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Injectable percutaneous bone biomaterials: an experimental study in a rabbit model

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New percutaneous filling techniques are beginning to be used in bone tumour pathology, because they are less aggressive than surgery. The purpose of this study was to test percutaneous injectable bone biomaterials with a reproducible model. A closed cancellous bone defect was created in the distal femoral extremities of 34 rabbits. Filling was done by a percutaneous injection made on the medial side, after the defect had been hollowed by a lateral approach. Three situations were tested: unfilled, filled with orthopaedic cement, filled with a soft collagen–hydroxyapatite material. Three time intervals (2, 4 and 8 weeks) were tested for the three situations. For the mixed hydroxyapatite–collagen material, each component was tested separately: the injectable collagen, and the hydroxyapatite powder. The quality of the defect was assessed by the variations of the defect area on sagittal sections. Bone formation in the defect was quantified for each group and time interval. A bone defect of reproducible size was obtained. Evolution of bone formation was different in each group. An unfilled defect was never completely filled by bone and the defect bone formation rate stayed between 9.9% and 15.1% without any statistical difference between the time intervals. The percutaneous injection of orthopaedic cement, which was progressive, was less complete than an opened surgical filling and explained a frequent low peripheral bone formation. A lower bone formation rate was observed in all the filled groups (orthopaedic cement, hydroxyapatite–collagen, hydroxyapatite and collagen) than in the control unfilled group. This study showed the use of bone biomaterials by an injectable percutaneous method, and a model to evaluate these materials is proposed.

1. Introduction

Biomaterials have a growing place in bone pathology, especially in orthopaedic surgery. New percutaneous techniques, which are less aggressive than classical surgical methods, have been developed in recent years in radiology and in spine pathology.

The percutaneous injection of biomaterials for filling bone defects is being reported more and more in the medical literature [1]: acrylic cementation by the percutaneous route is used in the treatment of spinal angiomas [2] and vertebral metastases [3], or for filling bone cysts [4]. The development of new bone cements such as bioactive phospho-calcic cements which can be used in liquid or paste form and injected before hardening, has opened new therapeutic possibilities for several bone diseases [5, 6].

No adequate experimental model was found in the literature, which would allow assessment of the induced bone ingrowth within a bone defect and where percutaneous materials could be injected in a reproducible manner.

Two preliminary conditions were noted: on the one hand, the bone defect might be located in an accessible site for injection and on the other hand, surgical procedures and material injection might be reproducible. An experimental bone defect model has been previously developed by Katthagen [7]. A bone defect was made in the rabbit distal femoral metaphysis, but remained open, and did not allow the use of injectable liquid biomaterials.

To test these injectable applications, the model of bone defect described by Katthagen [7] was modified by creation of a lateral square bone window to close the defect and controlateral injection of material in the defect.

The purpose of this study was to test several percutaneous injectable bone biomaterials with a reproducible model.

Two kinds of reference biomaterials were tested: orthopaedic acrylic cement, which is used for vertebroplasty [2], and hydroxyapatite (HA), which is the principal mineral bone component and is the most widely used calcium phosphate biomaterial. For this percutaneous use, HA cannot be employed in a solid sintered form, but as a powder mixed with a liquid vector. An association, as a mixture of HA and

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collagen, has previously been described in the literature [7] and used as a paste (C); we used a similar mixture of HA powder and collagen, which was injectable.

2. Materials and methods

2.1. Animals

Thirty-four adult female New Zealand white rabbits (controlled sanitary status) of 3.920 kg mean weight (range: 3.420–4.560 kg) were included in this study. Rabbits were fed with standard rabbit chow pellets and tap water *ad libitum*.

2.2. Biomaterials

2.2.1. Polymethylmethacrylate (PMMA)

The tested PMMA was an orthopaedic low viscosity cement (Cerafix* from Craver-Osteal corporation, France) used in human pathology for arthroplasties or percutaneous vertebroplasties [2]. This cement is obtained by mixing two premeasured sterilized components: a solid one and a liquid one.

Liquid component: methylmethacrylate: 16.89 ml; N-butyl methacrylate: 2.71 ml; N–N dimethyl-P-toluidine: 0.40 ml
Solid component (46.5 g): polymethylmethacrylate: 41.75 g; zirconium dioxyde: 4.30 g; active benzoyl peroxide: 0.45 g.

The liquid component was sterilized by ethylene oxide and packaged in an ampule. The solid one was sterilized by gamma irradiation and packaged in a pouch.

PMMA was injected during its liquid phase, 2 min after the beginning of mixing.

2.2.2. Hydroxyapatite (HA)

A pure heated (1000 °C) HA powder of 47 µm mean size, 0.85 g/cm^3 density and 50% porosity was used (furnished by C.E.R.E.M., Commissariat Energie Atomique, France). This HA powder was obtained by precipitation in a solution of calcium nitrate ($Ca(NO_3)_2$) with slow addition of diammonium phosphate ($(NH_4)_2(HP{-}PO_4)$). After filtration, the material was dried and heated at 1000 °C. Purity of HA was verified by X-ray diffraction. HA powder was sterilized by gamma irradiation.

