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Quantitative histomorphometric evaluation of spinal arthrodesis after biphasic calcium phosphate ceramic implantation in sheep

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The effectiveness of a macroporous biphasic calcium phosphate ceramic was studied after laterovertebral arthrodesis in sheep. A ceramic with a TCP/HAP ratio of 35/65 was compared with autogenous bone graft 1, 3, 6, 9 and 12 months after implantation. Surface, lengths, and relative pore parameters have been quantified by using light microscopy and image analysis. Quantitative analysis of the results indicated that the biphasic ceramic allows an arthrodesis after 12 months, although control graft is effective after six months. The implanted material is still present after 12 months. Degradation of the ceramic is initially fast, then slows down. The intra-implant new bone formation reaches 15% after three months and 19% after 12 months. In contrast, bone ingrowth increases from 9% after three months to 23% after 12 months in intertransverse zone. Bone formation was seen with a higher frequency in pores of 240–480 μm in average diameters. New bone fills only one part of the area of a pore. A spinal fusion can be obtained with this biphasic ceramic, but the use of this material cannot be currently recommended without further investigations.

1. Introduction

Bone grafting is frequently necessary in the practice of orthopaedics. In spinal surgery, scoliosis correction most often requires vertebral fusion. Currently, autogenous cancellous bone graft is still the most effective substance used to obtain spinal fusion. The main disadvantage of this technique is the difficulty to obtain a sufficient quantity of bone when performing a large spinal fusion. In addition removing bone for grafting, increases surgery duration, increases blood loss, and may cause complications [1, 2]. On the other hand bone allograft can be used as an alternative or supplement to autogenous bone. Meanwhile the management of a bone bank is sometimes difficult, allografts expose to the risk of bacterial and viral transmission, and the spine does not appear to be a good site for allografts [3]. Alternative materials for spinal surgery seem to be a promising field of biomaterials research.

Their similar chemical composition to the mineral part of bone and their excellent biocompatibility make the calcium phosphate ceramics good osseous substitutes [4]. To attain this end, ceramics must have some specific properties, particularly macroporosity with interconnected pores allowing bony formation within the implant, bioactivity defined by the property to induce specific biological activity [5], a sufficient physical strength and a bioresorption slower than the bony ingrowth [6–8].

Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HAP) and tricalcium orthophosphate $Ca_3(PO_4)_2$ (B-TCP) are bioactive ceramics with modulable porosity. Their degradabilities are very different: it is widely accepted that tricalcium phosphate is bioresorbable whereas degradability of HAP is very low.

Few animal studies have been done on the spine and they have not concerned osteosynthesized multidegree laterovertebral arthrodesis [8, 9, 19]. The osteogenesis and the bony resorption in these implants are still unknown and unquantified [10, 11]. Finally, no comparisons have been made between arthrodesis with ceramic implant and autologuous bone graft.

The aim of this study was to evaluate with light microscopy a biphasic ceramic (BPC) composed of 65% HAP and 35% TCP as a bone graft substitute in laterovertebral spinal arthrodesis in sheep.

A mixture of HAP and TCP combines the advantages of these two calcium phosphates: advanced exchanges between ceramic and bone, and stability with time [8]. A higher ratio of HAP than TCP seemed suitable for spinal arthrodesis which needs several months to be efficient. A high porosity has been chosen to increase cellular colonization and to facilitate bone ingrowth. A macropore size of 400–600 μm permit the onset of bone ingrowth into the areas in contact with host bone, particularly for large implants [12].

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2. Material

The biphasic ceramic used was a mixture of 65 ± 15% of HAP and 35 ± 15% of B-TCP with less than 15% of other phosphate components (pyrophosphates). Density was 24% of theoretical density. The Ca/P atomic ratio was 2 ± 0.2 with a minimal ratio equivalent to 35% of Ca and 17% of P. No fluorine, less than 100 p.p.m. of barium and less than 30 p.p.m. of heavy metals were found in the material. Porosity of the material was 400 ± 150 μm; 90% of pores measured between 100 and 1000 μm.

This biphasic ceramic was presented in parallelepipedic blocks measuring 20 × 5 × 5 mm.

3. Methods

3.1. Surgical procedure

Thirteen sheeps were implanted under general anaesthesia. After posterior access, the lumbar spine was exposed. Two lumbar segments (L3 to L5) were immobilized using Cotrel–Dubousset instrumentation [13]. The left laterovertebral groove was decorticated and two or three ceramic blocks were placed at each level close to a transverse position. In the right laterovertebral groove, autologuous graft from the right iliac crest was placed after decortication of the receiving bone. After cutaneous closing and wound healing, the sheep were returned to pasturage. No external restraint was used.

