Presentation of BIOBank and the Supercrit® process

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1. Presentation of BIOBank

Mission

BIOBank is a French tissue bank authorised by ANSM (French National Agency for the Safety of Medicines), whose mission is to offer bone allografts and innovative derived biomaterials.

With its innovative worldwide patented SUPERCRIT® technology (based on supercritical CO_2), BIOBank is able to offer to surgeons, bone grafts with unique properties for many surgical uses.

Type of activities

The key features of our activities, which have improved constantly since 2003, are:

- Tissue bank activity within the context of the bioethics law and consistent with the authorisations granted by ANSM from the management of the tissue processing stages to delivery of safe grafts;
- Distribution activities for orthopaedic, neurosurgery, maxillofacial and oral implantation surgery in France and in other countries;
- Industrial activities by providing viral inactivation technology (subcontracting the SUPERCRIT® process) to other French and foreign tissue banks which want to make use of its many advantages;
- Ambitious R&D activities based on partnerships with academic and hospital facilities, the purposes of which are to develop new materials and products;
- An industrial property portfolio which is regularly expanded by submission for new patents.

Organisation

The facilities including the bone tissue transformation laboratory, raw materials and finished product storage are grouped together on the same site, which is located in Presles en Brie (Seine et Marne).

BIOBank staff is composed of qualified people split between the R&D, production, Quality & Regulations and Customer Service departments.

Our products are distributed through a network of highly qualified professionals who are close to the surgeons who use the products for their patients.

Implementation of the Supercrit® process and all of the procedures on tissues are carried out in a laboratory which has been specially designed for tissue processing.

The working environment consists of cleaning rooms (ISO class 7), equipped with specific high technology materials, in particular special-purpose machines for supercritical CO_2 tissue process.

The stages of the Supercrit® process are implemented by trained, qualified staff, who work according to a quality system which complies with the most recent Good Tissue Practices. All handling is recorded by a computer system which complies with regulatory traceability requirements.

The R&D projects are supported by a scientific council, which is made up of orthopaedic and dental surgeon opinion leaders.

Quality System Management

BIOBank products are human bone grafts, in the European regulation (93/42/EC) they are excluded from the definition of a medical device.

BIOBank products comply with directive 2004/23/EC, BIOBank has a Quality Management System (QMS) according to the French Good Practice for tissue bank (2010-10-27).

Document system associated with monitoring tools allows the management of non-conformity, corrective action, change control and risk analysis. The BIOBank QMS is regularly evaluated and inspected by French health authorities (ANSM).

Training and accreditation of staff

The BIOBank staff are trained to meet the requirements of the tasks asked of them through an initial training plan which accredits the member of staff to take up his/her position.

The requisite level of qualification is maintained through an annually revised training plan.

Ambition

BIOBank is the bone tissue bank leader in France (Biomedicines Agency data) and a major player in Europe. The continuing growth in its tissue collection and distribution activities reflects its partners' satisfaction.

BIOBank's ambition over the coming years is to market new products with high added value, which are patent protected in order to extend its therapeutic offering and meet the needs of the reparative surgery of tomorrow, and through this, become one of the reference tissue banks in Europe.

