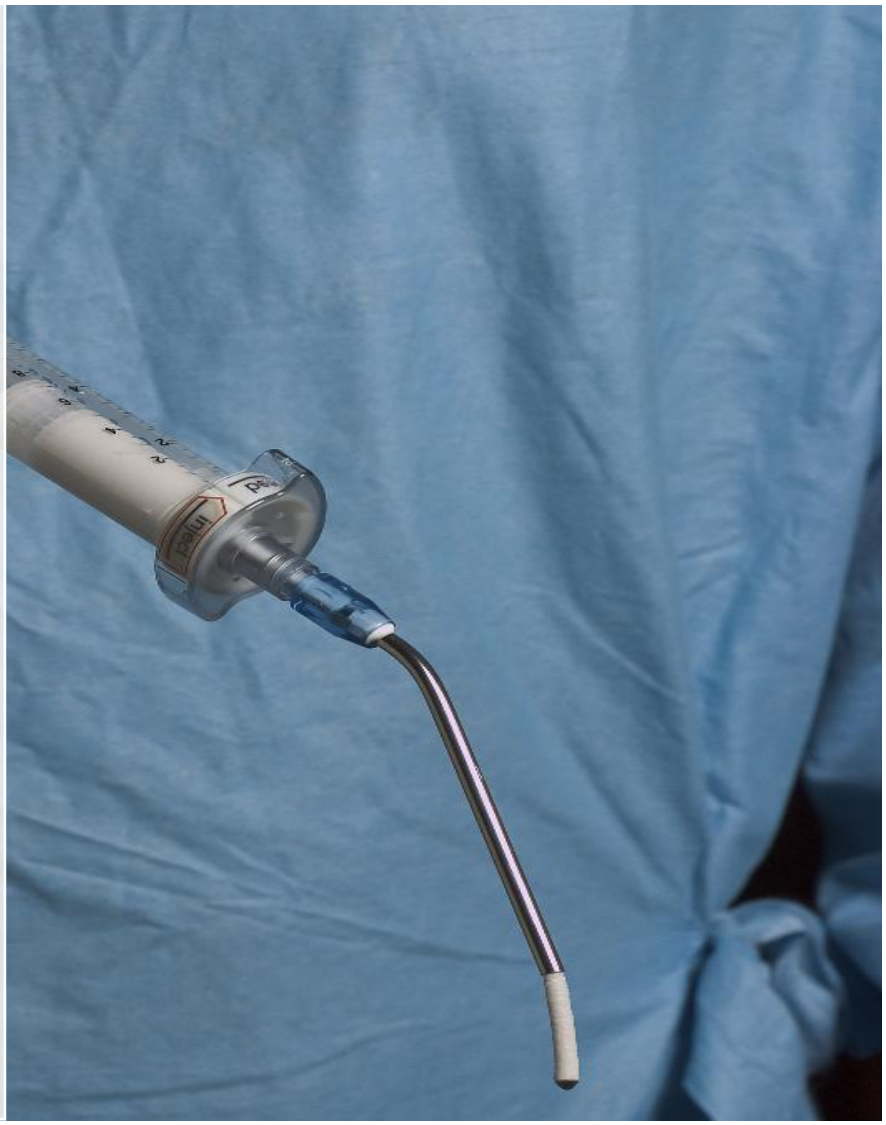
Summary of Preclinical data for Calcium Phosphate products: Graftys® HBS and Graftys® QuickSet

Graftys R&D Team

2011



A Graftys preclinical studies Review

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Special acknowledgment to Pr Olivier Gauthier for his contribution to the present document

Graftys®

Graftys was created in September 2005 in order to harness the potential of academic research to a proactive and dynamic commercial organization in the field of bone tissue engineering. We design, manufacture and market calcium phosphate-based synthetic bone substitutes to address the global orthopaedic and dental surgery markets. Through its unique portfolio of proprietary technology, Graftys provides innovative solutions to major unmet market needs. Our research and development projects include the latest generation of injectable and resorbable synthetic bone substitutes, biological repair of cartilage and of the spine, as well as bone substitutes combined with pharmaceutical agents.