2.2.3. Collagen (C)

Two different mixed forms of collagen were used: gel and microspheres. In the gel form, bovine unreticulated collagen type I was mixed with an ovine chondroïtin-4-sulfate (Coletica Corporation, France) and then buffered at pH 7.4 with a carbonate solution to obtain an injectable material. The final volume proportions were: bovine atelocollagen / chondroïtin-4-sulfate (11%), phosphate buffer 0.1 M solution (89%).

Collagen microspheres (Coletica Corporation, France) were obtained after emulsification in an ethyl-2-hexyl cocoate bath (stearineries Dubois, France) and reticulation by addition of a tereptaloyle chlorure in the solution. The collagen microspheres were recovered by centrifugation, washed in baths of ethyl-2-hexyl cocoate, ethanol and water, and finally lyophilized. Their final size ranged between 100 and 200 µm. The microspheres were placed in carbonate buffered solution to obtain an injectable mixture with the same volume proportions.

Sterilization of gel and microspheres was obtained by gamma irradiation.

2.2.4. Hydroxyapatite–Collagen (HA–C)

HA particles as previously described were coated with microspheres of collagen which floated in the liquid collagen to improve HA dispersion. HA particles were introduced at the rate of 5% in the initial chondroïtin-4-sulfate atecollagen solution.

The final volumic proportions of collasphere + HA was:

Bovine atelocollagen/chondroïtin-4-sulfate	11%
HA	23%
Phosphate buffer 0.1 M	66%

This material was sterilized by gamma irradiation.

2.3. Group constitution

Five groups were formed and compared in a randomized trial:

- *PMMA* group as an injectable reference bio-inert material.
- *HA–C* group: an injectable association of two materials copying bone composition.
- *C* group: to test the own effect of injectable collagen.
- *HA* group: to test the own effect of HA powder.
- *Control* group with an unfilled control defect, to study the spontaneous evolution of the bone defect.

For each group three time intervals were tested: 2 weeks (W2), 4 weeks (W4) and 8 weeks (W8).

Eighteen rabbits formed the "Control" and "PMMA" groups, with six femora at the 2 weeks interval, five at 4 weeks, and seven at 8 weeks. Sixteen rabbits were included in the "HA–C" group, with five femora at 2 weeks, six at 4 weeks and five at 8 weeks. Eight femora were included in the "C" group, with three at 2 weeks, three at 4 weeks and two at 8 weeks. In the "HA" group, eight femora were included: two at 2 weeks, three at 4 weeks, and three at 8 weeks.

2.4. Surgical procedure

2.4.1. Anaesthesia and preparation

Anaesthesia was induced by a dorsal subcutaneous injection using (Ketalar*: 50 mg/kg) and acepromazin (Calmivet*: 2.5 mg/kg). Surgery was performed under intravenous anaesthesia using ketamin (Ketalar*: 50 mg/kg).

Using a sterile technique, an external approach was made to access the external side of the lateral femoral condyle. The knee joint was opened during this

surgical approach only to guide the orientation of the defect and to prevent an articular effraction; medial patellar dislocation was avoided to minimize post-operative knee effusions.

Both femora were operated at the same surgical time.

2.4.2. Bone defect creation

The bone defect was made parallel to the joint line in the wider part of the femoral metaphysis, in the cancellous bone, and remained in the epiphyseal bone without effraction into the intramedullary canal or into the joint space.

An 8 mm square of bone (1 mm thick) was made on the lateral side of the femur to close the defect before the injection. This bone square must not be broken (Fig. 1).

Excavation of the defect was carried out with a succession of progressively higher diameter drills and reams. After several motor-drill-reamer trials, we finally adopted a manual excavation technique to create the bone defect. This procedure was more progressive, less blind, less traumatic and easier to control than a motorized method, even with a slow speed motor.

Hollowing began with a 5 mm diameter drill, followed by a 5.5 mm. A 6 mm ream was used to make a bone cylinder with regular borders. Drills and reams were marked at 10 mm depth. A gauge of 6 mm diameter and 10 mm length verified the volume of the defect.

The created defect was washed of bone debris and dried with a compress. An intramuscular needle was introduced into the defect by the lateral approach, punched through the internal cortex and crossed the internal skin (Fig. 2).

A large needle of 20 G size was threaded into the intramuscular needle which guided it into the defect percutaneously. The large needle was shortened (10 mm length) to prevent the risk of pushing the cortical square during injection.

