SODIUM FLUORIDE SUSTAINED-RELEASE BONE CEMENT : AN EXPERIMENTAL STUDY IN VITRO AND IN VIVO

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A new "low temperature" bone cement, consisting of polymethylmethacrylate (PMMA) and 6% sodium fluoride, was developed for use in orthopedics.
Fluoride is a well-known agent that may stimulate osteoblast activity and differentiation in vitro and in vivo ; for this reason fluoride has been used for 30 years in the treatment of osteoporotic diseases of the bone. A local effect obtained with a slow release of fluoride from bone cement at the interface between bone and prosthetic implants could potentially enhance new bone formation around the prosthesis. This material was investigated both "in vitro", by establishing the kinetics of fluoride release from the acrylic bone cement and its maximal compressive strength, and "in vivo", by fitting 24 rabbits, which were then killed after 4, 12 and 16 weeks, with femoral implants, following labelling with fluorescent stains to allow the histologic evaluation of bone remodelling.
The "in vitro" study revealed the release kinetics of fluoride from bone cement and the compressive strength of PMMA, that is not affected by the addition of fluoride. The "in vivo" investigation showed considerable healing ability after surgical and heat trauma, and new bone formation that appears larger on the surface in contact with fluoridated cement.

Keywords : bone cement ; fluoride.
Mots-clés : ciment à os ; fluorure.

INTRODUCTION

Among the major problems seen in clinical practice following prosthetic implantation is aseptic loosening of implants (5, 11), in which bone resorption around the prosthesis represents the most evident feature ; this complication is thought to be caused by biomechanical and biological factors.

A number of different approaches are taken to prevent or minimize this loosening, including the development of a system consisting of an acrylic bone cement with high mechanical strength and low polymerization heat, below the threshold of thermal damage to tissue proteins, which releases fluoride to the bone tissue (F-).

The ability of fluoride to promote bone densification is of a dual nature : *biochemical* : the fluoride rapidly becomes part of the bone hydroxyapatite mineral structure, thus forming fluoroapatite, which displays improved mechanical and biochemical properties (2, 18, 22) ; and *biological* : the fluoride ion exerts a direct action on osteoblasts, promoting their differentiation leading first to an enlarged osteoid wall of bone trabeculae, and in particular of trabecular bone, and subsequently to an increased volume of trabeculae (24). These effects, if locally achieved by slow release of fluoride from bone cement, should be very useful in the prevention of peri-prosthetic bone resorption.

The aim of our investigation was to test a controlled fluoride-releasing acrylic bone cement including its mechanical and physical properties,

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and the effects of experimental implantation of this cement in rabbits will be discussed.

MATERIALS AND METHODS

A polymethylmethacrylate (PMMA) bone cement, as is widely used in orthopedics, was investigated. A commercially available product was used (CEMEX — TECRES S.p.A.). Sodium fluoride in a proportion of 6% was then added to the bone cement.

Sodium fluoride in crystal form is added to the cement powder prior to polymerization ; it does not take part in this reaction, but remains dispersed, in its unchanged form, in the final polymer structure. Sodium fluoride molecules, however, display ionic bonds and are therefore highly soluble if exposed to a saline solution, where they release active F- ions.

The bone cement used shows a powder-to-liquid ratio of 3 : 1, the proportion of liquid monomer being smaller by one third than the cements exhibiting a "conventional" 2 : 1 ratio. This allows a proportional decrease in the amount of heat release and monomer leakage during polymerization.

A. **In vitro tests**

1. *Release kinetics*

A standard 40-g package of bone cement was used for testing the in vitro fluoride release. The total amount was divided into two 20-g doses ; 1.2 g (6%) sodium fluoride, mixed into the basic polymer, was added to each dose.

The grain size distributions of the sodium fluoride and the basic polymer powder employed in this study, tested according to the Standard DIN 4188, are reported in table I, and show no significant differences.

Table I. — Grain size distribution of NaF and PMMA powders employed

Powder size	NaF	PMMA
> 0.090 mm	12.6%	1.5%
> 0.063 mm	20.9%	19.8%
> 0.036 mm	52.5%	65.6%
< 0.036 mm	10.23%	13.1%

The powders thus obtained were polymerized, then 10 specimens for each cement were prepared according to the Standard ISO 5833/1 for mechanical strength tests (27), as will be discussed below.

Approximately 10 g by weight of each cement was set aside and used for sodium fluoride release tests.