Here, we describe the preclinical data for two major Graftys products.

Graftys® HBS and **Graftys® QUICKSET** are patented phosphocalcic, macroporous, injectable, hardening, resorbable cements. Their physicochemical structure confers osteoconductive properties adapted to surgery of reconstruction.

Graftys® HBS provides low viscosity, allowing excellent injectability along with a means to reach small, difficult to access and deep cavities (eg. use of screw holes in a tibial plateau). This capability ensures an excellent interface between both bone/material and implant/material. The slow setting time (15 min) allows a large injection time (which may be especially useful in revision procedures). Finally, Graftys® HBS facilitates injection into the trabecular structure, without damaging it.

Graftys® QUICKSET features high viscosity and excellent cohesiveness to address surgical situations with significant blood flow and ensure an excellent performance in uncontained cavities to minimize risk of implant displacement. The short initial setting time (8 min) avoids losing operative time and reinforces the cohesiveness properties of the material. Finally, all these properties give the possibility to position the material with confidence even when the gravity presents a challenge (eg. filling of acetabular defects).

Figure 1: Photograph of bone transverse section containing Graftys cement.

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Introduction

Graftys® HBS and **Graftys® QUICKSET** is intended for filling or renforcing bony voids or gaps of the skeletal system caused by trauma or surgery, that are not critical to the stability of the bone structure. The product provides bone void filler that resorbs and is replaced by bone during the healing process.

The powder mineral phase of Graftys® HBS and Graftys® QUICKSET is composed of calcium phosphates associated to an organic phase of hydroxypropylmethyl cellulose (HPMC) which confers wettability and porosity. This porosity facilitates percolation of the body fluids throughout the implant and improves the cohesion, elasticity, rheological properties, and injectability. The liquid phase (sodium phosphate solution) creates a homogeneous mix and according to the liquid/powder ratio establishes the setting time (crystallization) of the cement [1]. After complete hardening the final product (apatite crystals) is similar to native bone crystals. It is gradually resorbed by osteoclast cells which participate in the natural bone remodeling cycle. Conduction and formation of mineralized healthy bone occurs as the material resorbs without formation of voids (rapid dissolution leads to fibrosis and seroma).

Two recent studies conducted in rabbits and sheep to compare Graftys® QUICKSET to Graftys® HBS at short and long term are presented here. A critical size bone defect model in the femoral lateral condyles was used; this model was validated in Oniris at Nantes (Ecole Nationale Vétérinaire de Nantes, France) for these two species [2, 3, 4]. It consists in a bone cylindrical defect, created at the junction between the epiphysis and metaphysic, which is then filled with Graftys® HBS or Graftys® QUICKSET. These studies showed that Graftys® QUICKSET and Graftys® HBS have the same efficiency for bone reconstruction.

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Materials & Methods

Preparation of cements

Graftys® HBS

Graftys® HBS provides an easy, closed mixing system (which eliminates the bowl/spatula). Ring on injection end of syringe is shipped in "Closed" position. To initiate mixing, hold injection end upright and rotate ring to "Transfer". Press fluid into the powder side. Mix thoroughly for 2 minutes. Rotate ring to "Inject" and inject the material directly by hands or with injection gun.

Graftys® QUICKSET

In preclinical studies in rabbits and sheep Graftys® QUICKSET was produced and sterilized in the LIOAD laboratory (INSERM U791, Nantes). It was then prepared as follows: on a sterile field, the powder was poured into a sterile mortar and then the corresponding amount of liquid was added. The two components were mixed using a pestle for one minute then the cement was transferred with a spatula in a syringe for single use.