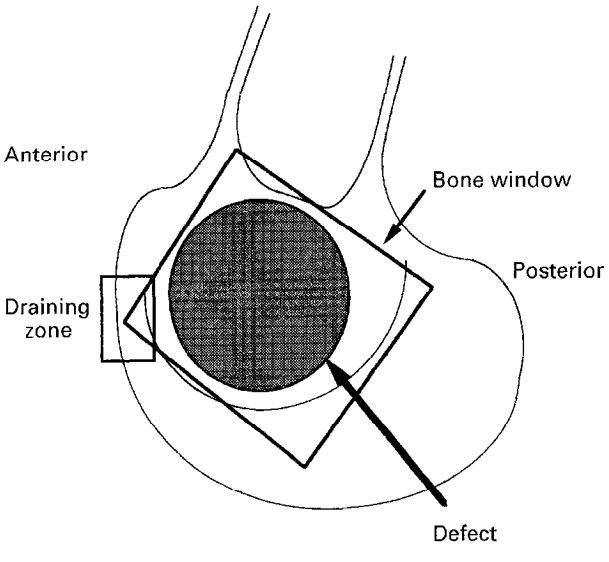

Figure 1 Lateral view of distal extremity of femur.

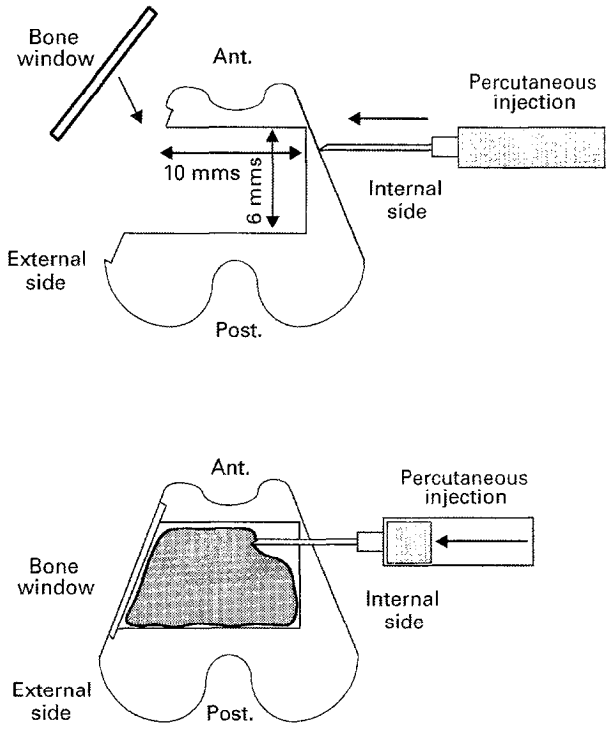

Figure 2 Percutaneous filling of the bone defect created in the distal femoral epiphysis of rabbit.

The created defect was then closed with the cortical square which was not fixed in place.

2.4.3. Bone defect filling (Fig. 2)

In the "HA" group, powder was directly put in the defect before its closure. In the "PMMA", "HA–C" and "C" groups a percutaneous injection was released with a needle introduced through the internal condyle.

Filling was made progressively, from the external to the internal part with a slow rotation of the needle to give a harmonious and as complete as possible filling of the defect. Air was flushed out through an upper corner of the bone square. Injection was stopped when the injected material began to appear at the drain point. During the injection, the bone square was kept in place without surgical fixation.

2.4.4. Closure and postoperative care

The muscular and underskin tissues were closed by separate suture points with Vicryl* (2/0). The skin was closed by an intra-dermic overcast of Ethicrin* (2/0).

After surgery, the animals were allowed to move freely in their cages without joint immobilization.

Animals were sacrificed by an overdose of sodium pentobarbital (Nesdonal*).

Post-sacrifice X-rays were carried out to look for pseudarthrosis of the bone window.

2.5. Microscopic study

2.5.1 Technical preparation

After sacrifice, the distal femurs were harvested, cleaned of soft tissues, resected and fixed in an alcoholic solution. The bone segment was dehydrated

through a graded series of ethanol baths and embedded in methylmethacrylate.

Undecalcified bone technique was used to obtain stained sections for qualitative analysis and microradiographs for quantitative histomorphometric evaluation.

Sagittal sections 7 μm thick were prepared with a Jung microtom (Jouan, France) and stained with May–Grünwald colouration.

Sagittal sections 100 μm thick were cut under cooling with a Leitz 1600 (Microm, France). Two sections were made in three sectors: internal (I1–I2), middle (M1-M2), and external (E1-E2) (Fig. 3).

Microradiographs of the sections were carried out on high resolution film Kodak SO343 (Kodak corporation).

2.5.2. Analytical procedures

In the qualitative analysis, local inflammatory reaction, vascular proliferation and new bone in defect were estimated.

In the quantitative analysis, histomorphometric parameters were measured by an image analysing computer (VIDAS 3D, Kontron, France). The magnification used was 6.3, and the scale was the millimetre. Some sections were excluded: sections with articular or intra-medullary communications, and the first sections of external and internal sectors.

