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Comparison of the ossification kinetics after implantation of a radioactivated coral and a natural coral

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The Corals that have been used as biomaterials in bone surgery consist of 98% calcium carbonate in the form of aragonite. However, physical analysis methods, such as neutronic radioactivation show that the coral, after its implantation *in vivo* achieves a mineral composition comparable to that of bone. In this paper, the behaviour of a radioactive implanted sample is compared to that of a non-radioactive sample. The composition in major elements of the implant tends towards that of bone. The kinetics of ossification varies depending on whether it is a radioactive or non-radioactive implant.

1. Introduction

The studies we have previously accomplished on coral used as a biomaterial in bone surgery, enable us to determine the ossification kinetics *in vivo* [1]. We now report on more extensive *in vivo* studies on the behaviour of these materials. The behaviour of a radioactive implanted sample in comparison with a non-radioactive one has been studied. Moreover, the radioactivated samples have been useful for other studies concerning the effect of various radioactive tracers [2] with the following characteristics:

$^{44}Ca(n,\gamma)^{45}Ca^*$ with a period of 163 days: $^{45}Ca^*$ desintegration provides β-rays of 256 keV at a neutron thermal flux of 2.10^{13} n cm^{-2} s^{-1}. The dose emission is 44 mGy/day for 0.5 h duration of irradiation.

2. Materials and methods

2.1. Samples

Coral is a naturally occurring calcium carbonate based material which has been shown to possess excellent biocompatibility with respect to bone [3].

Some histological studies show that a coral fragment implanted in spongy bone is progressively replaced by newly formed bone [4, 5].

For these experiments, coral of the type Porites Lutea obtained from New Caledonia, treated and purified to eliminate microorganisms and supplied by INOTEB in France, was used. The implants were fashioned with a saw in the form of cylinders (6 mm diameter and 14 mm length) are sterilized in an autoclave at 130°C for 1 h.

Porosity is an important factor for the penetration of bone cells [6]. With a picnometer, we have calculated the voluminal mass or density of the implants of each batch. It is the same for the two series of corals, but the volume of the non-radioactive implant is 1.5 higher than the radioactive implant.

2.2. Animal experiments

Animal experiments were carried out over a period of 7 months; mature male sheep were bred in the same conditions, before and during the experiment. The coral Porites Lutea was implanted in spongy bone of the femur metaphysis (Fig. 1a, b, c).

On each of the eight experiment animals used in these experiments, a cylinder of radioactive coral of 6 mm in diameter and 14 mm in length was implanted in the right femur, and a non-radioactive cylinder for control in the left femur. The cortex was removed and not replaced and the periosteal membrane sewn up.

Animals were kept for 1, 2, 3, 5, 7, 11 or 20 weeks (two animals for the 3 week interval). Samples were located by radiology are removed with a trephine 8 mm in diameter and shaped with a small grindstone to eliminate the bone around the intermediate part (initially the coral) of 6 mm in diameter. All biopsies were kept at -20 °C.

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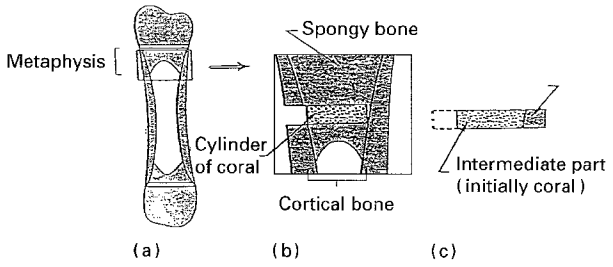

Figure 1 Implantation and sampling of biopsies in sheep.

Before any analysis, the samples were left to dry for an hour at 120 °C. The amount obtained refers to dry bone with fat not removed.

3. Physical analysis method

The analysis method, neutronic radioactivation, is based on the identification and measurement of radiations emitted by radionucleides obtained by nuclear reactions. The number of emitted radiations is proportional to the mass of the sample, which enables us to measure the amount of an element present in the sample. The advantages of this technique are its selectivity, its sensitivity, the dosage rapidity and the non-destructive character, which allows the samples to be kept intact for other physico-chemical and histological studies.

3.1. Choice of nuclear reactions

The choice of nuclear reactions to dose calcium, phosphorus, strontium and magnesium is guided by the corresponding cross-section [7], by the period of the produced radioelement and by the emitted γ ray energy [8].

We chose an irradiation duration of 0.5 h which allows us to obtain sufficient activity for all the measured radioelements in a sample, in one irradiation. The nuclear reactions used are summarized in Table I.

