As

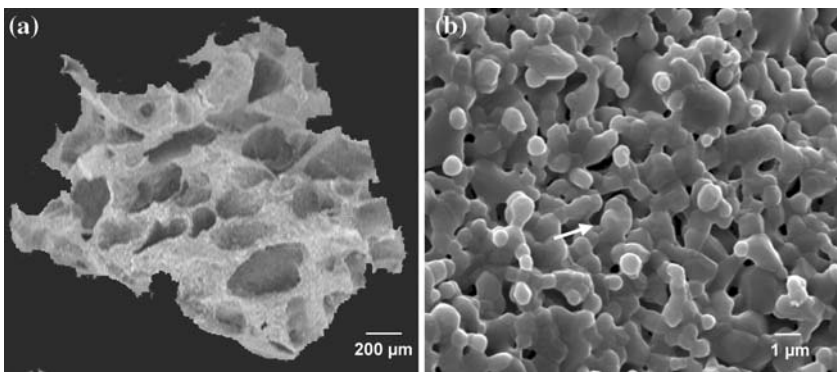

Fig. 1 SEM micrographs of the MBCP granules. (**a**) macropores, (**b**) microporous surface

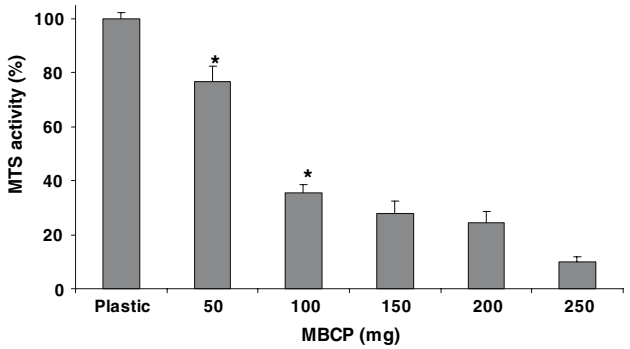

Fig. 2 MTS activity of total bone marrow cells cultured on to different amounts of MBCP granules for 8 days. MTS activity was determined as indicated in the Materials and methods section. Results are expressed in relative MTS activity compared with control conditions (plastic) (*: $p < 0.05$ compared with the control conditions)

shown in Fig. 3a, many red blood cells and rounded cells exhibiting short cytoplasmic extensions were observed on the material. After 4 h, the number of red blood cells on the surface of the MBCP granules was relatively lower than after 30 min (Fig. 3b). Adherent cells had spread over the surface with multiple peripheral filopodia and cytoplasmic extensions. After 8 h, only a few red blood cells were found on the material. Adherent cells had spread on to the surface with numerous cytoplasmic extensions (Fig. 3c). After 48 h, numerous adherent cells with fibroblastic-like morphology, rounded cells and a few red blood cells were observed (Fig. 3d). After culturing for 8 days, the surface of the MBCP granules was almost completely covered by a layer of adherent cells (Fig. 4a). At higher magnification, this layer was composed only of cells having a polygonal appearance (Fig. 4b).

In order to confirm the effect of the osteogenic factors, the differentiation of rat bone marrow cells on the ceramic material was studied. The ALP activity of these bone marrow cells cultured on 50 mg MBCP granules was measured after 21 days of culture (Fig. 5). The addition of osteogenic factors, such as dexamethasone, ascorbic acid and beta-glycerophosphate into the culture medium increased the ALP activity in comparison to the standard medium. This significant increase ($p < 0.05$) in ALP activity between the two culture conditions was about 200% compared with the same culture conditions without osteogenic factors.

In order to explain the decreased cell viability observed with measurement of MTS activity in Fig. 2, calcium concentration was measured in the culture media with different amounts of MBCP granules (Fig. 6). After 7 and 24 h of incubation without cells, we noticed a significant decrease in calcium concentration with increasing quantities of MBCP. Calcium concentration decreased 2.5 and 4 folds after 7 and 24 h of incubation respectively with 50 mg of granules as compared to the control group. With higher quantities of MBCP and regardless of the incubation period, the calcium concentrations in the culture media were extremely low (around 0.03 mM for 250 mg). This decrease in calcium concentration was also observed while the culture media was refreshed several times while high quantities of MBCP granules were used (data not shown).

