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# Bone Banks: Technical Aspects of the Preparation and Preservation of Articular Allografts

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For the technical aspects of this subject the Marseilles team collaborated with the Etablissement de Transfusion Sanguine Alpes-Provence (Alpes-Provence Blood Transfusion Service) to set up a bone bank on their premises because it has a competent cryobiology department equipped with storage tanks containing liquid nitrogen and a temperature-lowering programmer. This laboratory, which for a long time has been storing bone marrow, platelets, and various cryopreserved tissues, has the virology, bacteriology, quality control, and quality assurance laboratories of the Blood Transfusion Service and is accustomed to applying the transfusion safety standards. It was also one of the first in France to obtain the approval of the Microbiological Safety Committee of the Directorate General of Health in April 1996. Banks which were developed nationally have followed the same principles and currently more a hundred have been registered and some are awaiting authorization.

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## The Removal of Articular and Osteocartilaginous Grafts

Because the bone and cartilage fragments which are removed are not subjected to secondary sterilization, it is imperative that therapeutic maneuvers are performed in wholly sterile conditions.

## Selection of the Donors

Selection has to be rigorous so that there is no risk of the transmission of iatrogenic pathology to the host through the graft. This requires adequate knowledge of the history of the illness and the circumstances of the accident, as well as the medical history of the donor. There are many absolute contra-indications. Subjects with a cancerous condition, a systemic illness, collagenosis, an auto-immune disease, or bone dystrophy may not have tissue removed for grafts. We systematically eliminate from the list of donors those suffering from a viral, bacteriological, or parasitic infection, mycosis or tuberculosis as well as those with risk factors and those who have been on artificial ventilation for more than 72 h in intensive care, as they are potentially infected. As far as the validation of the grafting material is concerned, the banks have to conform to the legislation in force decreed by the French Graft Institute.

Bacterial decontamination is performed using a solution of antibiotics consisting of rifocine and chloramphenicol.

Samples are taken from each graft before and after decontamination and after thawing. Positive bacteriological results will mean that the tissues will have to be destroyed.

Blood samples have to be taken from each donor (live or deceased) in order to perform the obligatory virological examinations.

In accordance with the decrees of 25 February, 1992, of 24 May, 1994, and of 24, July 1996, medical biological analyses are performed to test for infection:

by the hepatitis B virus (HBs antigen and anti-HBc antibodies),  
 by HIV (antigen P24 and anti-HIV 1 and 2 antibodies),  
 by HTLV (anti-HTLV 1 and 2 antibodies),  
 by hepatitis C (anti-HCV antibodies),  
 for detecting syphilis in two different tests (VDRL and TPHA),  
 for assaying for transaminases for the live donors.

Decree No. 97-928 of 9 October, 1997 removes the obligation to carry out a search for the agent responsible for toxoplasmosis, for infection by cytomegalovirus, and by the Epstein-Barr virus. The Decree of 1 April, 1997 requires that the results of the examinations performed are examined before the patients have been transfused, as any hemodilution could falsify the tests. Finally, the French Blood Agency and the French Graft Institute recommend that the tissues are placed into quarantine and that the virological tests are repeated 4-6 months after the tissue has been removed. These samplings are performed either on the live donor or on the host of organs coming from the same donor and are performed to reduce the risk in the event of the tissue having been removed during a seroconversion phase. However, placing these products into quarantine is only one of the possible ways of ensuring safety. A decree will specify the conditions under which it is to be performed in the various situations according to the other methods which could be used, in particular directly testing

for the viruses by molecular biology techniques (PCR).

## Removal Techniques

As the risk of infection is the main concern in this surgery, the various stages of surgery must be performed under the strictest aseptic conditions possible. The tissue therefore has to be removed in an operating theatre, according to the same principles as regulated orthopedic surgery, and it is considered that a maximum time lapse of 6 h from the circulation stopping can be reasonably accepted. For joint removals, the whole of the joint capsule is preserved as well as the intra-articular ligaments and the menisci or labra. In the case of the knee, and in order to keep the extensor apparatus intact, we retain the whole of the patellar tendon continuously with the posterior half of the patellar joint. This is also continuous with the quadriceps tendon, the upper part of which is cut into an inverted V. The muscular insertions are scraped and the surgeon removing the tissue cleans all the bone attachments of the ligaments allowing the capsule to be refixed firmly. The part is then placed in a bag which is resistant to very low temperatures (captonteflon bags). The reconstruction of the skeleton is one of the important stages of the removal. It is a legal obligation and it has to be as perfect as possible.

## Coding and Measuring of the Parts

In order to find the desired bone fragment again easily in the bank, it is necessary to fill in the data sheet carefully and to perform X-rays without enlargement or with an enlargement control placed side-by-side with the bone part.