For the determination of the different concentrations of the studied elements, we used the following relation:

$$m_{sa} = m_{st} \cdot \frac{C_{sa}}{C_{st}} e^{-\lambda (t_{st} - t_{sa})} \quad (1)$$

where

m_{sa} = mass of element in the sample
m_{st} = mass of element in the standard
C_{sa} = count of impulses in sample element
C_{st} = count of impulses in standard element
λ = disintegration constant
t_{sa} = duration between the end of irradiation and the beginning of sample counting
t_{st} = duration between the end of irradiation and the beginning of standard counting.

3.2. Error calculation

The mass sample measurements, the mass standard measurements and the duration measurement between the end of irradiation and the beginning of counting are very accurate. We have therefore neglected errors in mass and times. The following relation is used for the error calculation:

$$\Delta\tau = \tau \sqrt{\frac{C_{sa} + 2B_{sa}}{C_{sa}^2} + \frac{C_{st} + 2B_{st}}{C_{st}^2} + 2(0.031)^2} \quad (2)$$

where

B_{sa} = background of sample measurement
B_{st} = background of standard measurement.

3.3. Experimental device

Considering the characteristics of the elements to be measured, our measurements require flux of fast neutrons. We carried out our analyses in the cyclotron of the CERI-CNRS (Orléans) with a neutronic flux of approximately 10^9 to 10^{11} n cm^{-2} s^{-1}. It is isochronal with variable energy. The fast neutrons are produced by the bombardment of a light target with charged particles. For our analyses, we used a beryllium target, because of its neutronic yield. This led us to use incident deuterons of 17.5 MeV energy, to obtain maximal neutronic flux.

After sampling for irradiation, the sample and the standards: $Ca_3(PO_4)_2$, $SrCO_3$ and MgO corresponding to each of the elements to be measured, are placed in polyethylene cylindrical sample containers and very slightly activated under a flux of neutrons. The whole (sample and standards) is held by a rod which turns continuously in front of the beryllium target, to achieve a homogeneous irradiation.

The measurement device consists of a semiconductor detector of Ge(Li) with 100 cm^3 of active volume and with a resolution of 2.5 to 3 keV, connected to a multichannel analyser with 4096 channels, and to a computer for automatic processing of the spectra.

The samples are placed in a teflon centring piece which permits the sample geometry reproductively in relation to the detector.

TABLE I Nuclear reactions

Nuclear reactions	Isotopic abundance (%)	Half-life	Cross section (mb)	Threshold energy (MeV)	γ energy, E_γ (keV)
$^{31}_{15}P(n,\alpha)^{28}_{13}Al^*$	100	2.3 min	30	2.0	1778 (100%)
$^{44}_{20}Ca(n,p)^{44}_{19}K^*$	2.1	22 min	3.5	5.4	1157 (88%)
$^{88}_{38}Sr(n,2n)^{87}_{38}Sr^*$	82.5	2.8 h	250	11.25	388 (83%)
$^{24}_{12}Mg(n,p)^{24}_{11}Na^*$	78.7	15.0 h	130	4.93	1368 (100%)

TABLE II Flow delivered by the radioactive coral versus its irradiation duration

Duration of irradiations (h)	Activity (Bq/mg)	Dose flow (mGy/day)	Dose accumulated over 30 days (Gy)
0.5	114 + 2	44 ± 6	1.2 ± 0.2
4	930 ± 40	400 ± 60	11 ± 2
5	1110 ± 40	460 ± 70	13 ± 2

4. Bone cell radiosensitivity

Among the research work [9–12] on this subject, Anderson's studies are the most appropriate to our experimentation, because the doses delivered *in vivo* to the animal are accumulated permanently over a period of 1 week to 2 months [13].

Anderson studies the survival rātio at different doses of bony cells at the level of the femur metaphysis of a mouse. Irradiations are made with an external isotopic source of Cesium 137 at a dose flow of 0.4 Gray/day. He has established that, at 12 Gray, the survival rate is about 80% for osteoblasts and 86% for osteoclasts. In our experiment, a mean dose of only 1.2 Gray per month has been used, considerably lower than the dose given by Anderson.

The more radiosensitive cells are the precursor of osteoblasts. Their mean dose varies from 1.15 Gray [13] to 1.70 Gray [14] and is defined as the ratio of radiations reducing the survival of cells.

Many parameters of our experimentation differ from the above mentioned works:

- the animal species is sheep,
- the type of irradiation is internal;
- the dose flow is weaker.

