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Culture of human bone marrow cells on non-porous calcium carbonate and plastic: preliminary results

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In this paper the possibility of growing human bone marrow cells (HBMC) on dense calcium carbonate in conditions driving these cells towards osteogenesis is explored. It was observed that HBMC were able to adhere and multiply and can be driven best towards osteogenesis in the presence of Dex and 1,25(OH)D3 (as regards ALP activity). Specific morphological features of cells were observed by scanning electron microscopy when cells were cultured on this material. Further experiments are needed to explain these characteristics.

1. Introduction

Coral is an interesting alternative to bone graft for skeletal reconstruction owing to its porous architecture, high compressive breaking stress, good biocompatibility and resorbability. Upon implantation in bone, five histological phases can be distinguished: invasion of coral by blood cells and extravasated bone marrow, vascularization, resorption of coral by osteoclast-like cells, bone neoformation with osteoblastic apposition and concomitant resorption and remodelling of neoformed tissue to reproduce the architecture of the recipient bone [1, 2]. Clinically, coral has been used with success in orthopaedic, dental and maxillofacial applications.

To obtain a better understanding of the complex biological events occurring at the bone–coral interface, an *in vitro* system has been used to study the interactions of bone-forming cells with calcium carbonate. Dense calcium carbonate was used instead of natural coral to simplify the system and to avoid the technical difficulties of analysis due to the three-dimensional structure of coral. They are both composed of calcium carbonate in the form of aragonite. However, dense calcium carbonate is not porous, in contrast to coral.

The rationale for using bone marrow is that it contains stem cells which have the potential to differentiate along various pathways including bone cells [3–5]. Rat bone marrow derived cells have been used in *in vitro* system to study the relationships between ceramic and the bone environment [6]. Culture conditions generally include dexamethasone (Dex) which stimulates osteogenesis in chick [7] or rat progenitor [8] cell population. Moreover, 1,25-dihydroxyvitamin D3 (1,25(OH)D3) is one of several humoral factors that may regulate osteoblast differentiation.

In this paper the possibility of growing human bone marrow cells (HBMC) on dense calcium carbonate in conditions driving these cells towards osteogenesis is explored. As a first step, HBMC was cultured on plastic and the effects of Dex and 1,25(OH)D3 on alkaline phosphatase activity studied to determine the optimal culture conditions for stimulation of osteogenesis. As a second step, cells were cultured on dense calcium carbonate in the presence of Dex and 1,25(OH)D3. It was observed that HBMC were able to adhere and multiply, and expressed ALP activity when cultured on calcium carbonate under appropriate conditions.

2. Materials and methods

2.1. Disc preparation

The dense calcium carbonate used was supplied by INOTEB, Saint-Gomery, France. Discs of 13 mm were sawn out of blocks of Tridacna shell. After sterilization by autoclave, discs were pre-incubated in 1 ml of medium overnight at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 before cell culture.

2.2. Bone marrow cell isolation and culture

HBMC were harvested from a 57-year old patient. A single cell suspension was prepared by repeatedly aspirating the cells through a 19 gauge and a 21 gauge needle. The cell suspension was filtered through sterile bolting cloth and cultured in alpha-MEM plus 15% fetal bovine serum in a humidified atmosphere of 95% air and 5% CO_2. First-passage cells were used to inoculate calcium carbonate discs or 24-well cell culture dishes and cultured for 20 days in alpha-MEM plus 15% fetal bovine serum.

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Initially, first-passage cells were cultured on plastic in alpha-MEM plus 15% fetal bovine serum at four different conditions: no addition (control), addition of 10^{-8} M 1,25(OH)D3, or 10^{-8} M Dex or 10^{-8} M Dex and 10^{-8} M 1,25(OH)D3 for 20 days in order to determine the optimal culture conditions for stimulation of osteogenesis.

First-passage cells were cultured on calcium carbonate discs with either no addition or both 10^{-8} M Dex and 10^{-8} M 1,25(OH)D3 for 20 days.

