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Influence of post-implantation physico-chemical changes in a macroporous ceramic on its mechanical strength

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The aim of this study was to determine if the compressive strength of a macroporous biphasic calcium phosphate ceramic (MBCP) changed with post-implantation physico-chemical transformations, in the absence of true bone deposition. MBCP cylinders were implanted in rabbit femurs and muscles. Compressive strength measurements and Fourier transform infrared (FTIR) spectroscopy measurements were performed after 1, 2, 3, 6 and 12 weeks. MBCP blocks implanted in a human and removed after 1 week due to a clinical problem were also submitted to the same analyses. Results showed that compressive strength increased in both osseous and muscular sites. Mechanical improvement was also measured on MBCP implanted in a human. Physico-chemical changes, particularly biological apatite precipitations, were evident in MBCP implanted in muscle and in a human. This study has shown that the compressive strength of MBCP increased after implantation, even in the absence of true bone deposition. This suggests that *in vivo* physico-chemical processes could improve the mechanical properties of MBCP.

1. Introduction

Because of their similarities with bone mineral, calcium phosphate ceramics are often used as bone substitutes [1–4]. Indeed, when in contact with biological fluids, physico-chemical reactions take place, leading to precipitation of biological apatitic crystals [5, 6]. This property, known as bioactivity, makes them able to bond directly with the host bone. Furthermore when calcium phosphate ceramics are in contact with bone and when they are in macroporous form, bone invades progressively the implant. The influence of bone ingrowth on the mechanical properties of the biomaterial has already been evidenced [7, 8] but according to the present authors' knowledge, no study has reported the mechanical consequences of physico-chemical changes.

The aim of this work was to determine if physico-chemical changes participate in the post-implantation modification of the mechanical properties of a macroporous biphasic calcium phosphate (MBCP).

2. Materials and methods

2.1. Materials

The ceramic used in this study was a macroporous biphasic calcium phosphate (Zimmer, France) consisting of about 70% hydroxyapatite (HA) and 30% β-tricalcium phosphate (TCP).

2.2. Animal experiments

Mature male New Zealand rabbits weighing between 2.5 and 3 kg were used. MBCP cylinders (5 × 5 mm and 6 × 6 mm) were sterilized and implanted intramuscularly and in bone tissue. Animals were sacrificed 1, 2, 3, 6, and 12 weeks after the operation. Bone around the cylinders was removed and the implants were fixed in 80% ethyl alcohol.

2.3. MBCP blocks implanted in a human

We had the opportunity to analyse MBCP blocks implanted in a human for femoral non-union and removed after 1 week due to a clinical problem. Surgical revision was made for hematoma. Samples were fixed in 80% ethyl alcohol and submitted to the same analyses as the specimens from the animal experiment.

2.4. Compression test

Specimens were prepared and tested as described in a previous work [9]. In order to determine the implant

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strength only, all surrounding bone was removed and the implants were sawn to 5×5 mm. Intramuscular implants were cleaned of soft tissues. An experimental device was then used to fix two metallic parts to the sample extremities so as to obtain parallel surfaces. The samples were tested for compressive strength on a MTS machine. The force was applied at a crosshead speed of 0.1 mm/min. The compressive strength was calculated from the load–deformation curve by dividing the failure load by the compressed area.

After the compression test, samples were stored in 80% ethyl alcohol and pulverized in a Spex Freezer/Mill (Spex Industries Inc., USA) using liquid nitrogen and stored at $-20\,^{\circ}C$.

2.5. Physico-chemical analysis

Fourier transform infrared (FTIR) spectroscopy was performed on powdered samples (1 mg sample/300 mg of KBr) with an FTIR Bruker IFS 28, 32 Scan spectrometer. Spectra covered 4000 to 400 cm^{-1}.

3. Results

3.1. Compressive strength

Fig. 1 shows that compressive strength of MBCP, not only in a bone site but also in muscle, increased linearly with implantation time. Analysis of variance and covariance showed that the difference between the two sites was significant ($p = 0.0001$). In the MBCP implanted in a human, no bone deposition was observed on histological sections, although the compressive strength significantly increased ($p < 0.05$, U Mann Whitney test) as compared with the non-implanted MBCP (7.1 MPa versus 4.2 MPa).