2. ORIGIN AND SELECTION OF TISSUES

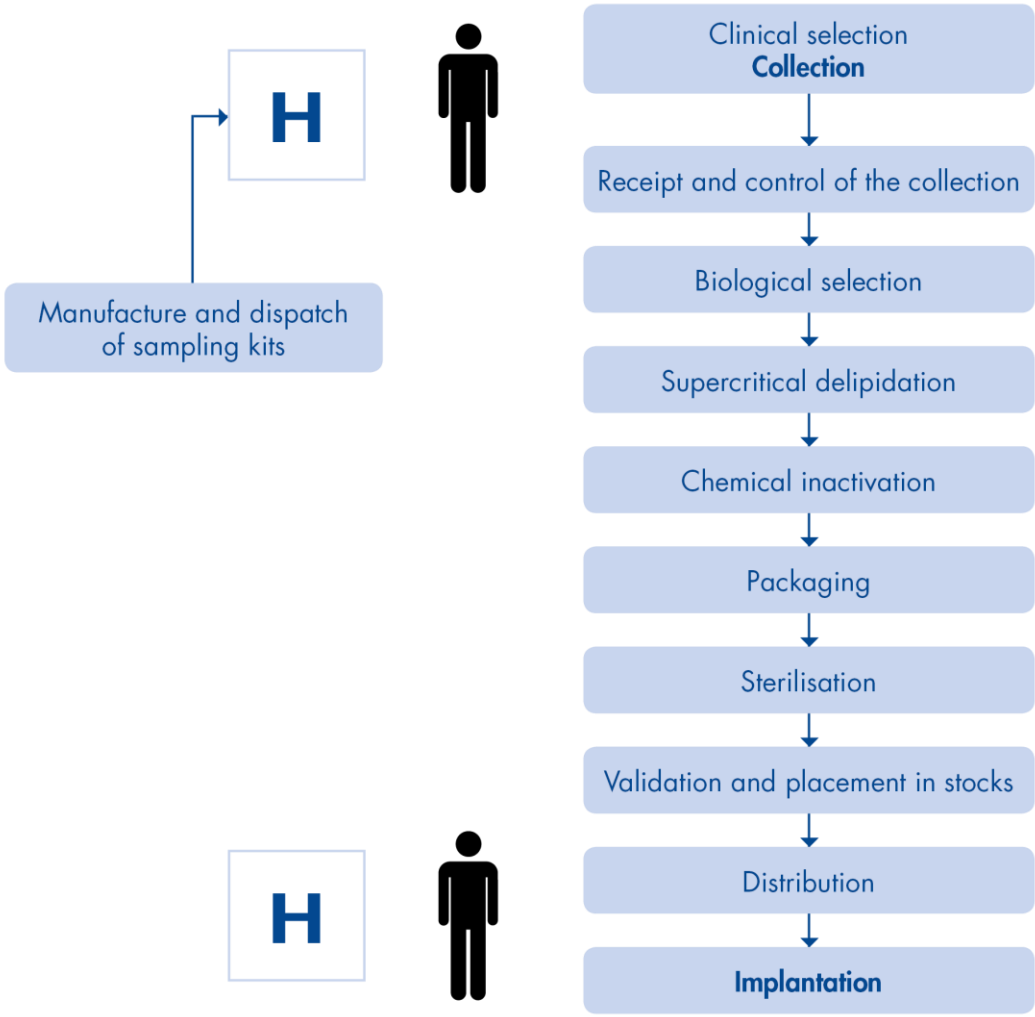
General presentation of the organisation of activities

The tissues processed by BIOBank are femoral heads harvested from patients having a hip arthroplasty. To do this, the tissues are removed during a surgical procedure in the operating theatre, guaranteeing optimal quality and asepsis. All femoral heads are harvested in France by orthopaedic surgeons in accredited health facilities.

BIOBank carries out all of the activities of a tissue bank from receipt of tissues to delivery of safe grafts. These procedures firstly involve donor biological selection and then the conversion of the bone grafts *per se*. All these procedures are carried out in compliance with French Good Practices for tissue banks in accordance to the European Directives.

As applies to all French tissue banks, BIOBank has a Unique Authorisation granted by ANSM which applies to its activities and bone grafts. Specific authorisation has been granted by ANSM for distribution activities in countries outside of the European Union.

The main stages of BIOBank's activities are shown in the flow diagram below.



Collection facility

Human bone tissues are collected by surgical teams in French health facilities with which BIOBank has made a tissue collection agreement. This agreement stipulates the responsibilities and undertakings of the parties involved and describes the donor selection and tissue collection procedures.

BIOBank provides the collection teams with an exclusive sterile kit (Medical Device) containing the parts required for tissue preservation and traceability. This includes the donor consent form and collection form listing the clinical selection criteria.

Donor consent and clinical selection

Clinical selection is an essential stage contributing to the safety of grafted patients. Its details are defined in the good practice requirements for collection of residual surgical material used for therapeutic purposes.

Each donor is therefore managed by the surgical team under the responsibility of the orthopaedic surgeon who is required to:

- Check the donor's medical file,
- Ensure that no regulatory contraindications to tissue collection are present,
- Inform the patient that their femoral head will be recovered for therapeutic purposes,
- Obtain the donor's consent for donation of his tissue, for serological screening and store of the patient's named data in the tissue bank,
- Complete and sign the collection form attached to the bone tissue.

Clinical donor selection observes the exclusion criteria defined by the French Biomedicines Agency (ABM) and set by the ministerial order of 4 November 2014. Compliance with these criteria and investigations for signs of a disease which could contraindicate collection are defined in order to avoid disease transmission.