The dose measurements were carried out by thermoluminescence. These measurements of activity are brought back to the sixtieth day after irradiation of the coral because radioactive coral implantation in the ovine femurs was made towards the date.

The activities by mass-unity of the coral are measured by liquid scintillation.

Table II shows the dose flow delivered by the radioactive coral together with its irradiation duration.

According to the bilbiographic study of bony cell radiosensitivity, which limits the dose to about 1 Gray, a duration of irradiation of the coral of 0.5 h at a thermal flux of 2.10^{13} n cm^{-2} s^{-1} is appropriate to our study.

5. Results and discussion

The concentration of elements Ca, P, Mg was determined by neutronic radioactivation. They represent major elements which constitute the biological matrix. Strontium exists in large quantities in coral in the form of aragonite and stabilizes its crystalline structure [15]. Analysis of these elements allows study of the kinetics of bone replacement of radioactive and non-radioactive coral, and their calcium to phosphorus rate.

The analyses are made in the intermediate parts (initially the coral) taken from the right femur where the implant was radioactive and from the left femur where the implant was non-radioactive. The same measurements were made for the cortical bone close to each implant.

The results we have obtained are presented in Tables III and IV, corresponding to Figs 2, 3, 4 and 5.

During the first 2 weeks after implantation, the mineral composition of the intermediate part does not undergo any modification. After this latent period, there is an increase of phosphorous and magnesium and a decrease in the amounts of calcium and strontium.

There are differences between the radioactive intermediate part and the non-radioactive part:

- The supply of phosphorous and of magnesium is faster in the radioactive intermediate part. In the course of time, the difference decreases.
- The amount of strontium in the radioactive intermediate part decreases more rapidly. Twenty weeks after implantation, the non-radioactive intermediate part has reached a strontium rate similar to that of mature bone. On the other hand, in the radioactive intermediate part, there is a fixation of an amount of strontium of 0.82 mg/g which is above the normal (0.38 mg/g). This phenomenon of fixation of strontium liberated by coral was also noticed in earlier studies of a non-radioactive implanted coral [13].
- The decrease in the calcium to phosphorous rate is more regular in the non-radioactive intermediate part than in the radioactive one.

TABLE III Concentration of minerals in the radioactive intermediate parts as a function of time after implantation

Time after implantation (weeks)	Ca (mg/g)	P (mg/g)	Sr (mg/g)	Mg (mg/g)
1	355 ± 17	0.7 ± 0.1	7.0 ± 0.3	1.0 ± 0.1
2	388 ± 19	3.4 ± 0.2	7.3 ± 0.3	1.4 ± 0.1
3a	247 ± 12	39 ± 2	4.0 ± 0.2	2.6 ± 0.1
3 3b	237 ± 11	58 ± 3	2.8 ± 0.1	3.2 ± 0.2
mean	242 ± 9	49 ± 10	3.4 ± 0.6	2.9 ± 0.3
5	259 ± 13	64 ± 3	2.6 ± 0.1	3.1 ± 0.2
7	266 ± 10	94 ± 4	2.3 ± 0.1	4.4 ± 0.2
11	257 ± 13	106 ± 5	1.1 ± 0.1	4.7 ± 0.2
20	246 ± 13	114 ± 6	0.82 ± 0.05	5.0 ± 0.2

TABLE IV Concentration of minerals in the non-radioactive intermediate parts as a function of time after implantation

Time after implantation (weeks)	Ca (mg/g)	P (mg/g)	Sr (mg/g)	Mg (mg/g)
1	366 ± 18	0.8 ± 0.1	7.2 ± 0.3	1.1 ± 0.1
2	392 ± 19	1.2 ± 0.1	7.3 ± 0.3	1.3 ± 0.1
3a	349 ± 19	29 ± 2	6.5 ± 0.3	2.4 ± 0.1
3 3b	300 ± 11	3.7 ± 0.1	6.4 ± 0.2	1.2 ± 0.1
mean	325 ± 25	16 ± 12	6.5 ± 0.2	1.8 ± 0.6
5	290 ± 8	28 ± 4	5.0 ± 0.1	2.2 ± 0.2
7	274 ± 14	63 ± 3	3.3 ± 0.2	3.2 ± 0.2
11	243 ± 14	103 ± 5	0.94 ± 0.04	4.5 ± 0.2
20	253 ± 13	123 ± 6	0.46 ± 0.03	5.1 ± 0.2