2.3. Alkaline phosphatase activity and DNA determination

At day 5, 10 and 20, cell layers were scraped in 1 ml of distilled water, sonicated three times for 10 s and transferred to microfuges tubes. Alkaline phosphatase activity and DNA content were determined as previously described [9, 10].

2.4. Scanning electron microscopy

At day 5, 10 and 20, cultures were fixed with glutaraldehyde cacodylate buffer, dehydrated through a graded ethanol series and critical point dried using CO_2 (Balzers Union CPD010). Samples were sputter-coated with gold and examined in a Joel (model JSM 840 A) scanning electron microscope (SEM) at an accelerating voltage of 10 kV.

3. Results

3.1. Culture of bone marrow cells on plastic

Cell growth. In order to establish the optimal cell culture conditions first-passage HBMC were cultured in the presence of either 1,25(OH)D3, or Dex or both, and DNA content and ALP activity were determined. Culture of HBMC in the presence of 1,25(OH)D3 or Dex + 1,25(OH)D3 had no effect on DNA content. However, Dex increased significantly the amount of DNA at day 20 (Fig. 1).

ALP activity. Culture of HBMC in the presence of 1,25(OH)D3, Dex or Dex + 1,25(OH)D3 led to an increase in ALP activity. Addition of Dex and 1,25(OH)D3 was the most potent. At day 20 an increase in the ALP activity by a factor 40 was observed in comparison to the control (Fig. 2). Consequently, HBMC were cultured on calcium carbonate discs in the presence of Dex and 1,25(OH)D3. Control samples cultured in alpha-MEM + 15% fetal bovine serum only were also prepared.

3.2. Culture of bone marrow cells on calcium carbonate

Scanning electron microscopy. A progressive invasion of the material by HBMC was observed. At day 5 and 10, cells appeared to be attached to calcium carbonate discs by very short processes. This result contrasted with that observed on plastic where cells exhibited long cytoplasmic processes. At day 10, some cells appeared to have a granular aspect when cultured on calcium carbonate discs. At day 20, cell layers were confluent and similar on plastic and calcium carbonate under all culture conditions (Fig. 3).

ALP activity. The kinetics of ALP activity of HBMC cultured with or without the addition of Dex and 1,25(OH)D3 showed that cells expressed ALP activity only in the presence of Dex and 1,25(OH)D3 (Fig. 4). Calcium carbonate alone did not induce any ALP activity. Between day 5 and 20 ALP activity increased by a factor 30 in the presence of Dex and 1,25(OH)D3. This result is similar to that observed on plastic.

4. Discussion

4.1. Culture of HBMC on plastic

ALP expression required Dex. Maximun activity was achieved in the presence of Dex and 1,25(OH)D3 and on this criterion osteogenesis was stimulated greatest in the combined presence of these two agents. However, no mineralized nodules were observed at any

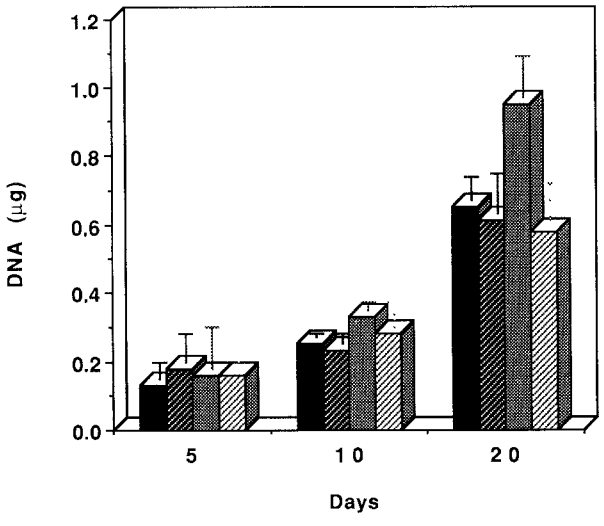

Figure 1 Effect of dexamethasone and 1,25(OH)D3 on HBMC cultured on plastic (■ control; ▨ 1,25(OH)D3; ■ Dex; ▨ Dex + 1,25(OH)D3).