For each femur three parameters were obtained:

- Defect area (mm^2).
- Bone area in the defect (mm^2).
- Bone formation in the defect (BF) (%), obtained by the ratio "bone area in defect/defect area".

With these parameters two analyses were performed:

Variations of bone defect area: a "mean defect area" (mm^2) was obtained for each topographic site (I1–I2–M1–M2–E1–E2) to assess model reproducibility.

Comparison of bone formation: a "mean bone area" (mm^2) was calculated for each group.

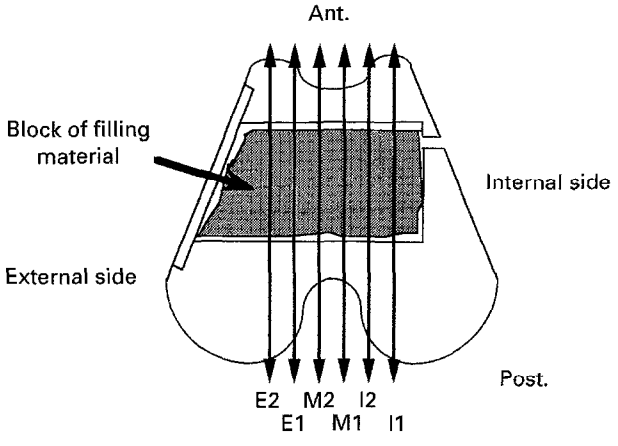

Figure 3 Distal femoral epiphysis of rabbit transversal view. Topography of sagittal sections E1: External section 1, E2: External section 2, M1: Middle section 1, M2: Middle section 2, I1: Internal section 1, I2: Internal section 2

2.5.3. Statistical analysis

All data were reported as the mean ± standard deviation.

Analysis of variance (ANOVA) and posthoc *t*-tests were used to evaluate significant differences between continuous variables in topographic sections comparison. A minimum of $p < 0.05$ was required for significance.

The Kruskall–Wallis median test was used to compare "mean defect area" and "mean bone formation" where samples were small. A posthoc Mann–Whitney *U-test* was done when significant differences appeared. A minimum of $p < 0.05$ was required for significance.

3. Results

3.1. Surgical results

Bone defect creation and the percutaneous injection were always easy to carry out.

No pseudarthrosis of the lateral bone window was seen in this experimentation. In the "PMMA" group, the presence of barium sulfate was used to check cement effraction into the intra-medullary canal or into the knee joint space. An important intra-medullary effraction was seen in three cases of the 18 analysed femora. No articular effraction was seen.

"Defect area" and "bone formation area" were calculated in 369 sections. Some sections were excluded as follows: when a part was fractured, or when sections were too irregular; often it was difficult to obtain good sections in the lateral condyle plane where the lateral side was oblique.

The "defect area", which was surrounded by cancellous bone, was always easily seen on microradiographs.

3.2. Microscopical results

3.2.1 Variation of bone defect area:

Defect area variability (Table I). A minimum of four analysed sections per femur was always obtained; a maximum of six sections was kept. Of 68 femora, 43 had six selected sections, 15 had five selected sections and 10 had four sections.

The mean surface area of the defect was 29.09 mm^2 ± 1.49 standard deviation, with extreme values of 22.24 mm^2 and 36.46 mm^2. Area values of 294 slices were found in the confidence interval.

Variations of defect area with different topographic sections (Table II). A significant difference was found between sections of different topography (I1-I2-M1-M2-E1-E2) with $p < 0.0001$.

TABLE I Variations of mean defect area (mm^2) with time

Parameter	Time interval 2 weeks	4 weeks	8 weeks
Mean	29.17	28.97	29.26
Std deviation	2.27	2.20	2.52
Std error	0.20	0.21	0.22
n	127	114	128

ANOVA: No significant difference was seen between delays

TABLE II Variations of mean defect area (mm^2) with topographic sections

Parameter	Section I1T	I2T	M1T	M2T	E1T	E2T	Mean T
Mean	29.44	29.32	28.46	28.20	29.89	29.85	29.09
Std deviation	2.15	2.17	2.33	2.27	2.31	2.32	1.49
Std error	0.26	0.28	0.28	0.28	0.32	0.31	0.18
n	67	59	68	67	53	55	68

I1T: Internal section 1, I2T: Internal section 2, M1T: Middle section 1, M2T: Middle section 2, E1T: External section 1, E2T: External section 2, Mean T: Total mean of all sections per femur.
ANOVA between sections: $p < 0.0001$.
Post-hoc analysis: M2T versus I1T*, M2T versus E1T*, M2T versus E2T*, M1T versus E1T*, M1T versus E2T*, * = Significantly different at this level.