## Quality Controls

All the bone banks have to be inspected periodically, and samplings are performed on a very regular basis (20 % of the grafts are rejected annually,

either immediately after having been removed on account of positive results being found in tests or subsequently at the 6-month checks).

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## Preservation Techniques

### Preservation Methods

Many preservation methods have been suggested since the use of allografts was first considered. The techniques of irradiation and sterilization by moist heat will be described in detail subsequently. Cryopreservation is the only current procedure which makes it possible to preserve bone fragments and in particular cartilage cells safely.

Preserving fluids (antiseptics, 1 % sodium methyolate,  $\beta$  propriolactoses), as well as ethylene oxide are cytotoxic and the problems involved in handling them have meant that they have been abandoned. The same applies for the methods involving sterilization by boiling.

Drying under vacuum or lyophilization uses plasma preservation processes. These grafts, which are theoretically usable indefinitely at ordinary temperatures, are fragile and are not sufficiently strong in mechanical terms to withstand the usual mechanical stresses and, in particular, all the cells are destroyed preventing the use of friction surfaces.

Irradiation of bone fragments taken under conditions which are not sterile is recommended by some teams who see a practical advantage for taking grafts. However, not only does this irradiation, conventionally performed at 25 K Gray, not guarantee perfect viral sterility but it also causes the destruction of all the cells.

Since 1981 we have been using cryopreservation of massive osteocartilaginous grafts in liquid nitrogen at  $-196^{\circ}\text{C}$ . This method makes it possible to preserve whole bones and complete joints over an extended period as it preserves the viability of the cartilaginous cells, the fibers, and fibroblasts contained in the ligaments and capsules.

Without going into the technical details of cryopreservation and storage, we would like to underline the fact that tissue preservation has to obey two essential rules:

uppression of cadaveric disintegration phenomena, preservation for an extended period of the architecture of the bone and preservation of the viability of the cartilaginous cells.

To avoid the formation of ice macrocrystals, it is necessary to impregnate the bone, cartilage, and ligament tissues with a suitable cryoprotector. At Marseilles, we use a mixture of macro-molecules (4 % human albumin which is to be replaced by Héloes) and 10 % final DMSO. This solution is maintained at a temperature of  $4^{\circ}\text{C}$  because DMSO is toxic. In theory, the "deep cold" should enable these objectives to be reached by stopping the action of the tissue enzymes. If relatively moderate freezing temperatures are used (higher than  $-20^{\circ}\text{C}$ ), the enzymes present in the tissue are not inactivated and will destroy the architecture of the graft in a few weeks. At  $-80^{\circ}\text{C}$  (limit of electric freezers), although the enzymatic activity is clearly reduced, it is not totally stopped, only collagenase is inactivated at this temperature. At  $-196^{\circ}\text{C}$  all the enzymes are inactivated and the proteins can be preserved over an extended period. On the other hand, DMSO has a eutectic point around  $-60^{\circ}\text{C}$ , at this temperature, the microcrystals of ice can recombine into macro-crystals and make the cells burst. For the same reasons, the temperature of the graft cannot be lowered in an haphazard fashion, and it is necessary to vary the rate at which this takes place in accordance with the temperature obtained. The optimum curve seems to us to be  $2^{\circ}\text{C}$  per minute down to  $-40^{\circ}\text{C}$  then  $5^{\circ}\text{C}$  per minute down to  $-140^{\circ}\text{C}$ , the temperature at which the graft is then placed in nitrogen vapor, directly in the tank where it is stored.

Thawing, on the other hand, has to be rapid so that the largest number of cells remain alive. Physiological serum or Ringer's lactate solution at  $40-41^{\circ}\text{C}$  will be used to thaw out and wash the grafts in order to eliminate the DMSO. Approximately 2 h after having removed the bone fragments from the liquid nitrogen tank, they are usable for a period of approximately 24–26 h, which means that they can be taken to any part of France and Europe. However, donors are becoming rarer and rarer and it is becoming increasingly difficult to obtain tissue.

## Biomechanics and Immunology

The mechanical strength of the cortical allograft is only 50–60 % of the strength of normal bone during a period ranging from the 8th to the 18th month after the graft has been implanted (on account of the revascularization of the bone). Maximum fragility is at around the 12th month and it is only after 2–3 years after the graft has been implanted that the bone regains normal density and biomechanical strength. The fixation of the graft therefore has to be complete in order for this period of fragility to come to an end. The mechanical properties of the allografts can be changed by the preservation and storage processes. Lyophilization, massive irradiation of the grafts (in excess of three megarads), or moist heat used for more than 60 min at 120 °C adversely affect the mechanical behavior of the grafts considerably. Cryopreservation, on the other hand, seems to improve the mechanical properties of the allograft, the strength of which is 110–120 % that of fresh bone but the graft, although it is stronger, does in fact become more brittle (in the mechanical sense of the term) and therefore breakable. In the case of articular allografts, the crucial point is preservation of the ligament structures, of the synovial fluid, and of the meniscus. The cells in these formations are preserved, as are their architectural and fundamental structure.