Tissue collection

During hip arthroplasty the surgeon removes the femoral head and places it in the collection kit following aseptic practice. The femoral head is labelled and then stored without delay at -20°C for a maximum period of 21 days.

Blood sample is taken during the procedure into the tube supplied and is stored without delay at +5°C for a maximum period of 21 days.

Tissue transport

BIOBank organises weekly transport of collected tissues from the health facility to the bank's site.

The tissues are given to the carrier by the operating theatre staff and are placed in a numbered transport container, which is then sealed. A temperature recording probe is incorporated.

The containers are transported by a specialist accredited company in vehicles at defined temperatures:

- Temperature of less than -20°C for femoral heads
- Temperature of +5°C (from +2°C to +8°C) for the blood tubes

BIOBank receives the tissues on D+1 (working days) and checks the transport conditions: integrity of the packs, presence of the seals and temperature compliance.

From reception, the femoral heads are stored at -70°C for a maximum period of 2 years before treatment. They are stored in freezers equipped with a continuous monitoring system and a back-up facility using dry ice in the event of breakdown.

Biological selection

Following validation of the harvested femoral head and transport procedures, the donors' blood is submitted to biological selection investigations.

The legal framework for this selection is governed by the health safety requirements laid down in the French Code of Public Health and the Order of 23 December 2010 on screening for infectious diseases.

The blood tests are performed by an accredited medical analytical laboratory, which is audited and COFRAC-certified. The investigation report is signed by a senior laboratory specialist and lists the methods and reagents used. It is sent to the orthopaedic surgeon to inform him/her so that he/she can inform the donor of the results.

Table of infection markers tested for biological selection:

Disease	Infectious agent	Marker
Hepatitis B	HBV	HBs Ag, Anti-HBc Ab, anti-HBs Ab
Hepatitis C	HCV	Anti-HCV Ab
HIV virus infection	HIV 1 and 2	Anti-HIV 1 + 2 Ab and P24 Ag
HTLV virus infection	HTLV I and II	Anti-HTLV I Ab
Syphilis	Treponema pallidum	Anti-Syphilis Ab

A serum archive is maintained by the analytical laboratory for each serum sample analysed, identified and stored at -20°C for a period of 1 year. The serum archive is used for confirmations or further laboratory investigations using new screening techniques.

The clinical selection and serological screening results are transcribed on the "graft identity form" and the traceability document which is provided with each graft delivered. This form is stored by the surgeon performing the graft in the recipient's medical file.

3. CONVERSION OF THE BONE GRAFTS

Bone graft processing difficulties

Cryopreserved allogenic bone grafts were and are still widely used in orthopaedic surgery for the treatment of large bone defects, particularly during revision surgery of hip and knee arthroplasty. Extension of their indications to small bone filling has promoted the development of techniques intended to offer appropriate forms. In this context, oral implantation bone surgery is an area of use for allografts which is expanding enormously.

Various processing methods have been developed since the end of the 1980s in order to devitalise bone tissue and render it “clean” with respect to three essential required properties: validated active viral safety processes (and not only by donor selection), storage at room temperature made possible by a state of dehydration and osteoconduction which is improved by cleaning the bone framework.

Fundamentally, removal of fats by deep cleaning of the trabecular bone network is the cornerstone for all of the subsequent processing stages and an essential prerequisite for the osteoconduction required by the surgeon.

An innovative technological process using supercritical carbon dioxide has been designed and developed in order to be applied to human bone tissue. This is known as the Supercrit® process.

Principle of the Supercrit® process

The novel innovative of the BIOBank Supercrit® process is that the bone tissue delipidation method is carried out using a non-toxic fluid and supercritical CO_2, combined with chemical oxidation of the residual proteins contained in the pores of the bone.

Apart from viral inactivation, this process aims to remove fats, cell debris and bone marrow proteins, at the same time preserving the organic and mineral structure of the bone tissue which consists of carbonated apatite and type I collagen.

Principle of the supercritical fluid technology:

Beyond a critical pressure and temperature, CO_2 no longer exists in a gas or liquid form but in another state known as a supercritical fluid. Like a liquid, it has a high density and therefore high solvent capacity. This property, which is broadly proportional to density, therefore varies depending on temperature and pressure.

The other beneficial property of supercritical CO_2 is that it has similar transport properties to that of gases because of its low viscosity and high diffusion capacity.