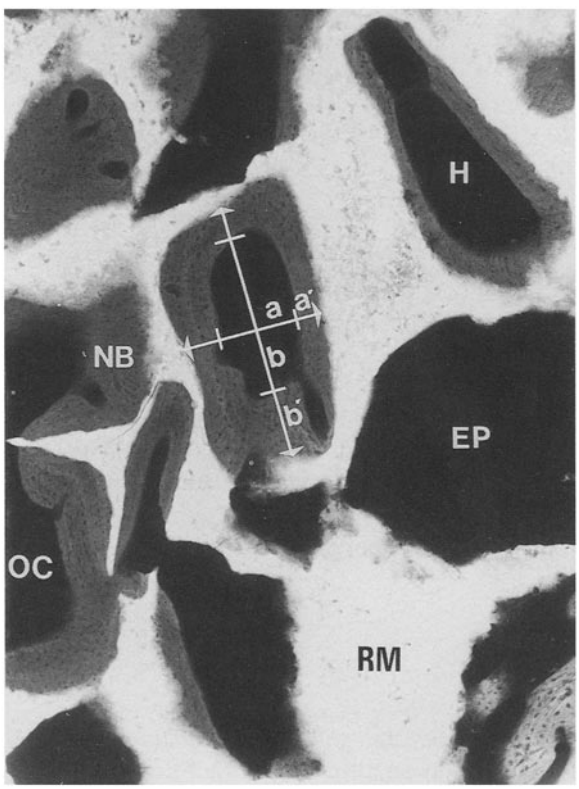

Figure 1 Microradiograph of a biphasic ceramic implanted for three months. EP, empty pore; RM, residual material; NB, newly formed bone; H, hollow (non-occupied area in the pores); OC, occupied cavity; a, b, parameters measured for hollow diameter calculation; a', b', parameters measured for pore diameter calculation.

3.2. Analytical procedure

X-rays were made before and after operation and at the times of slaughter, which were as follows: two after 1 month, four after 3 months, three after 6 months, two after 9 months and two after 12 months.

A double label was made by injection in the jugular vein of 25 mg kg^{-1} body mass of rolitetracycline (Transcycline*, Hoechst) 12 days before slaughter, and 30 mg kg^{-1} body weight of amino methyl-3 alizarine N.N diacétique (Alizarine Complexon Prolabo Paris) two days before slaughter.

After slaughter, the lumbar spine was harvested. X-rayed and cut longitudinally (anteroposterior section). The osteosynthesis material was then removed and a second X-ray of the specimen was taken before microscopic preparation.

Non implanted ceramic was also analysed and results were reported as time $T = 0$.

3.2.1. Microscopic study

Bone segments were fixed in 95% ethanol, dehydrated by graded alcohol, and embedded in methyl methacrylate without decalcification. 100 μm sections were obtained using an Isomet saw (Buelher Ltd, Evanston, Illinois) and ground further to a 50 μm thickness. Microradiographs of these sections were performed on a high-resolution emulsion S0343 by using a Machlett AEG 50 tube with a beryllium window. The exposure time was 15 mn for each section and distance between section and tube was 10 cm.

Sections used for microradiographs recording were mounted in Depex and observed under a fluorescence microscope.

Sections of 7 and 100 μm were stained with toluidine blue at pH 3.6 and with May Grünwald Giemsa.

3.2.2. Quantitative parameters

Each parameter was estimated on six sections of each implant, three on the transverse site and three on the intertransversal site at one lumbar segment (L3-L4). These parameters were measured semi-automatically on microradiographs with an image-analysing computer (IBAS 2).

3.2.2.1. Relative areas. The relative areas of internal newly formed bone (INBS), of residual implanted material and of "hollows" were evaluated. These areas were related to total implant area and expressed as a percentage (Fig. 1).

3.2.2.2. Osseous apposition on implant periphery. The lengths of free osseous appositions (appositions joining neither another implant nor the surrounding osseous tissue) were related to free edge lengths and called free osseous apposition (FOA). The lengths of inter-implant osseous appositions, related to inter-implant lengths were called inter-implant osseous apposition (IOA). The lengths of implant–receiving bone osseous appositions related to implant–receiving lengths were