After having been reinstated, the vascularization is linked to the revascularization of the bone insertion area. It is inadequate for several months (or years) which means that they have to be doubled with an artificial ligament during this period to avoid excess stresses which would lead to their being stretched or even ruptured. The extent of the immunologically competent tissue (synovial, capsule, etc.) also risks leading to the occurrence of immunological rejection phenomena justifying the use of immunosuppressants (such as Sandimmun) in the event of the hyperproduction of fluid indicating an immune response.

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## Transport

Transporting bone parts over long distances will require the use of special containers, in which grafts will be preserved at low temperatures

(liquid nitrogen or dry ice). When it is anticipated that these grafts will be used within 24 h following removal from the Bank, it is preferable to thaw the bone fragment and to dispatch it only after thawing. Once removed from liquid nitrogen, the graft has to be used within 24 h and it is not possible to refreeze it again if it is not used.

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## Use

The best indications for using these osteochondral or complete joint grafts are in the knee, ankle, shoulder, elbow, and wrist.

## Isolated Osteochondral Grafts

Partial or total graft of the femoral condyle,  
graft of the tibial plateau,  
graft of the patella,  
graft of the tibial pylon,  
partial graft of the humeral head,  
partial graft of the elbow,  
graft of the inferior radius.

The graft is fixed by osteosynthesis material and the ligaments of the host are refixed onto the graft. The functional results are generally excellent.

## Osteochondral Graft Plus Ligaments from the Donor

The excision is larger and the donor ligaments remain attached to the graft and are refixed onto the host bone or over the ligaments of the host. They have to be protected during the period of revascularization by artificial ligaments.

This applies to:

massive grafts of the inferior extremity of the femur,  
massive grafts of the superior or inferior extremity of the tibia,  
grafts of the humeral head,  
partial grafts of the elbow.

## Complete Articular Grafts

A total joint graft with its capsule, its synovial membrane, its ligaments, and the fibrocartilages it contains (meniscus or labrum) is indicated in the case of an extensive tumoral lesion in the joint cavity justifying extensive excision in a single piece.

The problems posed are twofold:

**Immunological:** connected with the size of the immunologically competent material implanted with apparently an inflammatory reaction which could result in a cutaneous fistula giving rise to an infection.

**Mechanical:** the ligaments must not be strained during the period of their revascularization and have to be doubled up by artificial ligaments.

## Reconstruction Prostheses Sheathed with Bone from a Bone Bank

The indications for using a prosthesis sheathed with bone from a bone bank are often present and

have to be analyzed according to the extent of the loss of musculo-ligament and cutaneous substance which requires tumoral excision or which has been produced by the trauma.

### Conclusion

It seems to us to be important to emphasize the fact that grafts have to be preserved without breaking the cold chain, without damaging the bags containing the graft, and under strictly technological conditions. The importance of the sterile environment for removing the graft has to be emphasized; for example, out of more than 5,000 parts preserved in the Blood Transfusion Center in Marseilles since 1981, we have had to destroy barely 2 % due to a super-infection being found. These encouraging results are due to extreme rigor in the ways in which the grafts are removed and the patients are selected. In our opinion, these preservation methods are the only ones which allow the bone, and particularly the cartilage, to still be guaranteed a normal structure and also good mechanical properties.

## Appendix

### Recommendations for Setting Up a Tissue Bank for the Locomotor Apparatus

These recommendations are those used by the Blood Transfusion Center of Marseilles to, among other things, preserve bone, cartilage, and ligament grafts.

### The Organization of a Tissue Bank

#### General

The need for:

a tissue removal team approved by the Ministry, a geographical location for treating, storing, and making the tissue available.

#### Equipment

A preservation department equipped with storage tanks or apparatus, a temperature-lowering programmer indispensable for preserving bone, cartilage, and ligament tissue. Laboratories experienced in the following quality controls:

donor control,  
tissue control,  
validation of the preservation techniques.

#### Personnel

At the hospital:

the person in charge of tissue removal checks that removal from a subject in a state of brain death is in line with the regulations, the surgical teams remove and treat the tissue.