3.2. Physico-chemical analysis

FTIR spectroscopy revealed physico-chemical changes in the MBCP after implantation (Fig. 2).

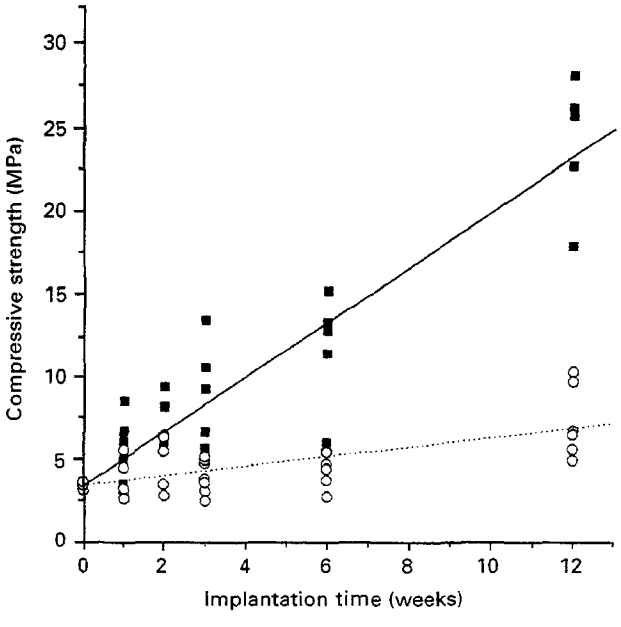

Figure 1 Compressive strength of the MBCP as a function of the implantation time at an osseous site (■) and muscle site (○).

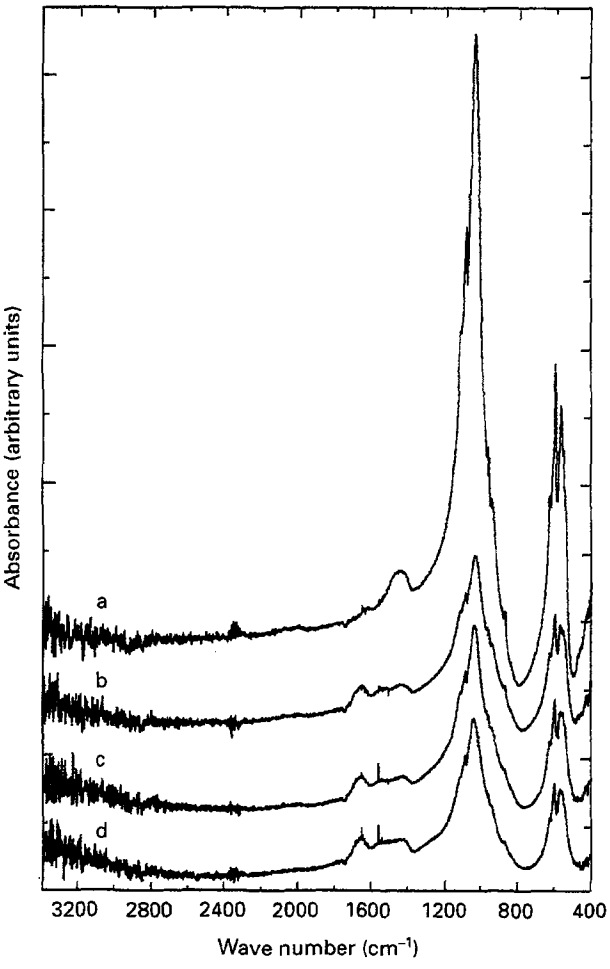

Figure 2 FTIR spectra of the MBCP (a) before and after (b) 1 week, (c) 3 weeks, (d) 6 weeks implantation in muscle.

Additional absorption peaks were identified as in previous studies [10–12]. New compounds were assigned to organic phases (particularly collagen, around 1660 cm^{-1} and above 3000 cm^{-1}). Moreover, carbonate ions (located at 1450 cm^{-1} and 1540 cm^{-1}) and an increase in the water content (over 3000 cm^{-1}) were found after implantation in both muscular and bone sites. In the MBCP implanted in a human, the same physico-chemical changes were found by FTIR spectroscopy (Fig. 3).