Cement fluoride-ion release was tested by repeatedly determining its concentration in a saline solution, in which the cement specimen had been immersed at the time of polymerization.

Fluorometric dosages were determined using a potentiometer (ORION — U.S.A. — model 720) with a fluoride-specific electrode (ORION — U.S.A. — 94-09). Using a YEV 3021 recorder, recordings were made continuously for the first 3 hours, then at regular intervals (24 hours) for the next 30 days after specimen preparation.

The experimental conditions were the following :

— specimen weight : 13.660 mg
— specimen surface area : 33.94 cm^2
— theoretical sodium fluoride content : 820 mg
— solution temperature : 37° C ± 1.

2. *Mechanical strength tests*

The cylindrical specimens, prepared as described above (Standard ISO 5833/1), were subjected to maximal compressive strength tests. This type of test is actually considered to be indicative of the cement mantle strength of a prosthesis implanted in humans under physiological working conditions.

Tests were performed on a first group of 5 specimens 24 hours after their preparation and repeated on a second group of 5 specimens which had been incubated at 37° C for 30 days in Ringer solution. Tests were conducted on two groups of 5 specimens each, both for the fluoridated cement and for the conventional control cement ; the mean of the 5 values was calculated along with its standard deviation.

B. **In vivo study**

The in vivo study was conducted on a series of 24 New Zealand White rabbits, approximately 3 kg in weight, divided into two groups. Under general anesthesia they received an unloaded polymethylmethacrylate femoral implant, using a low-polymerization temperature cement (CEMEX — TECRES S.p.A.) and the same cement fluoridated as described above.

The cement was injected into the femoral proximal metaphysis by means of a syringe system without pressurization (fig. 1). At the same time the maximum temperature on the cement mantle during polymerization was recorded by a thermocouple device.

Each group was then divided into 3 subgroups, which were to be killed after 4, 12 and 16 weeks.

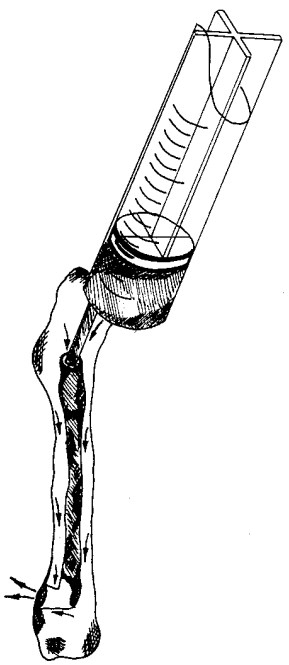

Fig. 1. — Detail of surgical procedure during the injection of polymethylmetacrylate.

Table II. — Labelling sequence in the postoperative period

Group	Label	Week(s)
4 weeks	XO	1
	CG	2
	TC	3
12 weeks	XO	4-5
	CG	7-8
	TC	10-11
16 weeks	XO	1-2-10-11
	CG	12-13
	TC	14-15

Labelling sequence.
XO : Xylenol orange ; CG : Calcein green ; TC : Tetracycline.

Postoperatively a multiple labelling procedure was carried out using three different stains : xylenol orange, calcein green and tetracycline, at the times given for each group in table II in preparation for ultraviolet light bone-remodelling histology.

After the animals were killed, the specimens were analyzed by standard x rays and then mounted for histology using two different types of embedding, i.e. they were embedded in 1) conventional methacrylate, which has the disadvantage of dissolving acrylic cement and 2) araldite (6, 17), which preserves PMMA unchanged and allows analysis of the cement-bone interface both by polarized and UV-light through the fluorescent enhancement of the labelled bands (20, 21).

Sections, including the PMMA fit, have been cut in all cases exactly perpendicular to the axis of the femur, so that similar cross sections of appositional bone surfaces close to the cement could be observed.

A histologic examination of the endosteal areas in direct contact with the implants was always carried out at the same time as the periosteal areas.