Characteristics of cements

Graftys® HBS	Graftys® QUICKSET
Mixing time (room temperature):	
2 min	2 min
Injection time (room temperature):	
15 min	2 min
Low sensitivity to temperature variations of operating room.	
Diameter of usable cannulas:	
14 Ga or larger	7 Ga or larger
Initial setting time (37°C/98°F body temperature):	
15 min	8 min
Complete hardening (37°C/98°F body temperature – simulating in vivo conditions):	
72 hours	24 hours

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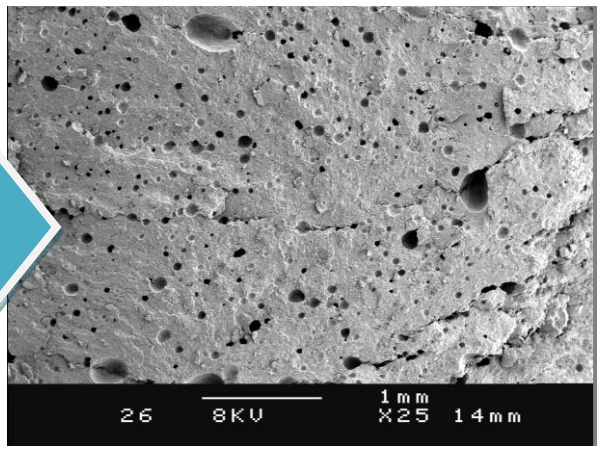
Physicochemical properties of cements

Graftys® HBS	Graftys® QUICKSET
Global Porosity 70% distributed as below:	
Micro porosity (<10 µm):	
84%	88%
Meso porosity (10 - 100 µm):	
8%	2%
Macro porosity (>100 µm):	
8%	10%
Available Initial Porosity measured after complete hardening at 37 °C (98°F body temperature) in biological conditions (Ringer's solution).	
No shrinkage during the crystallization process.	
Low exothermic reaction: 1-3 °C	
Mechanical compressive strength after complete hardening in biological conditions. This product has to be used in an intrinsic stable site or in association with material which will stabilize the surgical site.	
12Mpa	24MPa
Cohesiveness/Resistance to fluid:	
Good	Excellent

Porosity: general aspect after 72 hours at 37°C in Ringer solution



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Surgical implantation in bone defects

All animal handling and surgical procedures were conducted according to European Community guidelines for the care and use of laboratory animals (DE 86/609/ CEE) and were approved by the local animal care and safety committee. The experiment was performed according to Good Laboratory Practices at the Veterinary School of Nantes (Oniris).

In rabbits

Adult female New Zealand White (NZW) rabbits, weighing 3.2 to 3.8 kg were purchased from a professional breeder (Grimaud Frères Sélection, La Corbière, Roussay, France). General anesthesia is induced and prolonged using an intra-muscular injection of ketamine (Imalgène 1000®, Mérial, Lyon, France) and xylazine (Rompun®, Bayer Pharma, Puteaux, France).

The two limbs were shaved, skin was disinfected with iodine solution and the animal was sterile draped. After a lateral skin incision and lateral arthrotomy of the knee joint, the distal lateral femoral condyle was exposed. A critical cylindrical defect (6 mm diameter x 10 mm long) was created at the junction between the epiphysis and metaphysis using a motor-driven drill (Aesculap, Tuttlingen, Germany) and successive burs of 2, 4, and 6 mm diameter. During drilling, the site was irrigated using sterile saline solution. Bone debris was removed by saline irrigation and dried as possible. The femoral cavity was then filled with Graftys® HBS or Graftys® QUICKSET cement.

After implantation, articular, subcutaneous tissues and skin were closed in different layers using absorbable sutures (Monocryl déc.2®, Ethicon). The surgical site was finally covered with an adhesive bandage. No prophylactic antibacterial treatment was administrated before or after surgery. Peroperative analgesia was provided by morphine injections and prolonged postoperatively by non-steroidal anti-inflammatory drug injection (Métacam, ^{e}Métacam®, Boehringer Ingelheim, Reims, France). After 4 weeks, the animals were anaesthetized and sacrificed by intra-cardiac overdose of sodium pentobarbital.