4. Discussion

Calcium phosphate ceramics are widely used as bone graft substitutes for filling bone defects because of their similar chemical composition to the mineral part of bone and their excellent biocompatibility [1–4]. They undergo a resorption/bone substitution process, particularly when they are macroporous, and form a strong bond with the host bone. This property is known as bioactivity and has been described by several authors [13, 14]. Bioactivity includes biodegradation/dissolution of the ceramic and biological apatite precipitation. Many factors influence the degradation/dissolution property: physical form, composition and crystallinity. The process of degradation/dissolution results in physical changes (loss of mechanical strength) and chemical changes (pH reduction in the implant environment causing notably

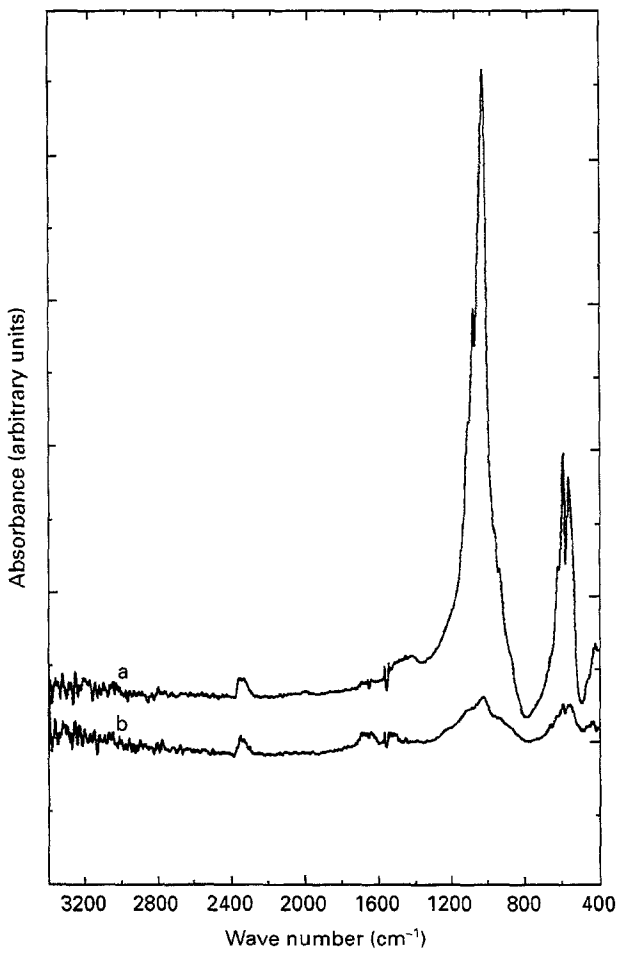

Figure 3 FTIR spectra of the MBCP (a) before and (b) after 1 week implantation in a human.

partial dissolution of the material). Dissolution provokes an elevation of the calcium and phosphate ion concentrations in the microenvironment, leading to the precipitation of a biological apatitic phase. These new microcrystals were evidenced regardless of the site of implantation (osseous or non-osseous) [6, 15]. However, no work has clearly demonstrated the presence of "true bone" in a non-osseous site and thus the osteoinductivity of calcium phosphates. Differences in features between implants from osseous and non-osseous sites are greater abundance of the new crystals in implants from the bone site and absence of mineralized collagen in implants from non-osseous sites [6].

To determine if physico-chemical changes participate in the modification of the mechanical properties of macroporous biphasic calcium phosphate, cylinders were implanted in a bone site (rabbit femurs) where physico-chemical changes and bone ingrowth take place, and in muscle where only physico-chemical transformations occurs. The presence of a new calcium phosphate phase and an organic matrix was evidenced by FTIR spectroscopy in both osseous and non-osseous site. This new mineral phase was assumed to be bone apatite-like crystals. At the same time compressive strength of MBCP, not only in bone but also in muscle, increased linearly with implantation duration. Mechanical improvement of the MBCP implanted in a human was also demonstrated simultaneously with biological apatite precipitation. Factors that might explain this enhancement are microporosity decrease and precipitation of needle-like crystals, but TEM and histological observations still have to be done to check that carbonated apatite precipitates are the major changes in MBCP after implantation in muscle.

5. Conclusions

This study has shown that the compressive strength of MBCP increased when implanted, even in the absence of true bone deposition. This suggests that physico-chemical processes, particularly biological apatite deposition, could improve the mechanical property of MBCP *in vivo*.

Acknowledgements

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