At the tissue bank:

the bank receives the tissue,  
the technical staff at the bank treats the tissue,  
the laboratories perform viral serological tests on the donor and a bacteriological examination of the tissue removed,  
the bank's medical supervisor oversees everything.

#### Techniques

For each tissue, all the technical aspects of the removal, treatment, storage, quality control, distribution, and results of examinations are recorded in a manual updated regularly.

#### Information

Information on the donor:

Identity, sex, age;  
Radiography of the tissue if necessary;  
Cause of death;  
Medical history;  
ABO blood group and HLA if known;  
Operating protocol of removal, therapies used;  
Results of any laboratory examinations;  
Results of the control examinations;  
Tissue removal center and department.

Information on the host:

Identity, sex, age;  
Origin of the graft;  
Attribution criteria;  
Identification of the use of the graft, site and date;  
Possible response to implantation of the graft;  
ABO blood group and HLA if known;  
Results of the culture at the time of the graft;  
Note any departure from the guidelines for handling and reconstitution;  
An estimate of the clinical results.

#### Quality Control

Each tissue preservation department has to take part in the development of the methods which make it possible to evaluate the indications of the tissue grafts preserved. Periodical monitoring of the bacteriological status has to be practiced, checking, before they are dispatched, at least 5 % of the grafts every 6 months and more if problems with bacterial contamination are suspected.

#### Tissue Removal

##### General Ethical and Legal Considerations

In general, acceptable sources of tissues are cadavers less than 6 h after circulation has stopped, patients in a state of brain death, and patients who

have had part of their tissue removed for therapeutic purposes (femoral head). The Caillavet law considers a donor to be any person who, during his lifetime, did not express any opposition to removal of his or her tissue. It is difficult, in practice, not to consider the pain suffered by those close to the person they have lost.

#### Selection Criteria

These vary depending on the tissue removed. Age may be a limiting factor following the use which will be made of the tissue taken. For cartilage in particular, it seems to be necessary to graft only normal joint surfaces which have been taken from healthy subjects.

Tissue may be removed from the cadaver for 5 h after his or her death if it is kept at ambient temperature and for approximately 12 h if the cadaver is stored at 4 °C immediately after death. The tissue removed from a live patient can be placed in a container, closed immediately and refrigerated at 4 °C. A valid preservation technique can be considered to be up to 12 h after the tissue has been removed and stored at the preservation temperature.

A medical history of the donor has to be sent. Potential donors will be excluded if their current medical history mentions:

- developing septicemia,
- a localized infection in the tissue to be removed,
- a slowly developing viral episode
- malignant neoplasia except for most of the cerebral tumors,
- the existence of active hepatitis or unexplained jaundice,
- systemic disease,
- a patient belonging to the risk groups,
- heavy irradiation on risk groups,
- treatment with drugs which are toxic to the tissue to be removed.

Laboratory tests have to be performed on the blood of the cadaver or on the live donor:

- a test for the hepatitis B virus,
- a test for syphilis,
- a test for HIV antibodies,

- the transaminase levels,
- a test for anti-HBc antibodies,
- a test for anti-HCV antibodies,
- a test for anti-HTL V1 and anti-HTL V2 antibodies
- a test for anti-CMV antibodies.

The erythrocyte blood groups and tissue groups should be used and a serum bank should be set up.

Wherever possible, the tissue should be removed under sterile conditions in an operating theatre. If the allografts are removed in a non-sterile manner, it should be ensured that effective sterilization techniques can be used without damaging the tissue structure.

If a collecting medium is used, it has to be sterile and physiological.

If antibiotics are used, the bacterial cultures have to be grown before they are added and the type of antibiotics has to be clearly recorded. A final bacteriological check is recommended before the tissues are dispatched.

Fragments of tissues to be grafted have to undergo bacteriological and fungal studies using current methods and media. Cultures of the donor blood have to be carried out when the tissue is removed as well as a urine culture and possibly also a culture of a pleural effusion.

Secondary sterilization. If it is carried out, the biological and biochemical integrity of the graft has to be maintained. The methods used for decontaminating surfaces are acceptable if only the surface can be contaminated.

#### Preservation and Storage

The methods used to preserve and store tissue allografts vary according to the type of tissue and the clinical application in which they are included. Although the optimum methods have not been defined, the best for long-term preservation would appear to be preservation at very low temperatures (−80 °C). Continuous monitoring of the temperature may be necessary. Storage for 12 h at the most at 4 °C may be practiced from the time the tissue was removed. Only materials which are resistant to low temperatures are suitable for this type of preservation. They have to be sterile. The culture media may vary and have to

be defined for each type of cell. Precautions have to be taken to check the persistence of the activity of the cells being cultured and for the absence of contamination.

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