In sheep

Adult female sheep were purchased from a professional stockbreeder. General anesthesia is induced and prolonged using an intravenous injection of ketamine (Imalgène 1000®, Mérial, Lyon, France) and propofol (Rapinovet, Schering-plough, France).

The two limbs were shaved, skin was disinfected with iodine solution and the animal was sterile draped. After a lateral skin incision and lateral arthrotomy of the knee joint, the distal lateral femoral condyle was exposed. A cylindrical defect (9 mm diameter x 20 mm long) was created at the junction between the epiphysis and metaphysis using a motor-driven drill (Aesculap, Tuttlingen, Germany) and successive burs of 2, 4, and 9 mm diameter. During drilling, the site was irrigated using sterile saline solution. Bone debris was removed by saline irrigation and dried as possible. The femoral cavity was then filled with Graftys® HBS or Graftys® QUICKSET cement.

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Explant harvesting procedure and analysis

Sample harvesting

At the end of experiment, femoral distal ends are dissected and immediately placed in fixation medium (10% neutral formalin). The samples, identified individually, are transmitted to the LIOAD histological platform (LIOAD, INSERM U791, Nantes, France) that has carried out the inclusion of samples according the following protocol:

Non-decalcified bone samples were dehydrated in ascending series of ethanol (70% -100%) and then in pure acetone for 24 h. The samples were impregnated in methyl methacrylate (VWR Prolabo, Fontenay-sous-Bois, France) for 4 days and then embedded in poly methyl methacrylate resin (PMMA). Blocks were cut in half with a circular diamond saw (Microtome 1600, Leica, Germany). One part was processed for histology. Thin, 7 µm sections were prepared using a hard tissue microtome (Reichert-Jung, Supercut 2050, Germany) and then stained with Hematoxylin-Eosin and Movat's pentachrome. The other part of the block was used for electron microscopy histomorphometrical measurements.

BSEM analysis

Samples were polished and sputtered with a thin layer of gold–palladium (EM Scope, England) for observation. Photographs were taken using backscattered electron microscopy at 15 kV (BSEM, LEO 1450 VP). The surface of the implant was divided into contiguous high-resolution images. Quantitative evaluation was performed with a semiautomatic image analyzer (Leica Quantimeter 500, Cambridge, UK). The areas of newly formed mineralized bone, cement, and non-mineralized tissue were identified by their grey levels.

Histological analysis

Histological analysis was performed from histological sections after Hematoxylin-Eosin and Movat staining by diagnostic platform and histopathological department (Oniris, Nantes).

Results

Implantation of Graftys® HBS and Graftys® QUICKSET in rabbit

BSEM analysis

Five adult females NZW rabbits were operated according to a predefined and validated model of critical bone defect in femoral condyle.
During the interventions, the Graftys® QUICKSET cement seemed to harden faster than the Graftys® HBS and presented also a less hydrated aspect.

As shown in Fig. 2, BSEM analysis of bone samples for the five rabbits revealed that after 4 weeks of implantation both cements are still in place; bone remodeling was observed around the cement, indicating good osseointegration of Graftys® HBS and Graftys® QUICKSET. Moreover, as shown in Fig. 2, induced porosity was present in both cement types. The quantification of this porosity did not show significant difference between Graftys® HBS and Graftys® QUICKSET.