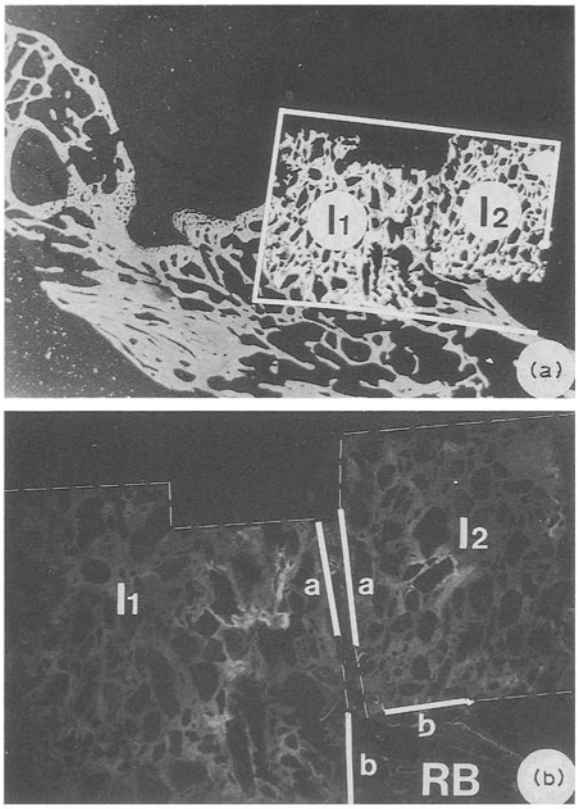

Figure 2 (a) Microradiograph of a biphasic ceramics implanted in an intratransversal site for three months. I1, enclosed implant; I2, free implant. RB, receiving bone, a = lengths of inter-implant osseous appositions, b = lengths of implant–receiving bone osseous appositions.

called implant–receiving bone osseous apposition (IROA).

Enclosed implants were distinguished from free implants distant from the receiving bone (Fig. 2a). To define active osseous appositions, fluorescence under UV light was observed. Only fluorescent zone lengths were measured (Fig. 2b).

3.2.2.3. Pore number within implant area. Occupied cavities were distinguished from empty pores and their number was expressed by an area unit (100 mm^2). The newly formed bone surface per pore is a mean obtained by the ratio of INBS to occupied cavities number.

3.2.2.4. Average diameter of pores (D_{ap}) and "hollows" (D_{ah}). Measured on microradiograph of a minimal sample of 50 pores, the diameter was measured by taking the average of longer axis and smaller perpendicular axis (Fig. 1).

$$D_{ap} = \tfrac{1}{2}[(a + a') + (b + b')], \quad D_{ah} = \tfrac{1}{2}(a + b)$$

The occupation rate of pores $(D_{ap} - D_{ah})/D_{ap}$ is the ratio of pore diameter occupied by osseous apposition.

3.2.2.5. Calcification rate. This was measured with a graticule under UV light as the distance (micrometres) between the two fluorescent lines divided by time (days) between injection of the two fluorescent compounds.

3.2.2.6. Extent of labelled zone and bone formation rate. This was measured with a graticule under UV light (Zeiss integration lines II). Simple and double labelled osseous length were distinguished. The bone formation rate was expressed by: calcification × labelled lengths.

3.2.2.7. Trabecular bone volume on autologuous graft side. On the autologuous graft side, essentially qualitative evaluation of arthrodesis was carried out.

Trabecular bone quantity obtained after complete arthrodesis (after six months) with autologuous graft was calculated (trabecular bone area divided by total bone area).

3.2.3. Qualitative analysis

Surface-stained thick sections were observed with normal light, UV light and polarized light. Different phenomena were investigated, including the inflammatory reaction, fibrous capsule around implant, remodelling of newly formed bone, cellular degradation of the implant and quality of newly formed bone (lamellar aspect, fibrous bone, mineralization).

3.2.4. Statistical analysis

For statistical calculation, comparison of standard deviations by the Fisher (variance ratio) test was performed for inter-delay study. Linear regression allowed the study of the change of parameters with time.

4. Results

4.1. Macroscopic radiology

4.1.1. Autologuous graft side

Arthrodesis begins at the third month and is completed after six months (Fig. 3 right). The trabecular bone volume is 21 ± 3%. This arthrodesis is continuous, laterovertebral at each lumbar segments (L3 to L5) and corresponds to what is required for an ideal intervertebral graft. After 12 months, there is a remodelling of the bone graft in the intertransversal zone.

4.1.2. Implant side

Implant blocks are still distinguishable six months after implantation although no radiologic arthrodesis is observed (Fig. 3 left). Some blocks appear fractured after six months. However, implant blocks are bound by osseous bridges 9 or 12 months after implantation. New bone formation does not appear to be as extensive as that observed in the autologuous bone graft sites.