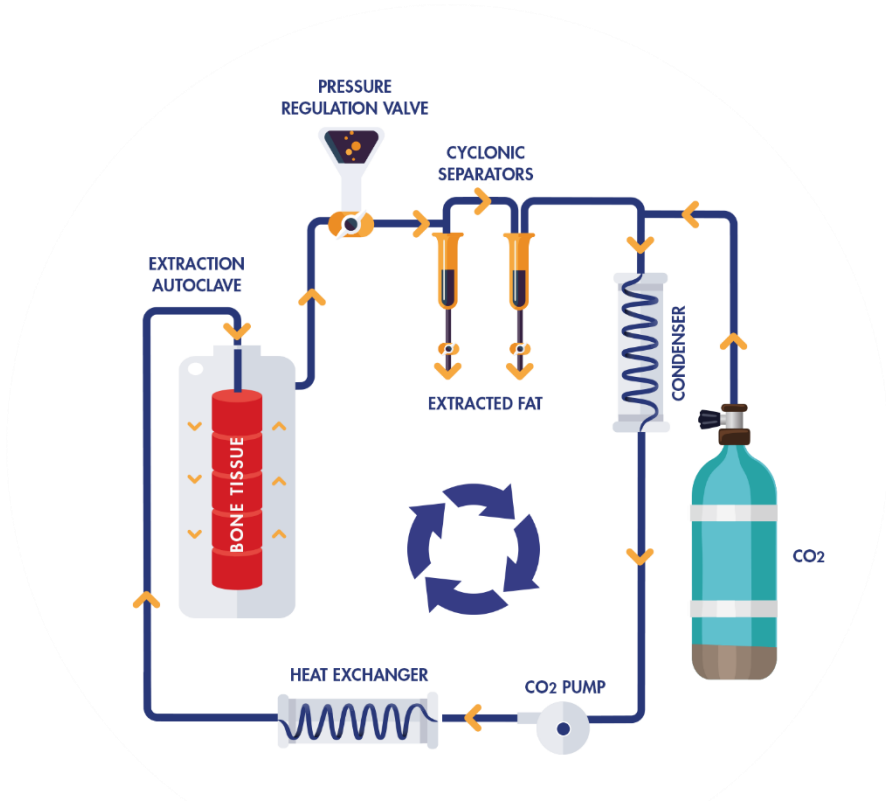
These two properties, high diffusion and solvent capacity, make this fluid extremely useful to extract compounds contained in a porous matrix such as cancellous bone tissue.

CO_2 in the supercritical state is often used as an extraction carrier as it is natural, safe (non-toxic, non-corrosive and non-flammable) and readily available.

In addition, its supercritical parameters are not demanding (critical pressure 7.38 Mpa, critical temperature 31°C) and are straightforward to achieve. The critical temperature also does not degrade the proteins encountered at these temperature and pressure conditions. This is an important property for bone tissue, as any denaturation of the bone protein matrix would result in a considerable reduction in its mechanical properties.

Non-polar molecules such as hydrogen carbides, oils and fats in general are soluble in the supercritical CO_2. Conversely, polar molecules, amino acids and proteins are poorly soluble.

Supercritical CO_2 is therefore a particularly well-suited compound for delipidation of cancellous bone.