RESULTS

A. In vitro tests

1. *Kinetics of fluoride release by acrylic cement*

The values measured by fluorometric analysis up to 30 days after specimen preparation are given in fig. 2. These findings reveal an immediate, remarkable fluoride release in the first 24 hours (2.8 mg in our specimen) ; after this, the release rate slows down, while acquiring a more regular pattern ; after 10 days 1.6 mg had been released with a mean value of 0.16 mg/day. On the 22nd day the mean daily release was down to 0.08 mg. After 30 days the daily release rate was 0.075 mg. In our specimen, with a surface area of about 34 cm^2, fluoride release was equal to :

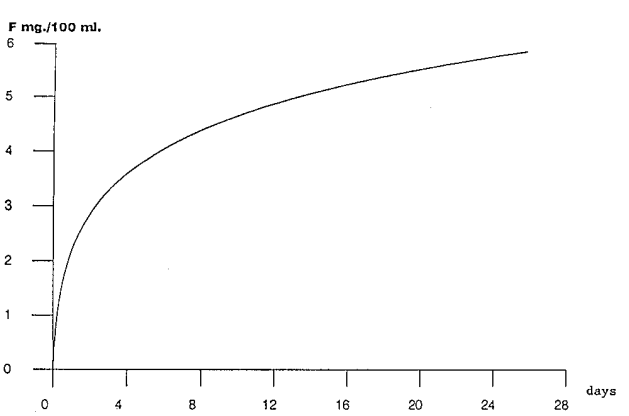

Fig. 2. — Fluoride release from fluoridated bone cement (mg/100 ml) recorded up to 30 days (for experimental conditions : see text).

— 0.082 mg/cm^2 : in the first 24 hours
— 0.0047 mg/cm^2/day : in the next 10 days
— 0.0024 mg/cm^2/day : in the next 10 days
— 0.0022 mg/cm^2/day : after 8 more days.

The amount of cement and the size of the bone-cement interface of an orthopedic prosthetic implant are known to vary considerably. For instance, the femoral site of a hip replacement can be expected to cover an average surface area of about 200 cm^2.

2. *Compressive strength tests* (Standard ISO 5833/1)

The following results were obtained (mean value ± SD) :

Nonfluoridated conventional cement

24 hours	107 ± 14 Mpa
30 days	111 ± 6 Mpa

Fluoridated cement

24 hours	110 ± 18 Mpa
30 days	113 ± 8 Mpa

B. In vivo study

Temperature

In vivo recordings of the polymerization temperature showed no significant differences between the two groups in which the same polymer had been used. In particular, the following polymerization temperatures were recorded on the cement surface :

— nonfluoridated conventional cement 43.1 ± 3.5°C
— fluoridated cement 41.9 ± 2.8°C

X ray examination

Conventional x rays of the harvested femurs showed no difference between the two types of implant to which no contrast medium had been added.

Histologic findings

1. Nonfluoridated conventional cement

As far as araldite-embedded specimens were concerned, a close bone-cement interface, with no interpositions, could be seen in most of the examined surfaces ; postoperatively-labelled areas were clearly visible, in close proximity to the cement, in all specimens. As is shown in fig. 3, in araldite-embedded specimens the fit of the cement-bone interface can be seen both under polarized and UV light. The fluorescent bands, which are indicative of new bone formation as early as the first week, are clearly visible under UV light. Some sections show thin fibrous tissue layers, thus proving that they do form even in unloaded implants, albeit in very limited areas (7).

The observation of the labelled bands under UV light made it possible to evaluate bone remodelling and new bone formation postoperatively by comparison between the endosteum, next to the cement, and the corresponding periosteum (fig. 4a, b).

2. Fluoridated cements

The histologic examination of the specimens mounted using the two techniques, as regards the femoral samples with fluoridated cement, led to the following conclusions :

— There is no difference in cement-bone bonding, fibrous tissue interposition and new bone-forming ability.
— A small amount of osteoid tissue can be seen, in methacrylate-embedded specimens, on the surfaces against cement ; however the amount of this osteoid is simiiar to that found in contact with the conventional, nonfluoridated cement.
— Under UV-light examination, a sequence of clearcut, sharply defined labelled patches with unblurred staining and no spreading to the surrounding layers can be seen in the specimens with fluoridated cement, and these are indicative of better organized, newly formed bone tissue.
— In addition a greater distance between labelled areas seems to be evident in the samples fitted