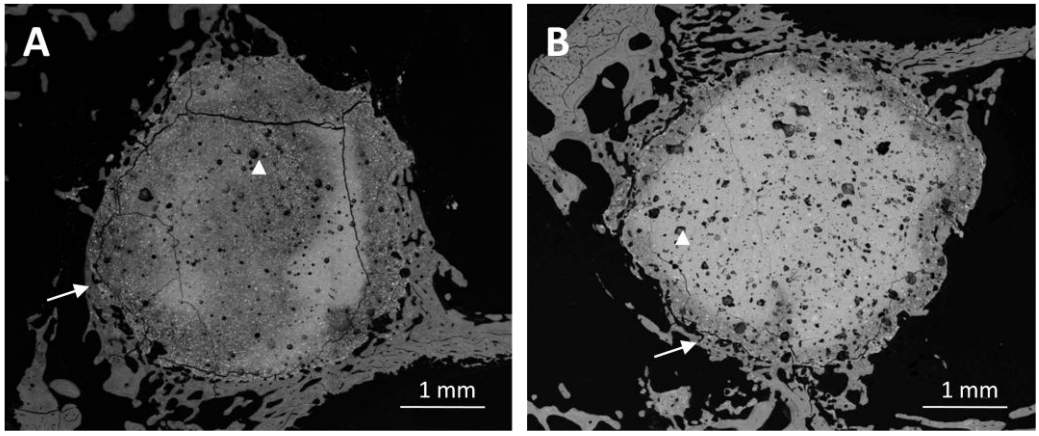

Figure 2: Representative BSEM pictures of Graftys® HBS (A) and Graftys® QUICKSET (B) after 4 weeks of implantation in rabbit showing peripheral bone remodeling (white arrow) and incuded porosity in cements (white arrowhead)

Histological analysis

Histological analysis of bone samples for the five rabbits implanted during 4 weeks did not show bone lesions of significant intensity (no inflammatory lesion, necrotic or fibrosis) around of the implanted biomaterials, indicating the biocompatibility of the implants. In these animals, a moderate deposition of osteoid tissue at the periphery of the biomaterials was noted, signing a reaction of bone to implanted cements and activation of ossification in the peri-implant tissue (Fig. 3). No significant difference was observed between samples of the Graftys® HBS group and those in Graftys® QUICKSET group.

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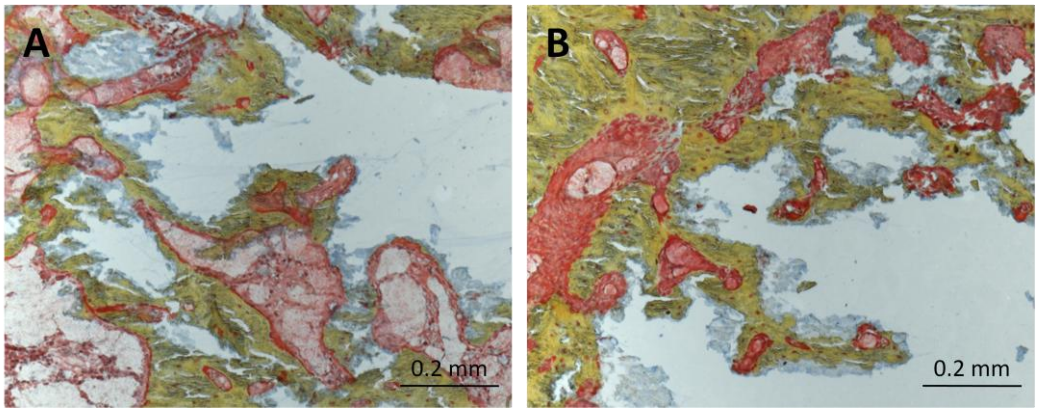

Figure 3: Movat pentachrome staining picture of Graftys® HBS (A) and Graftys® QUICKSET (B) implanted in rabbit showing a bone deposition (in yellow brow) at the periphery of the cements (in blue green and white parts)

Implantation of Graftys® HBS and Graftys® QUICKSET in sheep

X-ray and BSEM analysis

Two adult female sheep were operated according to a predefined and validated model of critical bone defect in femoral condyle.
During the interventions, both cements, Graftys® QUICKSET and Graftys® HBS, were easy to implant. The Graftys® QUICKSET cement seemed to harden faster than the Graftys® HBS.

As shown in Fig. 4, X-ray and BSEM analysis of bone samples for the two sheep revealed that after 6 months of implantation both cements were still in place. No radiolucent line was noted around of the implanted biomaterials (Fig. 4A, B). On BSEM pictures (Fig. 4C, D, F), bone remodeling was observed all around of the implants of Graftys® HBS and Graftys® QUICKSET, indicating good osseointegration.