After both incubation in culture media for 1 week and culturing of bone marrow cells for another week, the surface of the MBCP granules was covered with a typical apatite

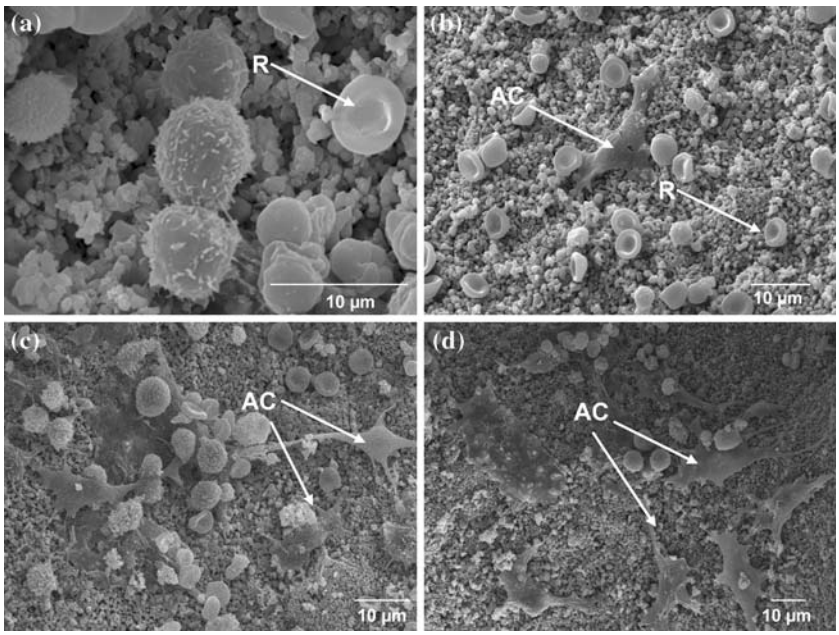

Fig. 3 SEM images of total bone marrow cell adhesion and morphology on the MBCP granules with increasing culture period. Fixed and dehydrated samples were observed after (**a**) 30 min, (**b**) 4 h, (**c**) 8 h and (**d**) 48 h of culture on biphasic calcium phosphate ceramic granules. The SEM images showed a heterogeneous cell population on the MBCP granules (R: red blood cell; AC: adherent cell). Note that the proportion of adherent cells (AC) on the ceramic surface increased with culture period

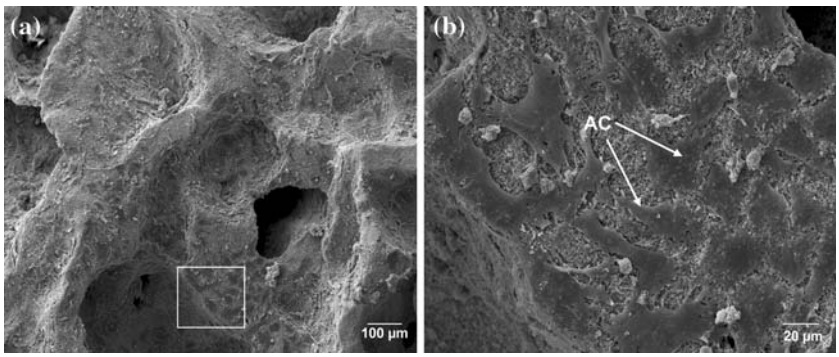

Fig. 4 SEM pictures of total bone marrow cells cultured on to MBCP granules for 8 days. (**a**) Low magnification image showing cells spreading over the whole surface of the calcium phosphate scaffold and (**b**) magnified detail showing polygonal appearance of adherent cells (AC)

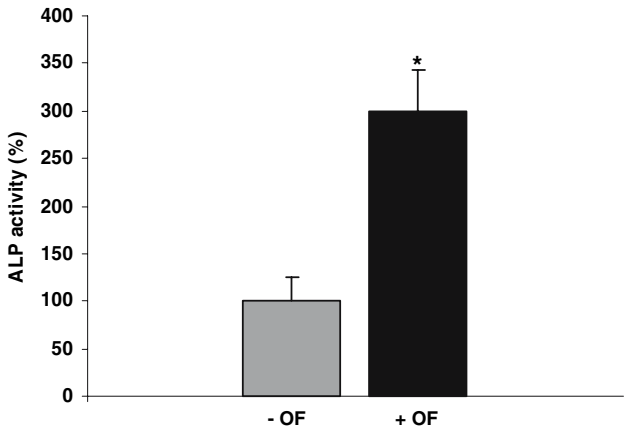

Fig. 5 ALP activity of total bone marrow cells after a 21-day culture in direct contact with the MBCP granules with or without addition of osteogenic factors (OF). ALP activity was measured as described in the Materials and methods section. Results are expressed in relative ALP activity compared with culture conditions without OF (*: $p < 0.05$ compared with conditions without OF)

layer. As shown in Fig. 7, adherent cells were also present on this apatite layer indicating that this precipitation occurred prior to the adhesion of the bone marrow cells.