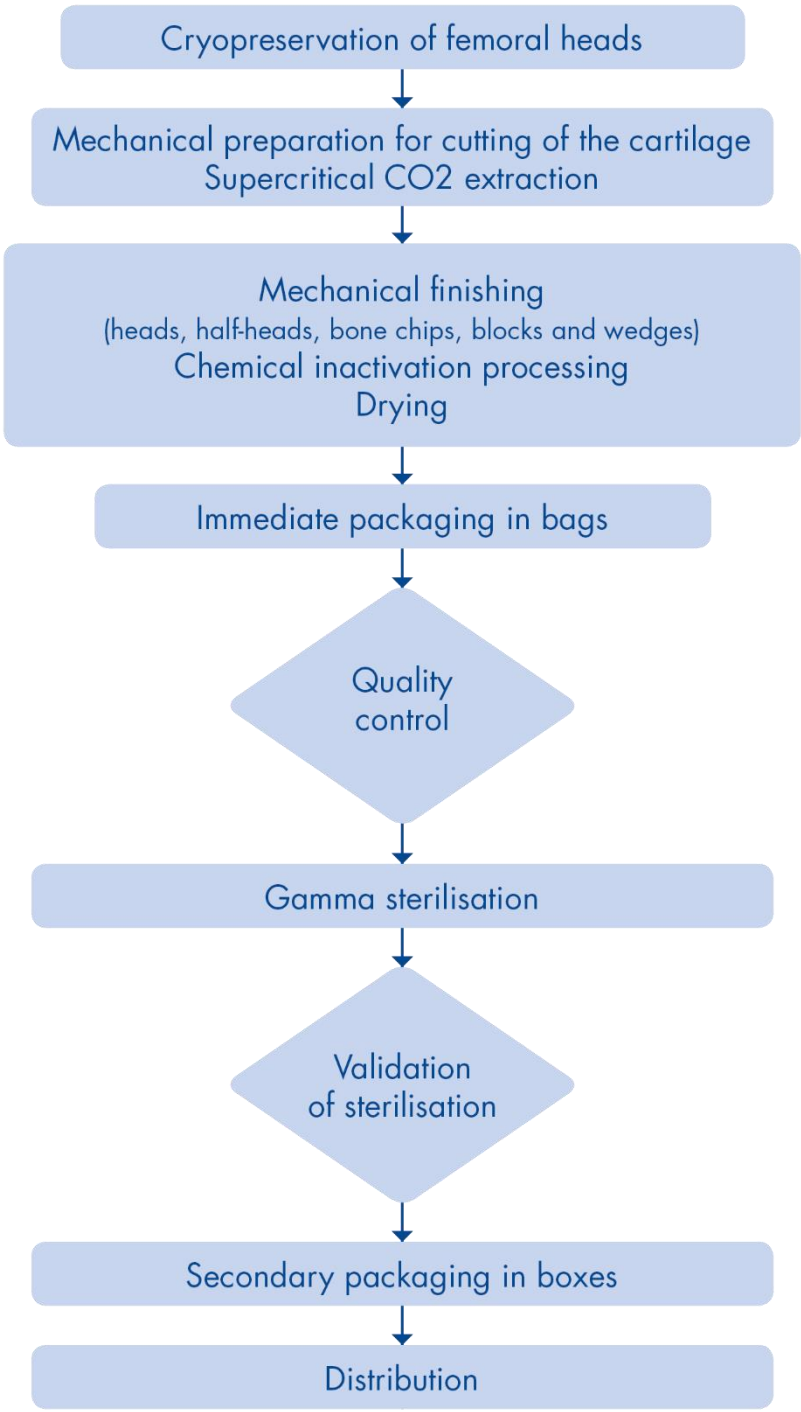
Plan showing the principle of a supercritical CO_2 extraction machine

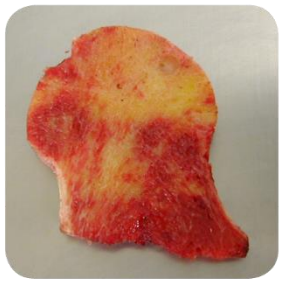
Use of the process

The tissues are prepared in batches of several combined parts, in which each unit is rigorously identified. No “pools” are produced during the process. Between each operation, a routine visual check on tissues is carried out by a senior qualified member of staff.

All tissues are handled in a laboratory which has been specially designed for aseptic tissue processing. The working environment consists of class D/C controlled atmosphere clean rooms (ISO 7), equipped with specific, highly technical material. All of the stages of the BIOBank process, except for gamma sterilisation, are carried out by trained, qualified staff.

Overview of operations:





Frozen half-femoral head

Processed half-femoral head

Mechanical preparation and delipidation with supercritical CO_2

The femoral heads are prepared in order to remove cartilage and osteophytes. This procedure is intended to remove the cartilaginous layer in order to promote penetration and the viral inactivation activity of the solvents.

The bone tissues are then placed in the supercritical CO_2 extraction reactor.

Under the BIOBank process conditions, the supercritical CO_2 is applied at 50°C and at 260 bars. These parameters are checked and managed by an ad hoc industrial machine. The processing time is the time required to pass a sufficient amount of CO_2 to completely degrease the tissues.

At the end of this stage, the femoral heads have lost over 55% of their initial weight and their residual fat content is reduced to under 0.5%.