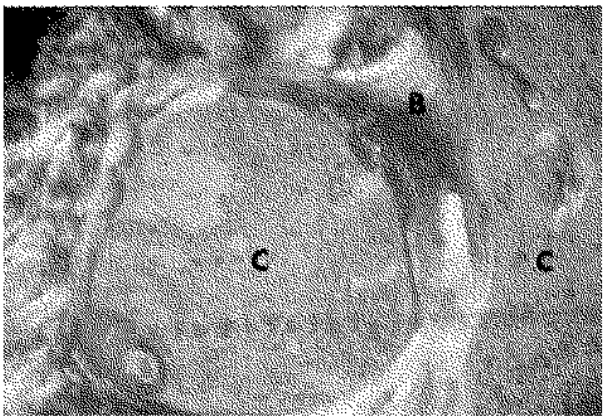

Fig. 3a

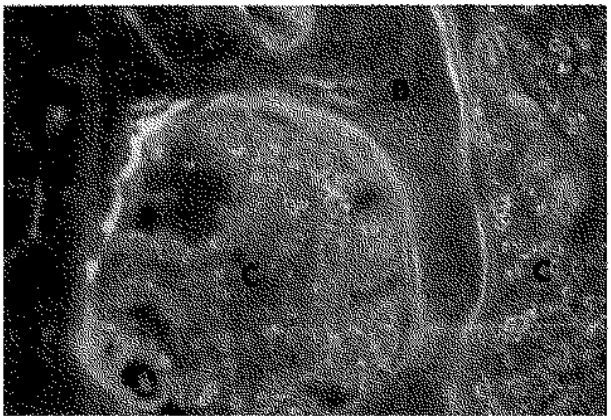

Fig. 3b

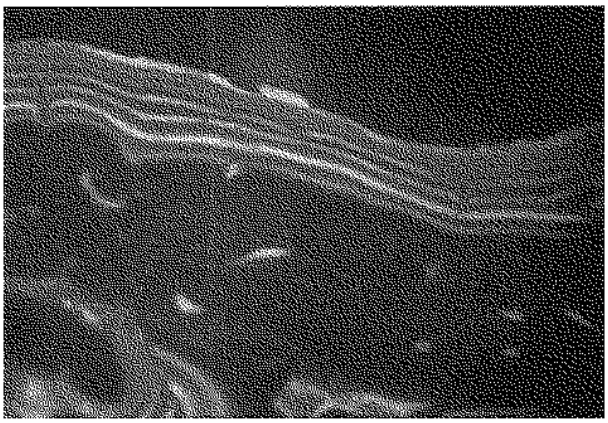

Fig. 4a

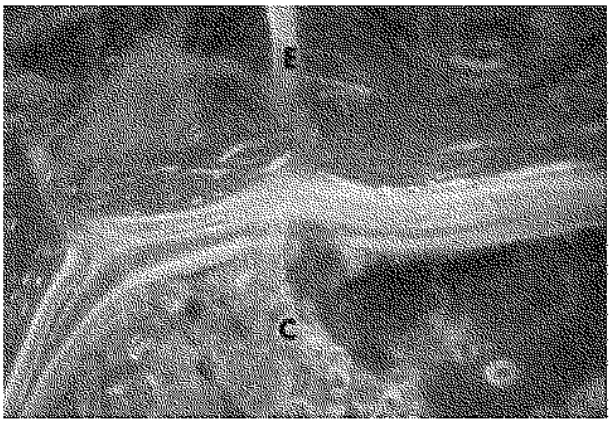

Fig. 4b

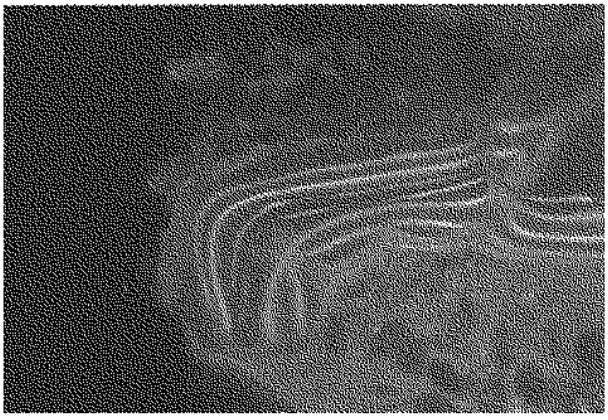

Fig. 5a

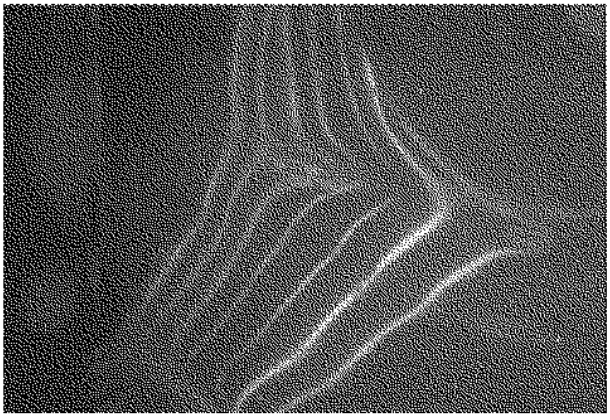

Fig. 5b

with fluoridated cement, up to about three times larger than the conventional cement controls (fig. 5).