TABLE III Variations of bone formation in defect: ratio "mean new bone area/mean defect area" (%)

Group	Time interval 2 weeks	4 weeks	8 weeks
Control	0.151 ± 0.082	0.124 ± 0.019	0.099 ± 0.03
n	6	5	7
PMMA	0.076 ± 0.04	0.075 ± 0.012	0.073 ± 0.018
n	6	5	7
HA-C	0.04 ± 0.012	0.077 ± 0.049	0.065 ± 0.037
n	5	6	5
C	0.072 ± 0.023	0.029 ± 0.011	0.036 ± 0.007
n	3	3	2
HA	0.034 ± 0.01	0.053 ± 0.007	0.051 ± 0.039
n	2	3	3

Control: Unfilled defect; PMMA: Defect filled with an orthopaedic polymethylmethacrylate cement; HA–C: Filling with an association of hydroxyapatite and collagen; C: Filling with collagen; HA: Defect filled with HA particles; Results = Mean ± standard deviation, *n* = number of femora.

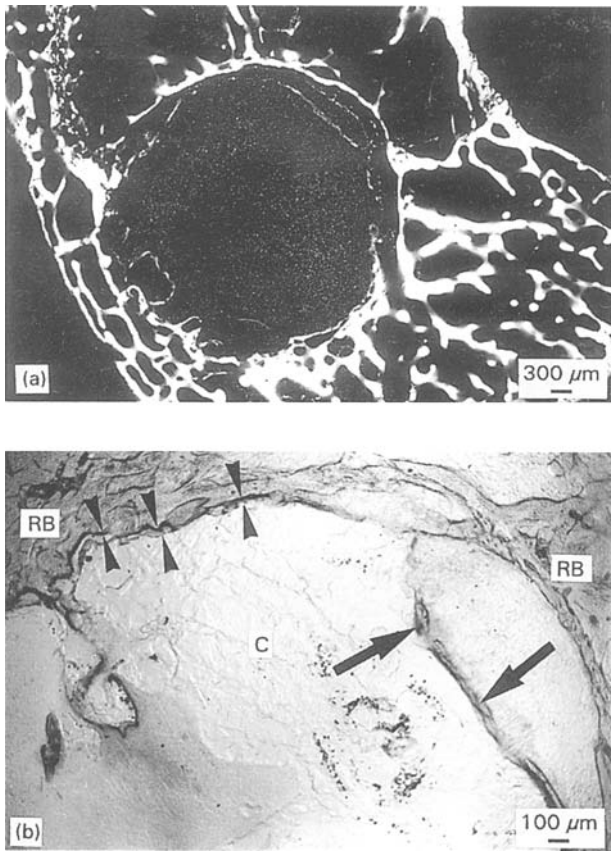

Figure 4 PMMA group at 8 weeks: (a) No bone edification into the cavity filled with PMMA, except in the folds, appeared during the injection. They let a pathway to the biological fluids (white arrows) and allowed the presence of thin new trabecular bone. (b) A dark line of fibrous connective tissue (black head arrows), mixed with new trabecular bone (black arrows), is seen between the receiving bone (RB) and the filled cavity (C).

A post-hoc analysis between each topographic section revealed a difference between the middle (M1 and M2) and the external (E1, E2) or medial sections (I1, I2). Mean defect area was larger in the external and medial sections than in the middle sections.

Comparison between the time intervals revealed no statistically significant difference.

3.2.2 *Variations of bone formation between the groups* (Table III)

"*PMMA*" *group* (Fig. 4). The bone defect was never completely filled by PMMA. The injection was carried out slowly with a small needle at a low injection pressure: the PMMA mass was irregular with folds, and empty spaces where new bone was growing. A low fibrosis reaction with the connective tissue was seen at the bone–PMMA interface on qualitative histological study. This small fibrosis reaction did not stop the invasion of PMMA gaps by new mature bone.

Bone invasion was limited by the central cement mass, and for the three time intervals, the mean bone formation was constant: 7.6% at 2 weeks, 7.5% at 4 weeks, 7.3% at 8 weeks.

"*HA-C*" *group* (Fig. 5). An optical study of material repartition in the defect area had shown a variability in the repartition and density of the couple "hydroxyapatite–collagen". The dispersion of the HAP particles was not as homogeneous as expected.

In the histological study, no inflammatory response and no vascular reaction was observed. Bone formation was peripheral.

No significant difference of bone formation, between each time interval, was found. Bone formation was very low after 2 weeks and remained small, although a little secondary bony reaction was seen at 4 weeks.

The filled defect was not invaded by bone although hydroxyapatite was employed as a bone conductive material.

"*C*" *group* (Fig. 6). Histological qualitative analysis did not reveal any inflammatory reaction or vascular proliferation. The action of this type of collagen was negative with regard to bone formation but presented no intolerance reaction. The bone reaction was lower than after a filling with orthopaedic cement.

A small bone reaction was seen at 2 weeks, but bone formation decreased with time and bone invasion was very weak, with the lowest rate in this study: from 7.2% at 2 weeks, bone rate area reduced to 2.9% at 4 weeks and 3.6% at 8 weeks.