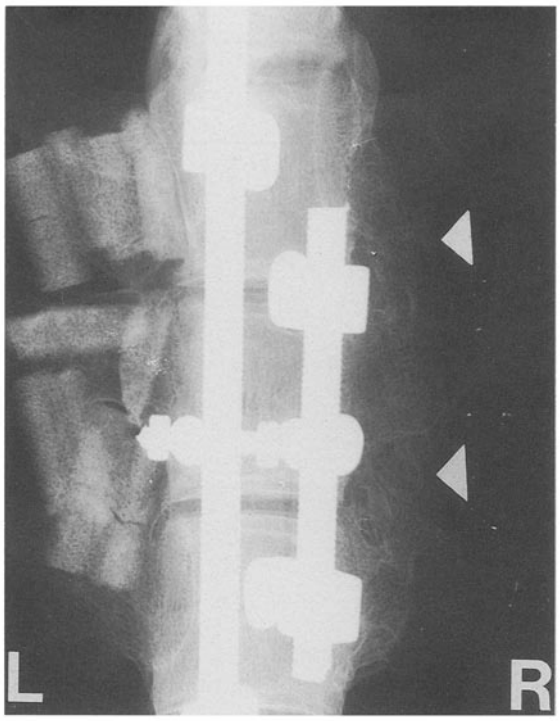

Figure 3 Radiograph of arthrodesis six months after implantation. Right (R) is the autologue graft side, left (L) is the implant side. Arrowheads point to the effective arthrodesis in intertransversal zone.

4.2. Bone apposition

4.2.1. Bone apposition within the implant (intra-implant evolution)

Evolution of the relative areas of newly formed bone is shown in Fig. 4. Replacement by new bone begins at one month and reaches a mean value of 11.9% after three months.

After three months, the evolution is different in the transverse site than in the intertransverse site. For the former, the new bone formation is stable until the twelfth month. For the latter, the new bone formation increases from 8.86% after three months to 23.22% after 12 months ($p < 0.001$). In these two sites, the bone formation is significantly correlated with time.

The percentage of occupied cavities increases with time (Fig. 5) in the transverse site. The correlation coefficient is 0.59 ($p = 0.03$). The occupation ratio of pores (72.11 $\pm$ 2.29%) and newly formed bone surface per pore (0.175 $\pm$ 0.013 mm^2 pore) do not significantly change with time, whatever the implantation site.

The distribution of diameters of empty pores and occupied cavities after 3 and 12 months is shown in Fig. 6. The occupation rate is highest for average diameters from 240 to 480 µm ($p < 0.005$).

4.2.2. Bone apposition between implants (inter-implant) evolution

The rates of inter-implant apposition (IOA), implant–receiving bone apposition (IROA), free osseous apposition (FOA) and internal newly formed bone surface (INBS) are reported in Table I.

When free and enclosed implants are not distinguished, inter-implants osseous appositions are correlated with time ($r = 0.64$; $p < 0.005$). Free osseous apposition is slower and less significantly correlated with time ($r = 0.55$; $p < 0.05$) and the 100% of free osseous apposition would be reached after 34 months.

When enclosed and free implants are distinguished, we note that IROA are significantly higher at the same time in enclosed implants than in free implants. On the other hand, the other parameters (FOA, IOA, INBS) show no significant difference between inclosed and free implants. From regression equations, we show that the internal newly formed bone surface is significantly correlated with the implant–receiving bone osseous apposition rate in enclosed implants ($r = 0.68$; $p < 0.02$) although it is not the case in free implants.

In the same way, free osseous apposition are significantly correlated with implant–receiving bone osseous appositions rate only in enclosed implants ($r = 0.66$; $p < 0.03$). We failed to find a correlation between inter-implant osseous apposition rate and implant–receiving bone osseous apposition rate.