Mechanical finishing and chemical treatment

Mechanical finishing

The quality of bone tissue varies greatly depending on the age and sex of the donor and on the etiology of the disease which led to the hip arthroplasty. As a result, detailed examination of each tissue is required. This examination is facilitated by the delipidation process and helps to remove damaged or excessively fragile parts of the femoral head. Some tissues are reduced into geometrical blocks or converted into bone granules.

Chemical oxidation with hydrogen peroxide

After delipidation, some membrane debris remains within the cancellous framework cavities which may cause specific antigenicity. An oxidation stage using 35% hydrogen peroxide is used to clean the pores of protein and cell residues.

Molar soda treatment

The maceration stage using molar soda (4% w/v) at room temperature is used in order to meet the French guidelines: DGS/VS2/SQ3/93/26 which restates a recommendation of the European Commission directive: *guidelines for minimizing the risk of transmitting agents causing spongiform encephalopathy via medicinal products*.

Molar soda is known to inactivate prions in infected tissue and transmission of the infectious agent. This treatment is theoretical for the bone tissue as bone tissue is a WHO classification class IV tissue, i.e. inoculation of a prion does not result in the tissue becoming contagious and therefore does not enable an assessment of the efficacy of treatment on this tissue.

Ethanol treatment

Rinsing in ethanol solutions is intended to dehydrate the bone tissue and leave a low bioburden. Ethanol is conventionally used in all inactivation procedures for bone allografts.

Drying

Drying in hot air (40°C) then enables the bone tissue to be dehydrated in depth, without adversely affecting the structure and mechanical properties of the bone.

Packaging and quality control

The grafts are packaged in a double packaging which excludes entry of microorganisms.

Production quality control is carried out to guarantee that product specifications are met.

The production batches are released with a view to end-stage sterilisation following a measurement of bioburden in compliance with the terms of Standard ISO 11737-1.

Gamma sterilisation

Sterile products are obtained through the use of gamma irradiation at a sterilising dose of 25 to 30 kGy on grafts which have been protected by a double immediate packaging. This stage is performed by a service provider which is accredited for the sterilisation of medical devices.

Sterilisation is validated in accordance with Standards ISO 11137 and ISO 11737.

The lack of heating of the material and the absence of toxic residues are additional evidence justifying the choice of this method.

Finally, the BIOBank Supercrit® process is particularly suitable for bone tissue and specifically for femoral heads in order to achieve maximal microbiological graft safety, at the same time preserving the intrinsic properties of the human bone tissue.

Validation of grafts before distribution

A systematic review of the production files is carried out by BIOBank's Senior Pharmacist. This leads to approval for placement in stock and the grafts being made available for therapeutic use.

Outer packaging and storage of grafts

The sterile finished products are packaged in a single unit box containing the regulatory traceability documents and instructions for use leaflet.

A label showing the required details is placed on each box.

All of the documents and labels refer to the unique identification number for each graft, or Single European Code (SEC), in accordance with the European Directive 2015/565.

The BIOBank grafts are stored at room temperature (+10°C to +30°C) for a maximum period of 5 years after sterilisation.

The finished products are stored in a protected place at a controlled temperature, access to which is restricted to authorised members of staff.

Distribution and transport

The grafts are distributed to surgeons in France on a nominative medical prescription, following Good Tissue Practice requirements.

Distribution to the tissue banks in both France and the European Union are based on a request form sent by the senior medical officer of the partner facility.

For countries outside of the European Union, the grafts are exported according to specific requirements.

The grafts are transported to final users at between 0°C and +40°C.

4. PRODUCT TRACEABILITY

Information system

Tissue traceability is an essential component in the management of tissue banks. This is managed by a specially designed information system with locks and rights of access which are essential for the activities of a tissue bank.

The “tissue and cell banks” software is from a company which specialises in producing software solutions for health professionals, designed for blood transfusion centres and medical analytical laboratories.

All of the tissue data from sampling to implantation are stored by BIOBank for 30 years after grafting.

Tissue traceability during conversion

Tissue traceability has three purposes:

- Ensuring traceability from the donor to the recipient,
- Preserving the anonymity of the donor and the recipient,
- Guaranteeing the history of the BIOBank activity data.

Registration of named data by BIOBank is subject to authorisation granted by the French National Data Protection Commission (CNIL).

Tissue traceability is centred around a unique, anonymous identification number, which is allocated at the time of collection and associated by a product code depending on the procedure.