Discussion

Bone tissue engineering using the patient's own bone marrow cells cultured on to synthetic scaffolds would provide an innovative approach for the reconstruction of large bone defects. Many studies have already shown that MSC cultured on to bioceramics possess osteogenic properties both in vitro and in vivo [30–32]. In view of these results, bone tissue engineering will certainly play a major role in clinical bone reconstructions. However, this approach needs to be optimized and standardized before it can be used in clinical practice. A major drawback for bone tissue engineering is related to the long culture times needed before bone grafting material is available. In most reports, MSC were first isolated from the total bone marrow cell population by their capacity to adhere to the tissue culture plastic [33]. The MSC were then expanded for several weeks before seeding on to the scaffolds. This approach thus requires considerable manipulation by qualified personnel in clean rooms making the bone grafting procedure extremely complex and expensive for clinical usage.

In the progress of bone tissue engineering, scaffolds suitable for ex vivo cell cultivation should be developed. The requirements for cell cultures are not necessarily similar to those for the in vivo performance of these scaffolds in bone reconstructive surgery. After filling bone defects, scaffold materials, such as calcium phosphate ceramics, interact with considerable amounts of body fluids containing a variety of proteins and cells which is far more complex than in cell culture conditions. On the other hand, scaffolds should maintain the cell viability and proliferation of MSC in order to support their differentiation into osteoblasts not only under in vitro conditions, but also under in vivo conditions.

The vast majority of studies on bone tissue engineering have used large numbers of isolated and expanded MSC with a small quantity of scaffold. For instance, Ogushi cultured 10 million MSC on to one HA disc of Ø5 × 2 mm [17]. Mendes seeded between 1 and 2.5×10^5 MSC per coralline HA particle of 3 × 2 × 2 mm [34]. Toquet and Rochet cultured a similar number of cells, namely 2×10^5 MSC, on MBCP ceramic discs of 5 × 2 and 15 × 1 mm, respectively [15, 18]. In most of these publications, pre incubation of the scaffold material in the culture media for several days prior to cell seeding was mentioned. However, the reasons for the pre incubation of scaffolds are poorly understood. In the perspective of clinical bone tissue engineering, large quantities of scaffold should be used for repairing large bone defects. Furthermore, certain clinical indications require immediate bone grafting (e.g. trauma) and large quantities of hybrid bone constructs should be available within a minimal time.

Our study examines the behaviour of total bone marrow cells directly seeded and cultured on relatively large

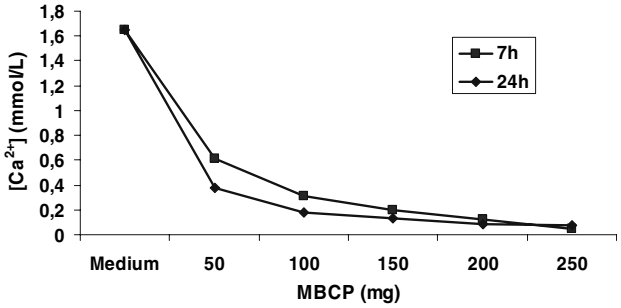

Fig. 6 Calcium concentration in the culture media with different amounts of MBCP granules. Calcium concentrations were measured as described in the Materials and methods section after (■) 7 h and (♦) 24 h

quantities of MBCP granules for 1 million nucleated cells. The MSC from bone marrow were not isolated and expanded prior to seeding on the scaffolds. Previous studies have established that total bone marrow contains 1–2% cells with osteogenic differentiation capacities [35]. In our approach, a complex mixture of haematopoietic and stromal cells was directly cultured on to the calcium phosphate ceramics. This heterogeneous cell population composed of red blood cells and adherent cells colonized the surface of the MBCP granules within 30 min. Within a few hours, only the adherent cells had attached and spread over the ceramic, exhibiting filopodia and cytoplasmic extensions. A fraction of these adherent cells on the ceramic could be considered to be MSC. It has been shown that the adhesion of cells on to materials depends on their surface energy, surface charge and roughness. Comparative studies have shown that calcium phosphate ceramics are the most suitable substrata for osteoblastic cell adhesion [23, 36]. Our study therefore corroborated that a fraction of the whole bone marrow cell population attached to the surface of the ceramic.