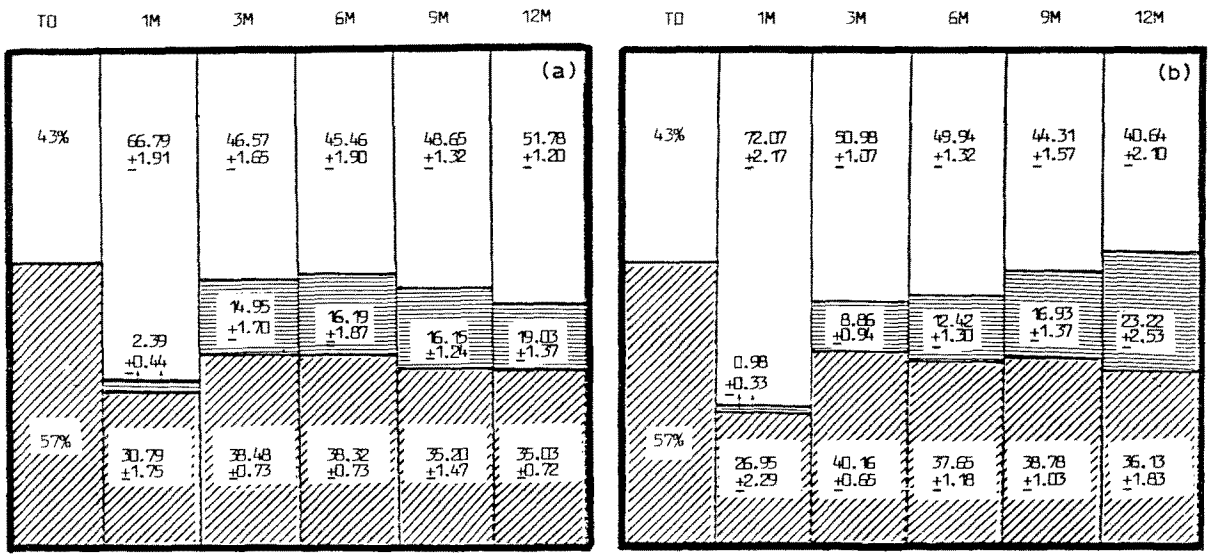

Figure 4 Evolution of the internal aspect of the implant. (a) Transversal site; (b) intertransversal site. □, Hollow; ▤, newly formed bone; ▨, residual material.

TABLE I Evolution of osseous apposition rate on implant peripheries (mean ± SD)

Time	Enclosed implant			Free implant		
	FOA[a]	IOA[b]	IROA[c]	FOA[a]	IOA[b]	IROA[c]
1 month	—	—	—	3.65 ± 2.1	1.15 ± 1.15	12.93 ± 4.51
3 months	7.86 ± 5.42	32.58 ± 15.85	60.51 ± 9.04	25.64 ± 10.48	42.50 ± 19.6	14.25 ± 5.97
6 months	54.19 ± 25.57	22.69 ± 10.33	72.94 ± 20.72	22.44 ± 9.69	31.83 ± 7.81	0
9 months	17.55 ± 13.55	17.97 ± 0	80.17 ± 8.5	16.81 ± 7.67	43.08 ± 4.88	2.18 ± 2.18
12 months	43.91 + 14.58	63.41 ± 16.84	74.21 ± 15.33	29.98 ± 16.87	43.29 + 9.95	0

[a] FOA: free osseous apposition (%).
[b] IOA: inter implant apposition (%).
[c] IROA: implant–receiving bone osseous apposition (%).

4.3. Implant resorption

4.3.1. Residual material evolution (Fig. 4)

The ceramic resorption is fast in the first month but is slowed down from the third to the twelfth month. The difference between residual implanted material percentage at one and three months can be explained by the animal variation after one month and image analysis performances.

4.3.2. Evolution of porosity

The pore number is reported on Fig. 5: the mean value is 158 ± 23 pores per 100 mm^2. The distribution of the pore size shows that they are ranged between 100 and 1000 μm (Fig. 6). At $t = 0$, the measured data are comparable with those of the manufacturer. No significant change of pore size distribution was observed with time.

4.4. Dynamic aspects

Results are shown in Table II. No significant differences are observed for the calcification rate, the extent of the labelled zone and bone formation rate, between the time periods.

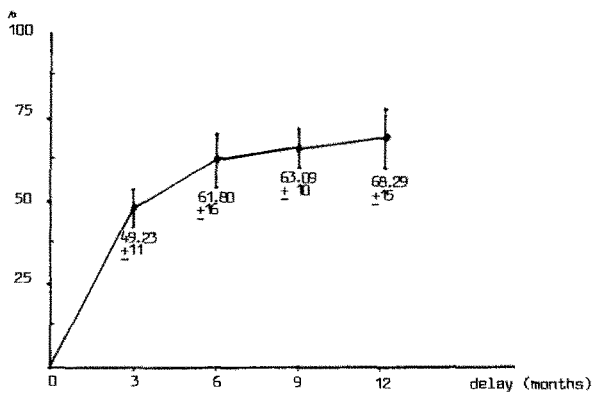

Figure 5 Ratio of occupied cavities in a 100 mm^2 area (transversal site).