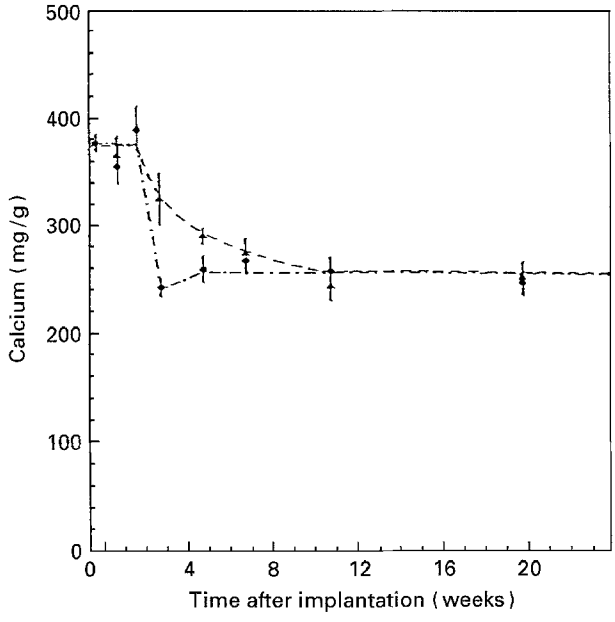

Figure 2 Amount of calcium in the intermediate parts as a function of time after implantation in sheep. • Radioactive; ▲ Non-radioactive.

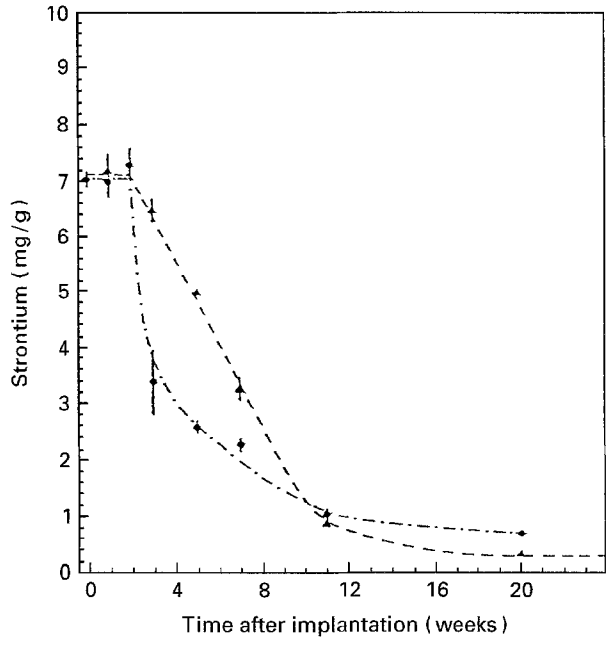

Figure 4 Amount of strontium in the intermediate parts as a function of time after implantation in sheep.

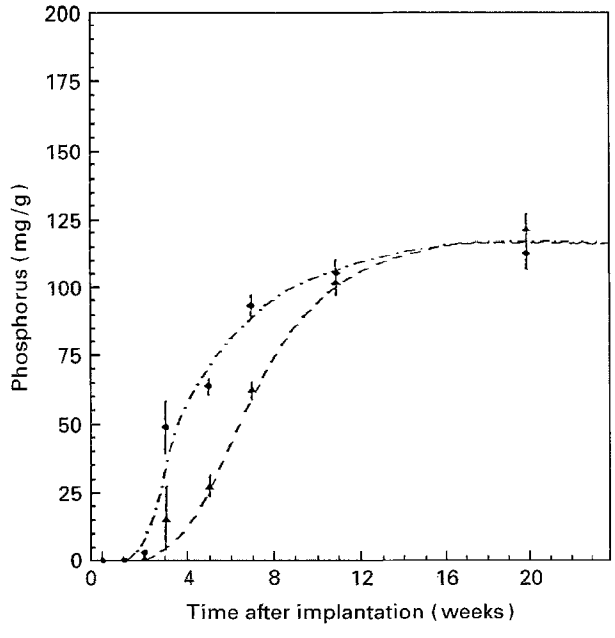

Figure 3 Amount of phosphorus in the intermediate parts as a function of time after implantation in sheep. • Radioactive; ▲ Non-radioactive.