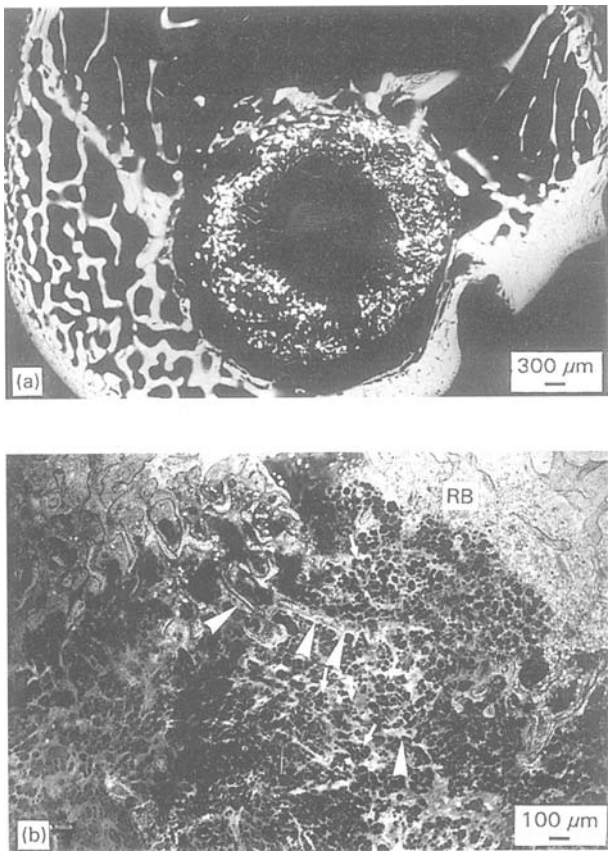

Figure 5 Hydroxyapatite–collasphere group at 8 weeks: (a) Variability in the distribution and the density of the hydroxyapatite is seen. The bone formation is weak in the defect. (b) Thin new trabeculae bone (white head arrows) surrounded the HA–C. Microbeads are sustained with the HA particles release (small white arrows). RB: receiving bone.

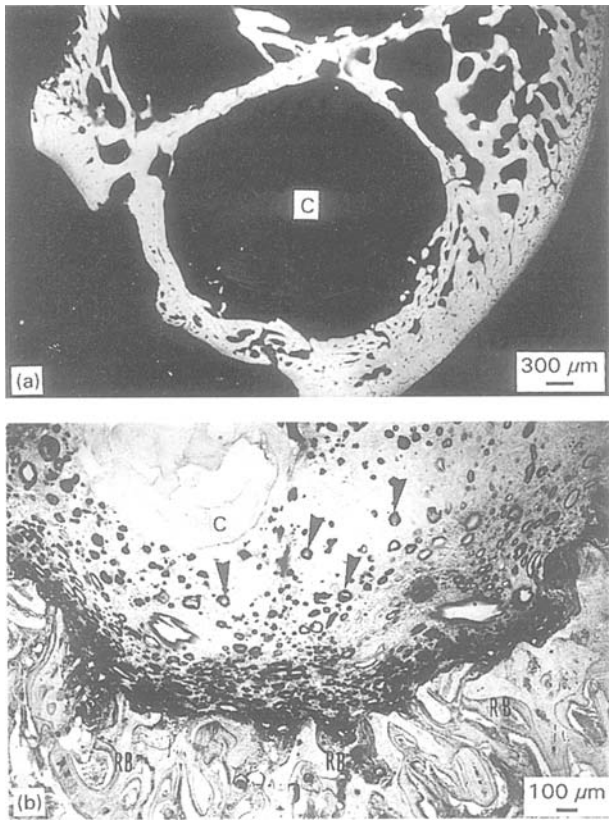

Figure 6 Collagen group at 8 weeks: (a) microradiograph; (b) stained section. At the interface between the cavity (C) and the receiving bone (RB), no bone formation is observed, presence of residual collagen (black head arrows).

This early bone reaction at 2 weeks was significantly higher than at 4 weeks ($p < 0.05$).

"*HA*" *group* (Fig. 7). Bone formation was seen only in the peripheral part of the defect, and the bone union with hydroxyapatite occurred without any inflammatory reaction. Quantitative results were characterized by the low levels of bone formation at every time interval: 3.4% at 2 weeks, 5.3% at 8 weeks and 5.1% at 8 weeks. No statistical difference of bone formation between each time interval was found.

"*Control*" group (Fig. 8). The "Control" group was the only group where the defect was not filled, and the defect never became completely filled by new bone. The bone defect limit could always be seen well on microradiographed sections, and differentiation with old cancellous bone was always possible.

Greater bone formation was seen after 2 weeks but the bone formation decreased with time. After an early bone invasion of the defect, bone formation was seen in the periphery of the defect but not in the central part.