4.5. Qualitative aspects

A lymphoplasmocytic proliferation was observed at the first time period and increased until 12 months. A thin and dense connective tissue was observed around implants but this connective tissue did not prevent osseous apposition. No important osteoclastic activity was observed. Some ceramic fragments were found in

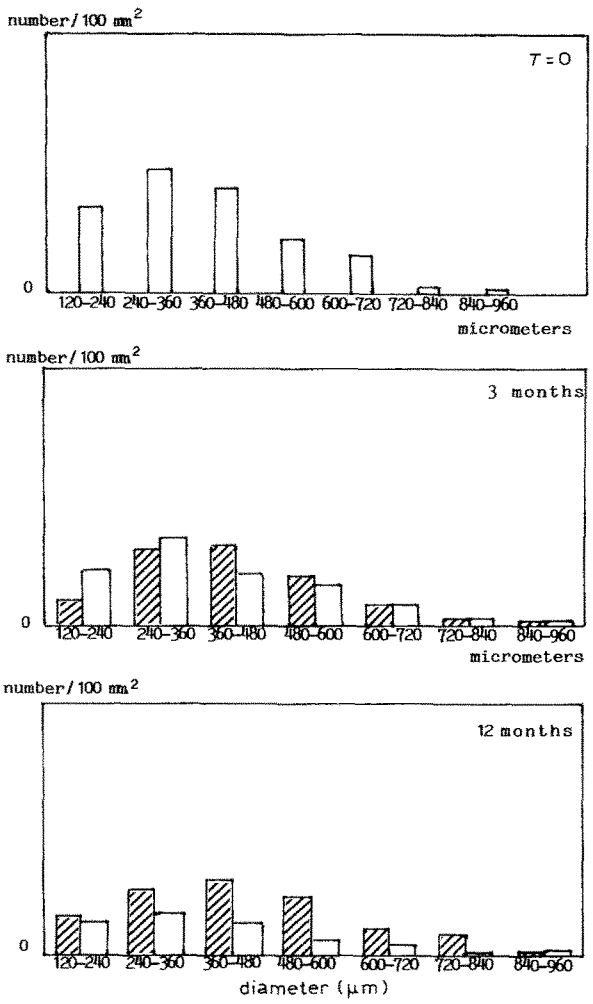

Figure 6 Distribution of diameter of empty pores and occupied cavities before and 3 and 12 months after implantation. ▨, Occupied cavities; □, empty pores.

TABLE II Dynamic aspects in fluorescence microscopy. (*T*, Transversal site; IT, intertransversal site (mean ± SD); ND, non-determined.)

Parameters		Time 1M	3M	6M	9M	12M	Significance
Extent of labelled zone[a] (μm)	T	0.315 ± 0.035	11.34 ± 1.32	10.83 ± 0.75	8.75 ± 0.45	9.44 ± 3.84	n.s.
(ELZ)	IT	0.86 ± 0.03	9.03 ± 1.78	9.99 ± 0.91	6.76 ± 2.16	15.27 ± 2.61	n.s.
Calcification Rate (μj^{-1})	T	ND	1.97 ± 0.11	2.07 ± 0.17	2.22 ± 0.19	1.83 ± 0.16	n.s.
(CR)	IT	ND	1.76 ± 0.09	2.32 ± 0.27	2.2 ± 0	2.13 ± 0.37	n.s.
Bone formation rate (BFR)	T	ND	20.05 ± 2.81	22.28 ± 2.33	19.26 ± 0.63	17.98 ± 8.63	n.s.
(BFR = ELZ × CR)	IT	ND	18.17 ± 4.4	19.96 ± 6.7	14.86 ± 4.74	31.56 ± 0.09	n.s.

[a] Simple and double label.

the cytoplasm of macrophages. After longer times, these fragments were also observed in marrow. A moth-eaten appearance of ceramic was observed after six months. This physical local change was seen without osteoclastic activity. The newly formed bone inserted near the implant shows a normal aspect. The newly formed bone within implant pores rapidly presents a lamellar configuration (after the first month) containing sometimes fibrous bone. The osseous growth occurs most often close to the ceramic but sometimes a dehiscence between bone and implant was observed. No remodelling of this bone was observed from resorption lacuna even after 12 months.

5. Discussion

Optical microscopy appears to be a good mean to quantify the *in vivo* integration of a bone substitute. In our study, we have used three different parameters: surface, lengths and relative pore parameters.

The surface parameters allow an evaluation of ceramic degradation, new bone formation, and residual cavities (hollows). By using these methods an implant resorption is observed, mainly occurring during the first three months. It is widely accepted that TCP [4, 6, 10, 14, 15] is more rapidly degraded than HAP and that resorption of biphasic ceramic increases with TCP/HAP ratio [16, 17]. Shamazaki and Mooney [14] note 46.4% of degradation for TCP after six months although Eggli *et al.* [15] report 85% of degradation. The use of radioactive TCP implants indicates a resorption rate of 25–30% in 22 weeks [10]. These findings suggest that the ceramic degradation observed during the first three months of our study may be assigned to the TCP degradation.