Links exist between these different numbering systems and between the tissues and the preparation conditions (tissue file, production file, ancillary therapeutic products, consumables etc.). These links enable the whole history of the collection, selection, storage, conversion and transfer of marketing rights of the product and biovigilance to be traced.

The information required for traceability are transcribed onto labels and documents provided with the tissues throughout their lifecycle, observing anonymity of the donor and of the recipient.

Traceability documents

Documents ensuring traceability consistent with regulatory requirements are divided in two categories.

Documents between sampling collection facilities and BIOBank for traceability of the femoral heads and blood samples:

- Collection form signed by the surgeon who collects the sample

- Traceability labels placed on the sample kit

Documents between BIOBank and the users for traceability of the validated bone grafts (documents included in the graft outer packaging box):

- The **"Graft identity form"**, certified by the responsible person of BIOBank staff restates the information required for traceability (origin, characteristics, clinical data, results of serological screening, preparation conditions, etc.)

  This form must be stored in the patient's medical file

- The **"Implantation form"**, which must be completed by the surgeon concerning the conditions of use and must be returned to BIOBank by email or fax;

  This form must be returned to BIOBank who manage tissue traceability

- The traceability labels placed on the graft to facilitate updating of the medical file, and to update a deposition register.

Biovigilance

According to French regulations, the national biovigilance system is based on reporting by health professionals of:

- Serious adverse events: incidents occurring during the chain from collection to patient administration,
- Adverse effects (regardless of severity) occurring in living donor patients and recipients.

The surgical team which identifies the incident must declare as soon as possible using the biovigilance form, with the assistance of his local facility's biovigilance representative. The form is sent to the French Biomedicines Agency (ABM) biovigilance unit. A copy of this form should be sent to the BIOBank local biovigilance representative.

As soon as BIOBank has been informed of the incident, an investigation is triggered in order to establish the causes, correct the problem and avoid any repetition. If non-compliant products are involved, the tissue recall procedure is then done under the authority of the responsible person of BIOBank staff and the results are submitted to the health authorities.

5. In vitro characterisation and preclinical validation

Viral inactivation

Two viral inactivation studies have been conducted by the Pasteur-Texcell laboratory. The results of these demonstrate the efficacy of the four stages of the Supercrit® process in inactivating the most resistant viruses including Parvovirus in the centre of the whole femoral head.

Integrity of bone tissue characteristics

- Biochemical analysis: mineral composition (Ca, P, Mg, Na) and organic composition (hydroxyproline, glycine, alanine and proline) which are identical to unprocessed bone tissue
- Scanning electron microscopy examination: complete removal of cellular and bone marrow components and correct conformation of the collagen fibres
- Histological analysis: unchanged organic mineral framework and bone architecture
- Immunohistochemical analysis: no denaturation of type I collagen

Effectiveness of cleaning

- Fat content reduced from 60% to less than 0.5%
- Water content under 4%

Preservation of mechanical properties

Three studies have been conducted in order to assess the influence of the Supercrit® process on the mechanical properties of the canalicular bone tissue:

- Compression tests, performed on 2 paired series of 100 treated bone samples compared to fresh bone determined the biomechanical indices of the BIOBank bone grafts prior to radio-sterilisation, with a maximum rupture resistance of 10.2 ± 5.2 MPa and an elasticity module of 412 ± 149 MPa. No statistically significant differences were found with fresh bone (6).
- Ultrasound analysis of the influence of gamma irradiation dose concluded that there is no difference between 10 and 25 kGy on the elasticity module of the dehydrated canalicular bone tissue compared to fresh unirradiated bone. These results help to demonstrate the efficacy of the end stage cell sterilisation method for bone allographs in light of its effectiveness (7).

- Different cancellous bone treatment processes have been compared by paired sample ultrasound analysis, before and after processing. These demonstrate that the Supercrit® process has no significant effect on the elasticity of the cancellous bone tissue and optimally preserves the structural and architectural qualities of the bone (8).

Evaluation of biocompatibility and bio-functionality

Toxicity tests have shown no toxic residues to be present on the products after the process:

- Negative cytotoxicity test
- Negative systemic toxicity test
- No pyrogens present
- Heavy metal content compliant with ASTM Standard F 1088-87 (Pb≤30ppm-Hg≤5ppm)

Osteoconduction and in situ intraosseous tolerability have been assessed in an animal implantation study. Bones from allogenic ewes (a treated series compared to an untreated series) were implanted in the acetabular site in 12 animals and examined histologically at 1, 4 and 8 months (4 animals per period) in order to assess bone healing and tissue tolerability. The results of this study showed better tolerability and both greater and faster bone apposition for the treated samples. The quantitative indices were all greater for the treated series, demonstrating more active bone remodelling (4, 5).