A comparison between the different time intervals (W2–W4–W8) was not statistically significant.

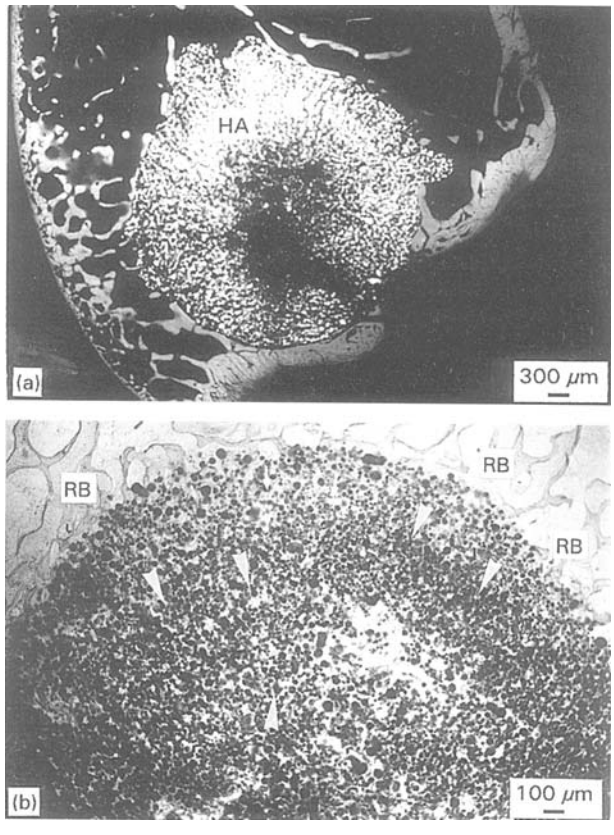

Figure 7 Hydroxyapatite group at 8 weeks: (a) Good contact between HA particles and receiving bone (RB) is noted. The small size of HA particles and the small spaces between them prevented new bone formation in the centre of the cavity. (b) White head arrows indicate the high density HA particles. Therefore bone formation was stopped and remained near the receiving bone (RB).

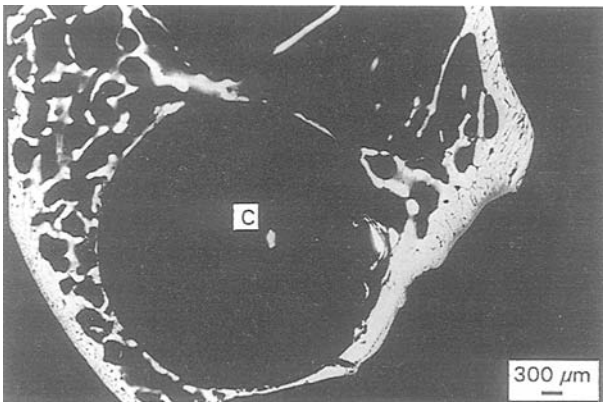

Figure 8 Control group at 8 weeks. No bone formation is observed in the cavity, only a little peripheral bone reaction.

4. Discussion

This study showed the use of bone biomaterials by an injectable percutaneous method, and a model to evaluate these materials is proposed.

4.1. The model

Our percutaneous filling procedure was surgically easy to realize in the distal femoral rabbit extremity. Our choice of model was different from other cement filling models in tibia of rabbits [8] or in femora of dogs [9], where the created defect was intramedullary. Our defect was created in a closed cancellous bone site, where the bone reaction was supposed more important . The defect volume was small in comparison to the intramedullary filling of other models, and closer to human conditions, as in the percutaneous filling of vertebral tumors.

The surgical closed bone defect allowed us to study bone formation following the percutaneous filling of the defect with liquid biomaterials.

The study of defect area variability (Table III) showed differences between the topographic sections, with lower area results for the central topographic sections compared to external and medial sections. We have attributed this variability to the frequent divergence between the orientation of the bone defect axis in the femoral intercondylar axis, which could not be strictly parallel. Bone sections were done perpendicular to this intercondylar axis, not to the defect axis: the defect was circular near the centre and oval in the internal and external sections.

Comparisons of "mean defect area" between groups or time intervals (Table II) showed no statistically significant difference. Low standard deviation and negative comparisons demonstrated the reproducibility of our surgical method.

As was observed by Katthagen [7], the calculation of defect area was always easy on microradiographed sagittal sections. But these sagittal sections gave only an analysis of the central part of the defect and excluded the defect extremities. In this experiment a bone window was placed on the external side, and a small hole for injection was made on the medial side. Bone behaviour in these topographic localizations was ignored.

4.2. The variations of bone formation

In this experiment, different bone behaviours were tested: what was the evolution of a large unfilled defect? What was the bone formation after different kinds of injected fillings?