Bony ingrowth within the implant occurs directly without capsule formation and without primary resorption [4, 18–20]. This bone ingrowth has been rarely quantitatively evaluated but appears more important with HAP than TCP [14]. Shamazaki and Mooney [14] report more than 50% of new bone formation with corraline HAP compared with 45% with TCP after 24 weeks. Holmes and Hagler [21] report an increase of intra-implant bone ingrowth from 17% after three months to 36% after six months. In our experiment, bony ingrowth reaches 12% after three months and 23% after twelve months.

This new bone formation goes from outer to inner of the implant [14, 15, 21, 22]. Whatever the delay, bone ingrowth fills only a part of the available pore space (30% maximum) [10, 18]. Hoogendorn *et al.* [18] find that bone growth into the implant pore depends on bone-ceramic contact and conclude that the ceramic material itself does not induce osteogenesis. Inside the pores the bone turnover is very low. The absence of mechanical stimulation could explain this inactive aspect of bone.

Osseous apposition around the implant has been measured with length parameters (FOA, IOA, IROA) to evaluate the elaboration of arthrodesis. The best osseous apposition rate is obtained when considering enclosed implants. The contact of the implant with bone, the location of the implant blocks and decortication of receiving bone depend on the surgical technique. A variation in the surgical technique could explain changes in results and some surgical rules can be identified: to favour bone implant contact [19], to apply a good internal contention [16, 23], to add osteogenic factors like split bone [7, 20].

We have calculated that 17 months would be necessary for bone ingrowth to reach the middle of the implant and that 100% of inter-implant osseous apposition would be reached after 27 months (if the phenomenon is supposed to be linear).

Some authors fail to find any inflammatory reaction [19, 22], but a quiescent fibrous tissue capsule is often noted [24]. The TCP dissolution and its transformation in small particles found in macrophages is a well-known phenomenon [15, 25]. In our study, the qualitative observation of the inflammatory reaction shows a maximum lympho-plasmocytic proliferation at 9 and 12 months. However, this inflammatory

reaction, especially after the longest period of time, cannot be correlated with the TCP degradation.

In conclusion, a spinal arthrodesis is obtained after biphasic, calcium ceramic implantation. This arthrodesis happens at a later time compared with autologuous graft. Nevertheless, current strong osteosynthesis material like Cotrel–Dobousset instrumentation allows arthrodesis to take place by six months. Inside the implant bone ingrowth occurs slowly, and fills only one part of the area of the pores. This new bone seems to have inferior qualities. Material remains inside the fusion and the strength of the remaining material is probably low.

Thus this study shows that a spinal fusion can be obtained with this biphasic ceramic, but currently the use of such material is not warranted and needs further evaluation and especially biomechanical investigations.