As shown in BSEM pictures, induced porosity was still present in both cement types after 6 months of implantation. The quantification of this porosity did not show significant difference between Graftys® HBS and Graftys® QUICKSET. Moreover, new bone formation was noted within these pores (Fig. 4E, black arrow).

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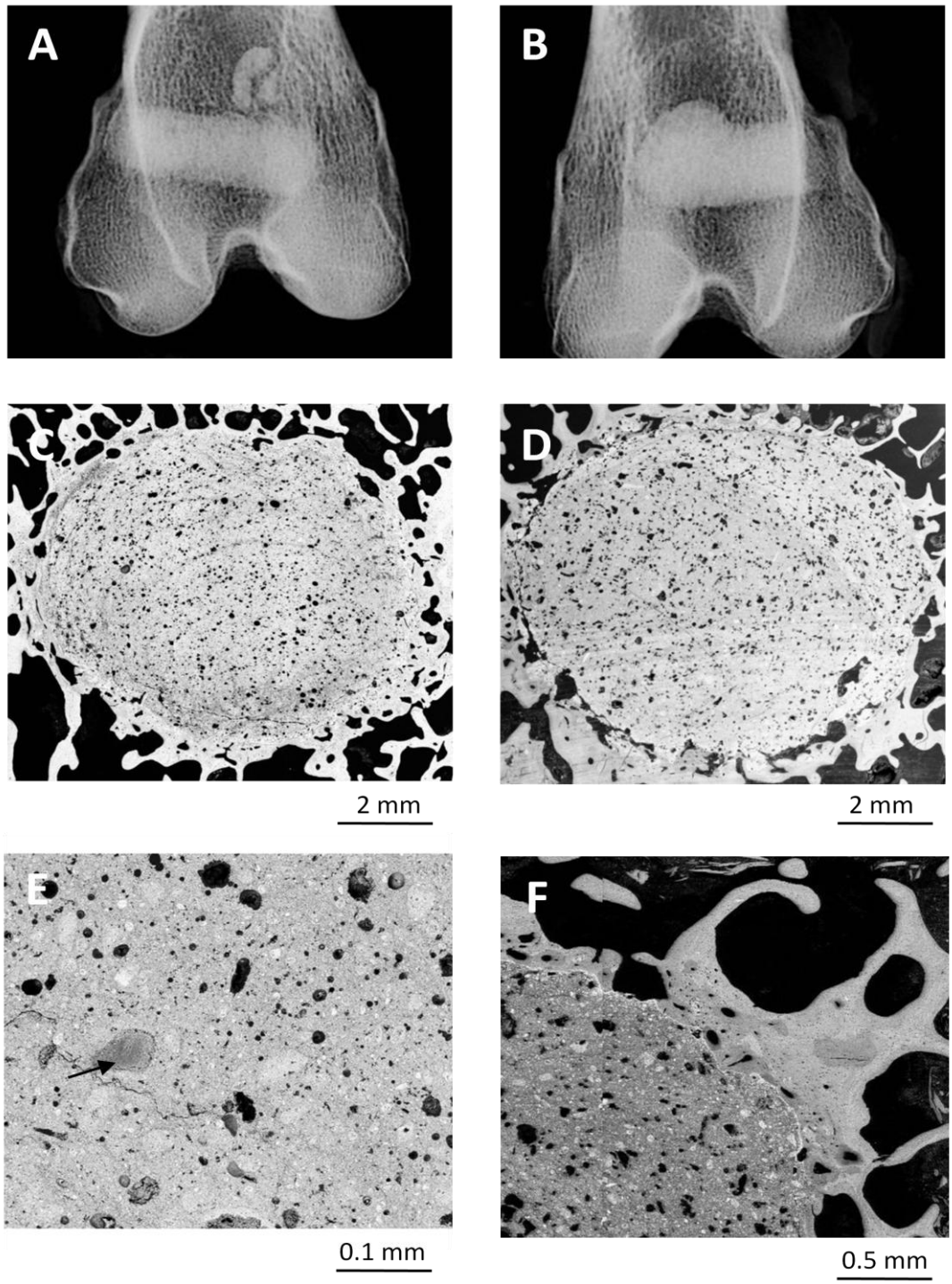