Numerous studies have shown that the addition of osteogenic factors into the culture media promoted the differentiation of bone marrow cells into osteoblastic-like cells [37, 38]. Of the various osteoblastic differentiation markers, ALP is one of the most widely used [39]. In our study, expression of ALP activity in total bone marrow cells cultured on MBCP granules suggested osteoblastic differentiation after 21 days of culture. The presence of osteogenic factors in the media increased the differentiation of the total bone marrow cell cultured on the granules.

The present study shows that increasing quantities of scaffold are detrimental to cell viability as compared to tissue culture plastic. Even a small amount of MBCP granules, such as 50 mg, affected cell viability by 20% for 1 million of nucleated cells from total bone marrow. This quantity of scaffold was nevertheless comparable to those employed in previous studies where isolated MSC at a cell density of 10×10^6 were seeded and cultured in a comparable volume of ceramic scaffold [15, 18, 21, 37]. Although the MBCP granules were washed and pre-incubated in culture media prior to cell seeding for 1 week, the cell viability was low after 8 days of culture. Bouler et al. studied the behaviour of macroporous calcium phosphate discs in culture media for 48 days [40, 41]. They found that a carbonated apatite layer precipitated on to the surface of the ceramics within a few hours. This apatite precipitation resulted in a 2- to 3-fold decrease in calcium and phosphate concentrations in the culture media. In this study, we also observed the precipitation apatite on to the surface of the calcium phosphate ceramic material (Fig. 7). The calcium concentration in the culture media decreased drastically resulting from the precipitation of this apatite layer at the surface of the MBCP granules (Fig. 6). As calcium is essential for cell viability and proliferation [42], its decrease may explain the present results of low cell viability with high amounts of scaffold.

Conclusion

In the development of bone tissue engineering, the scaffold plays a fundamental role in supporting bone marrow cell adhesion, proliferation and differentiation ex vivo. The clinical application of bone tissue engineering needs a relatively large amount of grafting material within a minimal time. Nevertheless, high amount of scaffold could be harmful for the viability of bone marrow cells. In this study, we found that 50 mg of MBCP scaffold was less detrimental for the viability of a whole population of rat bone marrow cells. Adherent cells were selected from the whole bone marrow by their capacity for adhesion to the

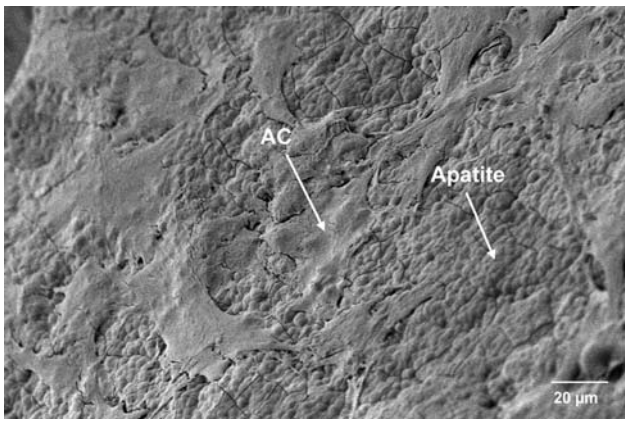

Fig. 7 BSEM picture after 8 days of total bone marrow cell culture on MBCP granules. A typical apatite precipitation was observed on the surface calcium phosphate ceramic granules. Polygonal adherent cells (AC) spreading on to the apatite were visible

MBCP granules. After culture, the cells differentiated towards an osteoblastic phenotype in the presence of osteogenic factors such as dexamethasone. The decrease in calcium concentration in the culture media resulted from the precipitation of apatite on to the scaffold. This decrease in calcium concentration could explain the low cell viability observed with the increased amount of scaffold. Total bone marrow cell adhesion, viability, proliferation and differentiation could be enhanced by using an optimal ratio of cell per surface of scaffold or by using adapted scaffolds for culturing bone marrow cells ex vivo.

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