DISCUSSION

Bone cement proved to be an appropriate medium for fluoride uptake and subsequent release, the latter occurring according to a well-defined, controllable and reproducible pattern, capable of maintaining local concentrations within the desired therapeutic range (0.21-0.4% in dry bone) (9).

The amount of released fluoride is directly correlated with the total amount present in the cement, the magnitude of the release interface and the temperature (1, 13) : in our experimental model a release of about 6 mg of fluoride ions was obtained in a 30-day period, equal to about 1.5% by weight of the total fluoride content. This procedure does not appear to involve any risk of uncontrolled or massive release, which might cause acute toxicity in an in vivo situation. An additive may, however, affect the polymerization of polymeric resins and bring about changes in the polymer spatial arrangement ; this possibility must be verified and quantitatively evaluated by mechanical strength testing.

It is reported in the literature (15, 19) that antibiotic-enriched cements may exhibit a 5 to 10% decrease in the PMMA strength ; these fluctuations, though, lie within the acceptable tolerance limits of the mean values of PMMA mechanical properties.

In this study adding sodium fluoride to the acrylic cement did not alter its mechanical strength features on compression testing. This finding, which is in partial agreement with previous findings reported (19) concerning the addition of antibiotics, may be explained by an appropriate grain size of the added NaF, which is dispersed in the polymer powder-presenting particles of very similar dimension and shape (spheric) to the polymer grains resulting in a high-density final polymer structure.

The clinical and experimental results reported in the literature confirm the existence of a direct and specific fluoride action (16, 26) on the cell populations of osteoblasts and osteoclasts. This effect is consistently seen at least in the early stages of exposure (25) and manifests itself through morphological and functional cell changes which are indicative of increased activity in the existing metabolic units.

The most distinguishing histomorphometric changes concerned the trabecular component (3, 4, 8, 10, 12) and were reflected in an increased total trabecular volume, both in mineralized and in the nonmineralized portion. An overwhelming proportion of the nonmineralized is still the sequel most feared by clinical investigators, since an excess of osteoid tissue may thwart the therapeutic goals or even increase the hazard of fractures of the long bones, particularly of the hip.

The findings in clinical studies, however, appear to bear out Kanis and Meunier's opinion (12), i.e. that such histomorphometric changes are closely related to the local concentration of fluoride

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Fig. 3. — Endosteal detail of a sample fitted with a standard cement implant after 4 weeks ; a cement fragment is surrounded by newly formed bone tissue, observed under polarized (a) and UV light (b) (× 300), (B-Bone ; C-Cement).

Fig. 4. — A) Periosteum of an araldite-embedded sample (12 weeks after surgery) observed under UV light : a double xylenol orange (orange), a double calcein green (green) and a double tetracyline (yellow) labelling band are clearly visible (× 200). B) The corresponding endosteum (E) in contact with the cement (C) showing the same labelling sequence as the periosteum (× 200).

Fig. 5. — Endosteal details of samples fitted with fluoridated cement implants, 16 weeks after surgery. seen under UV light after araldite embedding.
Labelling bands are extremely well defined and a greater distance between them than in samples with standard cement implants seems to be evident (A — × 200 ; B — × 300).

depending on daily dose and, secondarily, on the length of treatment.

The histomorphometric findings are confirmed by an increased experimental compressive strength of the rat vertebrae and long bones (16, 23). The dissimilarities between the experimental models do not allow us to define conclusively the mechanical properties of bone tissue after fluoridation, even though it is certain that a modified mineral structure, fluoropatite, is formed (14).

Although experimental compression tests of trabecular bone showed increased strength following exposure and Rich reported increased strength also in rat long bones with a predominant cortical component, no such increase was seen in terms of bending strength, which in a rat investigation (16) was indeed shown to decrease in the short-term. Compressive strength appears proportional to the fluoride amount in tested trabecular bone, which, in clinical practice, may essentially depend on the duration of treatment.

The present study in vivo showed a very close bone-cement interface, with no interpositions, in the tested unloaded implants and proved that bone remodelling and ingrowth do occur on the cement surface after surgical and thermal trauma ; in our model a greater bone formation seems to be present on the surface of fluoridated cement, likely resulting from osteoblast activation and resultant increased bone matrix production.