Figure 4: Representative radiographic (A, B) and BSEM (C, D, E, F) pictures of Graftys® HBS (A, C, E) and Graftys® QUICKSET (B, D, F) after 6 months of implantation in sheep

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Histological analysis

Histological analysis of bone samples for the two sheep implanted during 6 months did not show bone lesions of significant intensity around of the implanted biomaterials, indicating the biocompatibility of the implants. A moderate deposition of osteoid tissue at the periphery of the biomaterials and new bone formation into pores of cements were noted (Fig. 5). No significant difference was observed between samples of the group of Graftys® HBS and those in group Graftys® QUICKSET.

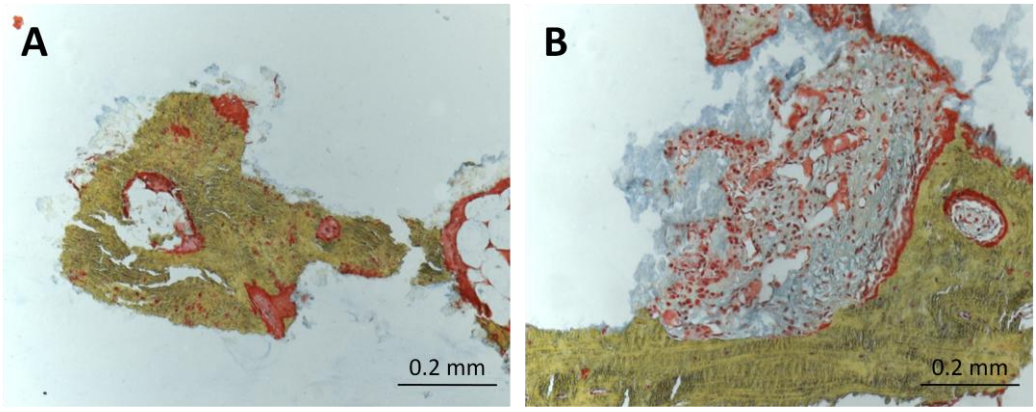

Figure 5: Movat pentachrome staining picture of Graftys® HBS (A) and Graftys® QUICKSET (B) implanted in sheep showing a deposition of osteoid tissue at the periphery of the cements (B) and new bone formation into pores of cements(A)

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Conclusion

Two recent studies were conducted in rabbits and sheep to compare Graftys® QUICKSET to Graftys® HBS at short and long term. A critical size bone defect model in the femoral lateral condyles was used; this model was validated in Oniris at Nantes for these two species.
The filling of bone loss using cements Graftys® HBS and Graftys® QUICKSET was very easy and in both studies it was confirmed that the Graftys® QUICKSET hardened faster than Graftys® HBS.

The results obtained have shown a very good osseointegration for both types of cements, Graftys® HBS and Graftys® QUICKSET. This osseointegration was evidenced by BSEM by the presence of bone remodeling in close contact with cements (bone remodeling which completely surrounds the implants) and by XRay with absence of radiolucent line at the bone-implant interface.

The results of histological analysis on thin sections stained with HES and Movat have shown that rabbits and sheep showed perfect biocompatibility for implants of Graftys® HBS and Graftys® QUICKSET. This biocompatibility was demonstrated by the presence in the periphery of the implants of osteoid matrix deposition showing an activation of the ossification in the peri-implant tissue.

In conclusion, Graftys® HBS and Graftys® QUICKSET offer two different timelines for their hardening rate, but they both show the same biocompatibility and the same efficiency for bone filling.

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