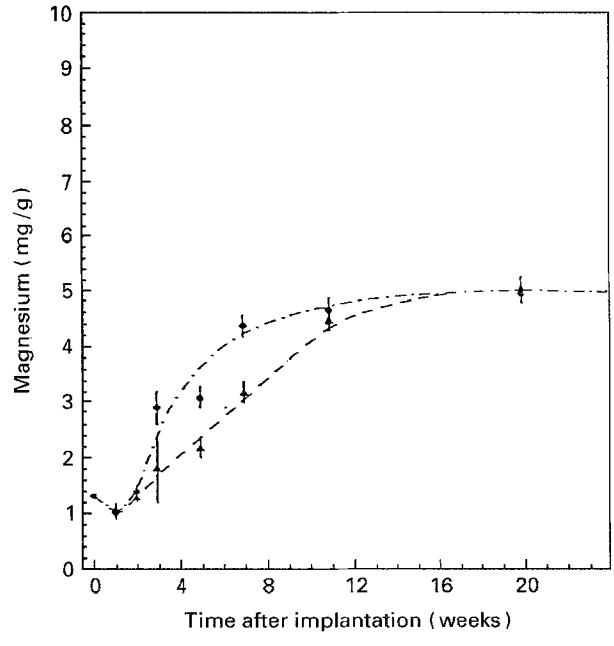

Figure 5 Amount of magnesium in the intermediate parts as a function of time after implantation in sheep.

In our previous work, we have shown that the mineral composition of natural coral, made up of calcium carbonate which crystallizes in an orthorhombic system, is progressively transformed to a mineral composition similar to that of the bone which crystallizes in a hexagonal system [16]. This is due to the calcium carbonate structure which dissolves and liberates the atoms which constitute it, after vascularization through the coral pores. A new bony mineral structure takes place. The difference of the kinetics of this bony reconstitution between non-radioactive natural coral and radioactive coral is due to one or all of following causes:

- Effects of the irradiation on the coral to be implanted: the diagram of X-ray diffraction of a fragment of irradiated coral at a very intense integrated neutronic flux (100 times higher than that used in our study) does not show any macroscopic modifications of the crystalline structure [2]. But the irradiation may create microscopic crystalline defects and then weaken the biomaterial. The consequences of bone irradiation depend on physical factors like the type of irradiation (internal or external), the debit of dose, and on biological factors such as the type of bone and animal species [17].
- Effects of the radioactivity on the bone cells: radoactivity can damage the bone cells and then alter the renewal of the bone. The results shown in other work [2] allow the toxic effect induced by radioactivity to be minimized.
- The implants of activated and non-activated coral are of the same type but do not come from the same batch.

All these factors may explain why the two series of implants (radioactive and non-radioactive) do not have the same kinetics of physico-chemical modifications. For the neighbouring cortical bone, the amounts of Ca and P are stable for the different implants. The amounts of Sr and Mg vary with the dates of sampling. These differences have repercussions on the corresponding calcium to phosphorous rate. As for the amounts of Ca and Sr, decrease in calcium to phosphorous rate is faster in the radioactive intermediate part. This ratio is stable for the corticals (Fig. 6).

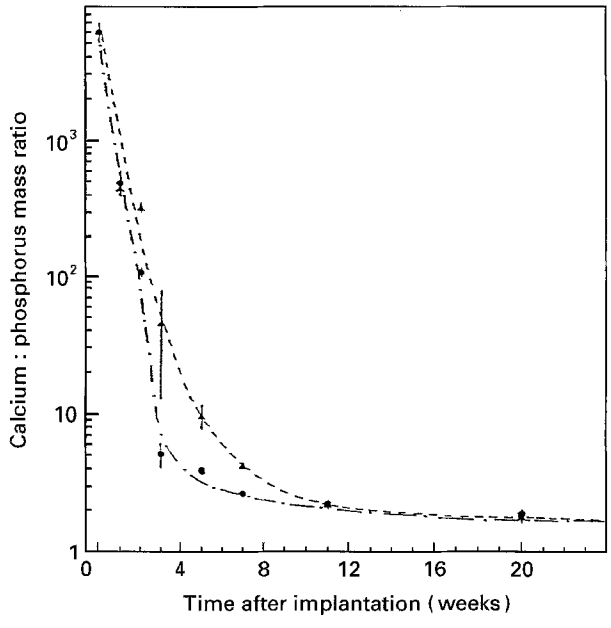

Figure 6 Calcium to phosphorus mass ratio as a function of time after implantation in the intermediate parts.

Neutronic radioactivation shows mineral element modifications of the coral during the time after implantation. The composition of the implant in terms of major elements (Ca, P, Sr and Mg) tends towards that of bone. The calcium to phosphorous mass ratio of the implant decreases from 6000 to 2.2 (normal value of a mature bone) after 5 months in spongy surrounding but the kinetics of ossification varies depending on whether it is a radioactive or a non-radioactive implant.

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