The evolution of the control group shows that a bone defect made in a rabbit femoral distal extremity does not spontaneously fill with new bone, even if this defect is closed. Bone reaction was similar to that of humans with an early peripheral bone formation process and a secondary bone remodelling at the periphery of the defect without colonization of the central part.

None of the materials tested improved the filling of the bony defect above that of the control. Since there was no filling in the control group, contrary to the other groups, the bone invasion was probably easier. However, this bone formation in the defect decreased with time.

A bone defect substitute is employed for two reasons [10]: to fill the defect space (mechanical effect), and to enhance bone formation in the defect space (biologic effect). Two types of percutaneous injected materials were tested in this study:

- PMMA which has good mechanical characteristics. It is a reference bio-inert material, used as orthopaedic acrylic cement. This material is currently the most widely employed material for percutaneous bone filling in human pathology.
- HA which is the reference bioactive material, is usually employed in porous ceramic form obtained after sintering. Porous HA is widely used, its biocompatibility, its bioactivity, and osteoconduction inside the pores are well known [11]. This kind of ceramic cannot be used by injection. The low diameter of HA powder is compatible with a narrow needle diameter, however, HA powder cannot be injected alone because of its rheological properties. Collagen type I, which is the second common component in bone [12], can be used as a vector. Furthermore, collagen is supposed to bring interesting properties [12] and this association has been previously described in the literature. Mehlisch [13], in human odontology, obtained good results with an association of hydroxyapatite and collagen.

In the "PMMA" group, bone formation was stable over time. This was considered to be an effect of filling: the volume filled with PMMA limited bone invasion in contrast with the "empty" control group.

In the "PMMA" group injected by the percutaneous route, the filling was not complete even if blood was drained during the injection and even if the defect was dried before closure. The percutaneous injection of PMMA was probably incomplete because of the presence of blood cells which arrived during the interval of external closure and complete injection: PMMA was injected progressively under low pressure and using a small needle. The injection was made slowly as in human pathology when vertebral bodies are filled percutaneously. This phenomenon could explain the gaps found in the cement mass and the irregularities of bone invasion. If the injection was

incomplete, the space between PMMA and the defect limit was well invaded by bone. Acrylic cement, which is bioinert, was well tolerated by bone which colonized the cement gaps. Incomplete cement filling was not a factor in disorders of bone defect evolution.

Results of the group "HAP + C" were a sum of the results of the "HAP" and "Collagen" groups, but with better results after 2 weeks than the "HAP" group. "HAP + C" filling material had the mixed and additive effect of each component. This increase was probably due to a specific effect of the mixture after 2 weeks. The "HAP + C" group did not provide the good results described by Katthagen [7] or Iwano [14], but our materials were different; they were not chosen for their potential bone bioactivity but for their particularities: HAP and Collagen mixing, with an original presentation of collagen (collaspheres). Ono [10] used an original bone filler: an apatite–wollastonite granule–fibrin mixture which was tested with different proportions of fibrin, and this ratio between fibrin and apatite–wollastonite influenced bone formation. Iwano *et al.* analysed the tissue reaction to collagen-coated porous hydroxyapatite [14]; the compression strength was better with collagen-coated hyroxyapatite, but a multinucleated giant cell reaction was more frequent in this group than in hydroxyapatite alone. For this author, this reaction decreased with time. No difference was found on newly formed bone ratio between the groups.

HAP alone had a late effect which appeared at 4 weeks and remained strong at 8 weeks. The HAP which was tested, was an experimental product with apparently weak bone bioactivity. The "HAP" group also had a weak bone formation rate, which is surprising. The hydroxyapatite used in this experiment was an experimental heated hydroxyapatite and not a sintered referenced hydroxyapatite. Its presentation was not a porous ceramic block but a fine compact powder. Its bone induction capacity was unknown. The final results appeared weak when compared to other sintered ceramics. This weak bone induction was not in accordance with bone attachment to hydroxyapatite which is often described in the literature [15].

Collagen alone had a very slight bone stimulation effect, even if no inflammatory and vascular reaction was observed. Collagen behaved with bone as an inert material. At every time interval, the mean percentage of new bone formation was less than in the "Control" or "PMMA" groups. Collagen had a global negative effect on bone formation and slowed it down. The "Collagen" group had the lowest rate of new bone formation, without any inflammatory reaction. Our analysis technique, with the comparison of behaviour of each component taken separately, allows us to conclude that a poor bone reaction was obtained with the collagen used in this experiment. We have not found, as was described by Joos and Ochs [16] in rabbit mandible or by Speer *et al.* [17] in the healing of knee osteochondral defects, a quicker ossification.

5. Conclusion

Even if bone formation obtained with bone materials obtained in this study were fair, this experiment confirms that the clinical concept of percutaneous injectable bone materials is realistic.

Further studies are needed to improve the properties of these materials.

In the future, bone filling with little surgical agression appears to be an important method of research into tumors, or axial bone localizations as in the spine.

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