In addition no disproportionate osteoid increase was seen in our samples up to 16 weeks after implantation ; this observation should ensure that no implant will loosen due to an excess of non-mineralized bone caused by fluoride, as, in oral treatment, excess osteoid tissue is typically found in the early stages and at high doses (3, 10, 12).

At the present stage, however, the mechanical features of the bone surrounding a fluoridated-cemented implant in humans is not predictable, needing further experimental studies and clinical applications over the time. Available data suggest a well-defined correlation between bone structure and local amount of fluoride (9, 12, 14), that appears to be safely affected with local administration by drug delivery systems.

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SAMENVATTING

B. MAGNAN, C. GABBI, D. REGIS. Botcement met natriumfluoride. Experimentele studie in vitro en in vivo.

Een nieuw "lage temperatuur" botcement, bestaande uit polymethylmethacrylaat (PMMA) en 6% natriumfluoride werd ontwikkeld voor gebruik in orthopedische heelkunde.
Het is gekend dat fluoride "in vitro" en "in vivo" de osteoblastische aktiviteit en differentiatie kan stimuleren : sedert 30 jaar werd fluoride dan ook gebruikt bij de behandeling van osteoporose. Een lokaal effekt, bekomen met het traag vrijkomen van fluoride uit botcement t.h.v. de interface tussen bot en prothetische implantaten, zou potentieel nieuwe botformatie om de prothese kunnen begunstigen. Dit materiaal werd "in vitro" onderzocht met bepaling van de snelheid van loskomen van fluoride uit het botcement en studie van de biomechanische eigenschappen ; "in vivo" werden femorale implantaten, na markering met fluorescente kleuren, ter histologische evaluatie van de botformatie, bij 24 konijnen geplaatst ; de dieren werden gesacrifieerd na 4, 12 en 16 weken. De "in vitro" studie bezorgde gegevens over de snelheid van loskomen van fluoride uit het botcement en de mechanische eigenschappen van PMMA, die door het aanbrengen van fluoride niet beïnvloëd worden. De investigatie "in vivo" toonde een hoge genezingscapaciteit na chirurgisch en thermisch trauma. De botformatie was vrij belangrijk op de oppervlakten die met fluoride cement in kontakt waren.

RÉSUMÉ

B. MAGNAN, C. GABBI, D. REGIS. Ciment à os au fluorure de sodium. Étude expérimentale in vitro et in vivo.

Un nouveau ciment à os "basse température" composé de polyméthylméthacrylate (PMMA) et de 6% de fluorure de sodium fut mis au point pour l'orthopédie. Le fluorure de sodium est un stimulateur bien connu de l'activité ostéoblastique et de la différentiation in vitro et in vivo ; cette propriété l'a fait employer depuis 30 ans dans le traitement de l'ostéoporose. Un effet local obtenu par une libération lente du fluorure à l'interface du ciment et de l'implant prothétique pourrait, semble-t-il, favoriser la formation d'os nouveau autour de la prothèse. Ce matériel a été expérimenté "in vitro", en définissant la cinétique de la libération de fluorure au départ du ciment acrylique et ses caractéristiques biomécaniques et également "in vivo" en plaçant chez 24 lapins des implants fémoraux après marquage à l'aide de colorants fluorescents pour permettre l'étude histologique du remodelage osseux ; les lapins furent sacrifiés à 4, 12 et 16 semaines.
L'étude "in vitro" documenta la cinétique de libération du fluorure au départ du ciment osseux et définit les propriétés biomécaniques de ce PMMA qui ne sont pas altérées par l'addition de fluorure. Les recherches "in vivo" montrèrent une importante capacité de guérison après traumatisme chirurgical ou thermique. La quantité d'os qui apparaît sur les surfaces en contact avec le ciment fluorescent est nettement plus importante.

EDITORIAL NOTE

The methylmethacrylate cement has been one of the most ancient drug delivery systems and its value as an antibiotic carrier has been widely recognized. The above paper is another original application of such a system in attempting to improve osteogenesis through the sustained release of fluoride ions. The authors showed with multilabeled cross-sections the apparent mineral apposition rate was greater with fluoride.

However, to confirm such apparent results, additional studies are necessary such as morphometrical investigations of strictly perpendicular sections with statistical analysis.

It is the only method to validate these results.