6. PRODUCT RANGE

The grafts are available in different forms belonging to 5 product categories approved by ANSM:

Anatomical forms

Granule forms

Geometrical forms

Powder forms

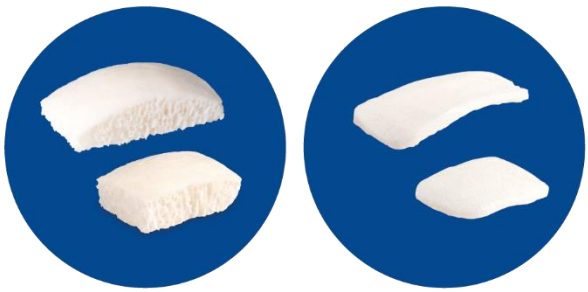


Plate forms

The BIOBank grafts are available in various forms suitable for different indications which surgeons encounter in restoring their patients' bone mass.

Category	Form
Anatomical forms	- Whole femoral head - Half-femoral head
Geometrical forms	- Osteotomy wedges, 6°, 8°, 10°, 12° and 14° - Blocks 20x10x10 mm and 30x20x10 mm - Dowels 9, 10, 11, 12, 13 and 14 mm
Granule forms	- Vial presentation: 7, 18 and 25 cc - Syringe presentation: 7, 18 and 25 cc
Powder of cancellous and cortico-cancellous bone forms	- Vial presentation: powder of cancellous and cortico-cancellous bone, 0.5 mm in 0.5, 1, 2 and 4 cc - Vial presentation: powder of cancellous bone, 1 mm in 0.5, 1, 2 and 4 cc - Syringe presentation: powder of cancellous and cortico-cancellous bone, 0.5 mm in 0.5, 1 and 2 cc
Plate forms	- Cortico-cancellous plate, 15x10x4 mm and 22x12x4 mm - Cancellous bone plate, 12x10 mm and 18x10 mm

7. REGULATORY AUTHORISATIONS

Authorisations relating to BIOBank's activities

BIOBank is a human bone tissue bank authorised by the French National Agency for the Safety of Medicines (ANSM) to prepare, store, distribute and offer virally inactivated bone allografts required for orthopaedic surgery and dental surgery.

BIOBank carries out all of the activities of a tissue bank as stipulated in article L.1243-2 of the French Code of Public Health (it has been authorised since 29 September 2003) from the receipt of tissues harvested to the delivery of safe grafts. These stages involve in particular, donor selection using regulated clinical and biological criteria and viral inactivation *per se* of the bone grafts through the implementation of the Supercrit® process.

All of these operations are carried out in compliance with Good Tissue Practice requirements laid down by the ANSM decision of 27 October 2010 and the French Code of Public Health, the articles in which transpose European Directives 2004/23/EC, 2006/17/EC and 2006/86/EC.

The BIOBank grafts are obtained from femoral heads harvested exclusively from living donors during hip arthroplasty. All of the femoral heads are taken exclusively in France by orthopaedic surgeons in accredited health facilities.

As for any French tissue bank, BIOBank holds a Unique Authorisation granted by ANSM which firstly governs the activity of the facility and secondly governs the preparation processes used, grouped together by families in terms of type of graft (anatomical, geometrical, granules, powders or plates).

BIOBank's Unique Authorisation granted on 10 February 2017 carries the number: BT/16/O/014.

Authorisations relating to importation and exportation

As a European Tissue Facility, BIOBank is authorised to export its products to member states of the European Union (EU) belonging to the European Economic Area (EEA). This authorisation has been extended to some third party countries outside of the EU and EEA.

BIOBank also has authorisation to import femoral heads taken from living donors with a view to re-exportation of the bone grafts which have been virally inactivated by the Supercrit® process to the country of origin of the bone grafts. This authorisation is restricted to tissue facilities which have agreed a contract with BIOBank and for which implementation is subject to prior authorisation from ANSM.

Biobank is listed on the European register of tissue facilities under the number: FR07701T (https://webgate.ec.europa.eu/eucoding/reports/te/index.xhtml).

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