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References

1. M. B. COVENTRY and E. M. TAPPER, Pelvic instability. A consequence of removing iliac bone for grafting. *J. Bone Joint Surg.* **A54** (1972) 83–101.
2. B. N. SUMMERS and S. M. EISENSTEIN, Donor site pain from the ilium. A complication of lumbar spine fusion. *J. Bone Joint Surg.* **B71** (1989) 677–680.
3. Y. GERARD, C. DELLOYE, D. GOUTALLIER, H. GUERIN-SURVILLE, C. HEDDE, P. HERNIGOU, D. HUTEN, B. LOTY, D. POITOUT and J. M. VITAL, Banques d'os (allogreffes). *Rev. Chir. Orthop.* **74** (1988) 109–153.
4. M. JARCHO, Calcium phosphate ceramics as hard tissue prosthetics. *Clin. Orthop.* **157** (1981) 259–278.
5. D. F. WILLIAMS, "Definitions in biomaterials" (Elsevier, Amsterdam, 1987).
6. N. PASSUTI and G. DACULSI, Céramiques en phosphate de calcium en chirurgie orthopédique. *Presse Med.* **18** (1989) 28–31.
7. A. UCHIDA, S. NADE, E. McCARTNEY and W. CHING, Bone ingrowth into three different porous ceramics implanted into the tibia of rats and rabbits. *J. Orthop. Res.* **3** (1985) 65–77.
8. G. DACULSI, N. PASSUTI, S. MARTIN, J. C. LE NIHOUANNEN, V. BRULLIARD, J. DELECRIN and B. KEREBEL, Etude comparative de céramiques bioactives en phosphate de calcium après implantation en site osseux spongieux chez le chien. Analyse histologique ultrastucturale et par microsonde électronique. *Rev. Chir. Orthop.* **75** (1989) 65–71.
9. T. SHIMA, J. T. KELLER, M. M. ALVIRA, F. H. MAYFIELD and S. B. DUNSKER, Anterior cervical discectomy and interbody fusion. *J. Neurosurg.* **51** (1979) 533–538.
10. W. RENOOIJ, H. A. HOOGENDOORN, W. J. VISSER, R. H. F. LENTFERINK, M. G. J. SCHMITZ, H. VAN IEPEREN, S. J. OLDENBURG, W. M. JANSEEN, L. M. A. AKKERMANS and P. WITTEBOL, Bioresorption of ceramic strontium-85-labeled calcium phosphate implants in dog femora. A pilot study to quantitative bioresorption of ceramic implants of hydroxyapatite and tricalcium orthophosphate *in vivo*. *Clin. Orthop.* **197** (1985) 272–285.
11. N. PASSUTI, G. DACULSI, J. M. ROGEZ, S. MARTIN and J. V. BAINVEL, Macroporous calcium phosphate ceramic performance in human spine fusion. *Clin. Orthop.* **248** (1989) 169–176.
12. G. DACULSI and N. PASSUTI, Effect of the macroporosity for osseous substitution of calcium phosphate ceramics. *Biomaterials* **11** (1989) 86–87.
13. Y. COTREL and J. DUBOUSSET, Nouvelle technique d'ostéosynthèse rachidienne segmentaire par voie postérieure. *Rev. Chir. Orthop.* **70** (1984) 489–495.
14. K. SHIMAZAKI and V. MOONEY, Comparative study of porous hydroxyapatite and tricalcium phosphate as bone substitute. *J. Orthop. Res.* **3** (1985) 301–310.
15. P. S. EGGLI, W. MULLER and R. K. SCHENK, Porous hydroxyapatite and tricalcium phosphate cylinders with two different pore size ranges implanted in the cancellous bone of rabbits. A comparative histomorphometric and histologic study of bone ingrowth and implant substitution. *Clin. Orthop.* **232** (1988) 127–138.
16. G. DACULSI, R. Z. LE GEROS, E. NERY, K. LYNCHAND and B. KEREBEL, Transformation of biphasic calcium phosphate ceramics *in vivo*: Ultrastructural and physicochemical characterisation. *J. Biomed. Mater. Res.* **23** (1989) 883–894.
17. R. Z. LE GEROS, J. R. PARSONS, G. DACULSI, F. DRIESSENS, D. LEE, J. T. LIU, S. METSGER, D. PETERSON and M. WALCKER, Significance of the porosity and physical chemistry of calcium phosphate ceramics. Biodegradation–bioresorption. *Ann. N.Y. Acad. Sci.* **523** (1988) 268–271.
18. H. A. HOOGENDOORN, W. RENOOIJ, L. M. A. AKKERMANS, W. VISSER and P. WITTEBOL, Long-term study of large ceramic implants (porous hydroxyapatite) in dog femora. *Clin. Orthop.* **187** (1984) 281–288.
19. T. J. FLATLEY, K. L. LYNCH and M. BENSON, Tissue response to implants of calcium phosphate ceramic in the rabbit spine. *Clin. Orthop.* **179** (1983) 246–252.
20. D. C. MOORE, M. W. CHAPMAN and D. MANSKE, The evaluation of a biphasic calcium phosphate ceramic for use in grafting long-bone diaphyseal defects. *J. Orthop. Res.* **5** (1987) 356–365.
21. R. E. HOLMES and H. K. HAGLER, Porous hydroxylapatite as a bone graft substitute in mandibular contour augmentation: a histometric study. *J. Oral Maxillofac. Surg.* **45** (1987) 421–429.
22. S. N. BHASKAR, J. M. BRADY, L. GETTER, M. F. GROWER and T. DRISKELL, Biodegradable ceramic implants in bone. Electron and light microscopic analysis. *Oral Surg.* **32** (1971) 336–346.
23. H. WAISBROD and H. U. GERBERSHAGEN, A pilot study of the value of ceramics for bone replacement. *Arch. Orthop. Trauma. Surg.* **105** (1986) 298–301.
24. M. JARCHO, Biomaterial aspects of calcium phosphates. Properties and applications. *Dental Clin. North Amer.* **30** (1986) 25–47.
25. C. P. A. T. KLEIN, K. DE GROOT, A. A. DRIESSEN and H. B. M. VAN DER LUBBE, Interaction of biodegradable B-whitlockite ceramics with bone tissue: an *in vivo* study. *Biomaterials* **6** (1985) 189–192.

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