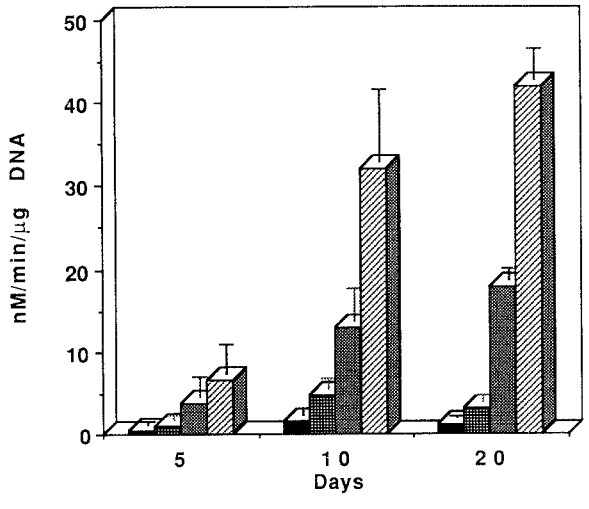

Figure 2 Effect of dexamethasone and 1,25(OH)D3 on alkaline phosphatase activity of HBMC cultured on plastic (■ control, ■ 1,25(OH)D3; ■ Dex, ▨ Dex + 1,25(OH)D3).

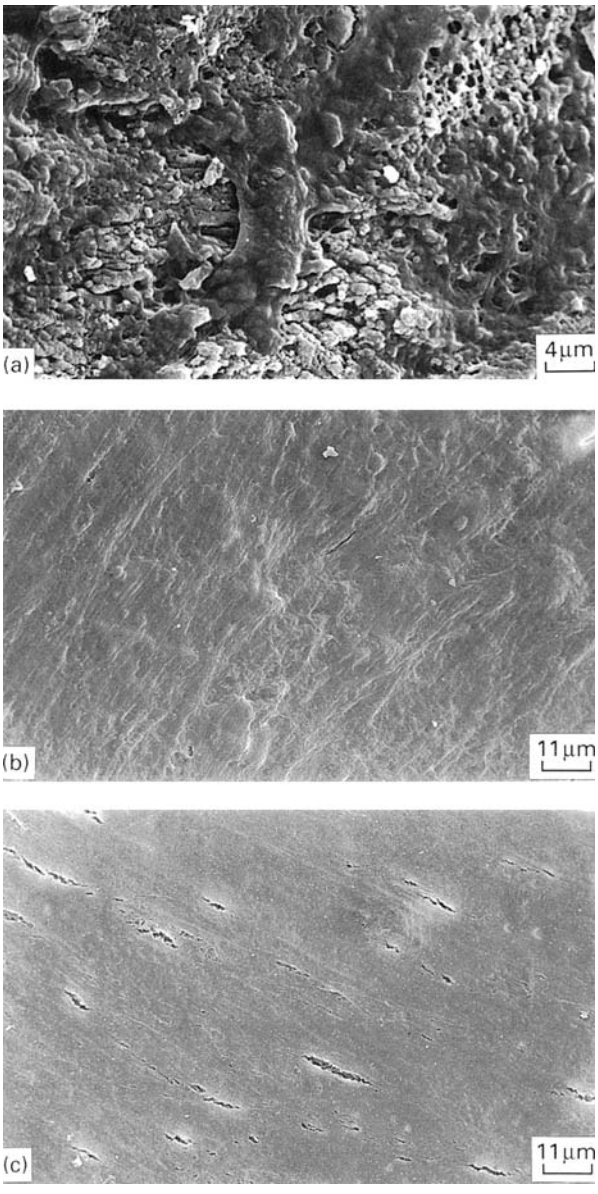

Figure 3 Scanning electron micrograph of human bone marrow cells cultured on calcium carbonate after (a) 10 days and (b) 20 days; and (c) on plastic after 20 days

time. The effect of Dex has previously been observed on rat [8]. Nevertheless, it is noteworthy that the addition of 1,25(OH)D3 to Dex led to a doubling of ALP activity.

4.2. Culture of HBMC on calcium carbonate

HBMC were able to grow on calcium carbonate. At day 5 and 10, these cells had short processes, and some of them a granular aspect. These two features were distinctive of cells growing on calcium carbonate. However, further experiments are needed to explain and characterize these phenomena. Cells appeared to reach confluency by day 20: at this time, morphology of HBMC was similar, whatever the material. Calcium carbonate did not have any inductive effect on ALP

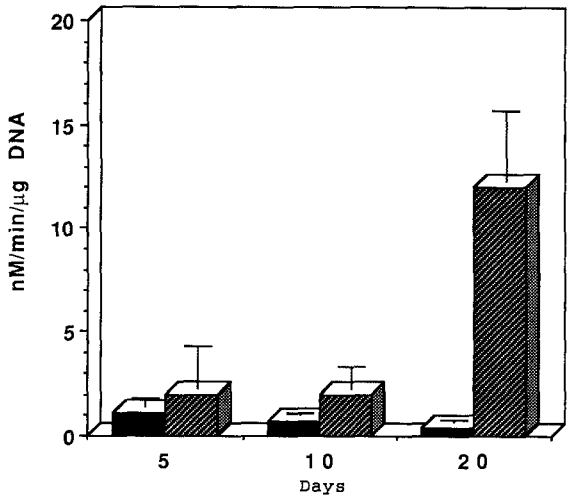

Figure 4 Effect of dexamethasone and 1,25(OH)D3 on alkaline phosphatase activity of HBMC cultured on calcium carbonate (■ FBS; ■ Dex + 1,25(OH)D3).

activity. Induction of the osteoblast marker, ALP, required supplementation of the medium with Dex and 1,25(OH)D3, as observed on plastic.

5. Conclusions

In conclusion, HBMC are able to adhere and multiply on dense calcium carbonate and can be driven best towards osteogenesis in the presence of Dex and 1,25(OH)D3 (as regards ALP activity). Specific morphological features of cells were observed by scanning electron microscopy when cells were cultured on this material. Further experiments are needed to explain the characteristics.

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References

1. G. GUILLEMIN, J. L. PATAT, J FOURNIE and M. CHETAIL, *J. Biomed. Mater. Res.* **21** (1987) 557.
2. G. GUILLEMIN, A. MEUNIER, P. DALLANT, P. CHRISTEL, J. C. POULIQUEN and L. SEDEL, *ibid.* **23** (1989) 765.
3. M. OWEN, in "Bone and mineral research/3", Edited by W. A. Peck (Elsevier Science, Amsterdam 1985) p. 1.
4. J. T TRIFFITT, *Acta Orthop. Scand.* **58** (1987) 673.
5. J. N. BERESFORD, *Clin. Orthop. Related Res.* **240** (1989) 270.
6. J E DAVIES, R. CHERNECKY, B LOWENBERG and A SHIGA, *Cells and Materials* **1** (1991) 3.
7. C. A G. McCULLOCH and H. C TENENBAUM, *Anat. Rec.* **215** (1986) 397.6. C.
8. C. MANIATOPOULOS, J. SODEK and A. H. MELCHER, *Cell Tissue Res.* **254** (1988) 317.
9. P. S. LEBOY, J N. BERESFORD, C. DELVIN and M OWEN, *J. Cellular Physiol.* **146** (1991) 370.
10. D. C WEST, A SATTAR and S KUMAR, *Anal. Biochem.* **147** (1987) 289.