Tissue and Cell Banking

Department of Transplantology and Central Tissue Bank, Center of Biostructure Research, Medical University of Warsaw, ul. Chalubinskiego 5, 02-004 Warsaw, Poland

Grafts

Autogenic - the transplantation of cells, tissues, or organs from one site to another on the same body (donor = recipient)

Izogenic - the transplantation of cells, tissues, or organs to a recipient from a genetically identical donor of the same species

Allogenic - the transplantation of cells, tissues, or organs to a recipient from a genetically non-identical donor of the same species

Xenogenic - the transplantation of cells, tissues, or organs to a recipient from a genetically non-identical donor of the different species

I. BIOVITAL = ALIFE

- must function immediately after transplantation

IA: BIOVITAL VASCULARISED - ORGANS
- cannot be preserved
- cannot be sterilised
kidney, liver, heart, pancreas \(\Rightarrow\) transplantation after few hours

IB: BIOVITAL NON-VASCULARISED
- can be preserved and stored,
- cannot be sterilised (decontamination with antibiotics)
cornea \(\Rightarrow\) few days, one month
heart valves, blood vessels \(\Rightarrow\) few months, years
cells \(\Rightarrow\) years
only stored: hematopoietic stem cells of bone marrow or cord blood
cultured in vitro: keratinocytes, chondrocytes, fibroblasts, osteoblasts, limbfibrocytes, monocytes, limbal cells, MSC, MNC, etc.

II. BIOSTATIC = WITHOUT LIVING CELLS

- during processing and preservation procedures cells are killed and often removed
- importance of extracellular matrix - scaffold of fibers (e.g., collagen type I) proteins, including cytokines.
- can be sterilised and stored for long time
- biological prosteses/biological dressing
- are gradually replace by recipient's own tissues
- connective tissue grafts:
  - bone, cartilage
  - tendons, ligaments, menisci
  - skin
  - amniotic membrane
  - pericardium

Allogenic grafts (allorgrafts)

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Tissue and cell banking

Europe
ok. 4,500 t&c banks
UE
-1,388 ART banks
-1,411 HSC banks
-1,115 t&c banks

Poland
-7 ART banks
-25 HSC banks
-18 t&c banks

USA
-261 tissue banks
-110 tissue banks AATB accedited
-unknown number of cell banks
Tissue and cell banking

I – donor identification, donation, initial screening, tissue and cell procurement (tissue bank or procurement organisation)

II – testing – further donor evaluation (biological testing of blood), testing of procured tissues and cells. Decision of release of procured tissue for processing or its disqualification and utilisation

III – processing – preparation of tissue and cell grafts (incl. sterilisation)

IV – conservation and storage

V – distribution of grafts

VI – feedback information / traceability

Cell and Tissue Banks

Article 25
Cell and tissue banks shall be established with the aim of gathering, processing, sterilizing, storing and distributing tissues and cells appropriated for transplantation.

Article 26
1. The duties referred to in article 25 shall be performed by a cell and tissue bank after obtaining of a permission given by the minister competent to do with health matters to perform these duties.
2. A cell and tissue bank shall submit an application for the permission referred to in paragraph 1 to the National Centre for Tissue and Cell Banking. The minister competent to do with health matters upon an application from the National Centre for Tissue and Cell Banking and after the National Transplantation Council pronounces its opinion shall give the permission referred to in paragraph 1.
3. The permission referred to in paragraph 1 shall be given for five years.

Multitissue banks

Monotissue banks
### Public cord blood banking in Poland

#### 2013 - allo related

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### Private cord blood banking in Poland

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### Private cord blood banking in Poland

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Donor concentration

Donor evaluation

- Social and behavioural information, including travel history
- Medical history
- Family history of genetic disease
- Physical examination
- Serology and microbiological testing and other additional complementary tests
- Additional information such as the autopsy report, that provides additional information from internal examination in the case of deceased donors.

A donor’s medical history includes specific information regarding:
- current clinical information
- medication
- infusions/transfusions
- medical history (diseases, surgeries, medication)
- family history of genetic disease
- cause of death (deceased donor)

Current clinical information

The attending physician or medical staff caring for the patient shall need to answer the questions regarding the patient’s clinical history; in case of a deceased donor specifically relating to events leading to death.

If a potential donor appears eligible after initial screening, available records should be obtained, reviewed and evaluated as soon as possible.

- if it is a living donor: taking into account that some special issues have different implications if it is allogenic or autologous donation;
- if is a deceased donor whether death occurred within or outside a healthcare facility.

The following records may have been produced and, if so, should be obtained and reviewed:
- emergency room and emergency medical transport (ambulance) records, if applicable;
- admission records, progress notes, physician’s orders/notes and nursing observations;
- laboratory test results (microbiology, chemistry, haematology, virology, urinalysis, toxicology, genetic screening, pathology);
- transfusion and infusion information (to be used for hemodilution evaluation, Chapter 5/Appendix 6);
- X-rays, scans;
- surgical records (review for additional transfusions and infusions that may have taken place and any biopsy reports);
- records of consultations, such as psychiatry, infectious disease, neurological, orthopaedic, oncology, rheumatology, counselling;
- discharge summary or death record (assess whether an autopsy is planned).

Direct communication with the attending physician or the medical staff caring for the patient is recommended as it often provides valuable information.

Information can be obtained from outside of a healthcare facility:
- police records, if available;
- medical examiner or coroner records;
- extended care facility records (assisted living facility);
- funeral home records.
Medication

Treatment with immunosuppressive agents which can weaken the immune system and thereby can be of influence on the outcome of the serological tests; all other medication, always perform a risk assessment per tissue/cell, e.g. corticosteroids can have an impact on the quality of bone tissue.

Transfusions/infusions

When hemodilution is more than 50%, the serology testing will not be reliable on blood samples withdrawn at the time of the procurement. Blood samples before the hemodilution, if available, must be used for the serology testing of the donor. Potential hemodilution should be considered in donors with massive trauma, blood loss during operation, ruptured abdominal aneurysm.

Family history of genetic disease

- If a blood relative of the donor was definitively diagnosed by a physician to have CJD, then the donor may have a small, genetic predisposition to this disease if their relative’s CJD did not have an iatrogenic cause. Deceased donors having a family history of non-iatrogenic CJD should be excluded;
- Genetic disorders that can transmitted by gamete or embryo donation;
- The risk associated with HTLV-1 in a donor may be higher if the donor or his parents originated from a high prevalence area.

Medical history

All medical events, diseases, surgeries should be evaluated in potential donors to determine if they qualify for donation. This evaluation should include histories related to:
- Malignancy;
- Auto immune disease;
- Genetic disease;
- Chronically persistent infection: history of bacterial and protozoic diseases that can lead to chronically persistent infections, including tuberculosis, brucellosis, leprosy, melioidosis, Q fever, chlamydiosis, salmonellosis and tularemia. Specific attention should be paid to tick/arthropod-borne diseases such as borreliosis, bartonellosis, rickettsiosis, trypanosomiasis, leishmaniasis, babesiosis and ehrlichiosis.

Cause of death (deceased donors)

The cause of death (COD) is important and can indicate if the deceased donor had, or is suspected of having had, a transmissible disease, risk of hemodilution, or it can point to other concerns regarding tissue quality, including contamination. If the COD is unknown, donation cannot be permitted because the death may have been due to a disease that could be transmitted to cell/tissue recipients.

The only exceptions are when:
- The differential diagnosis includes no risk of transmitable disease and all generic contraindications are ruled out, or
- An autopsy clarifies the definitive cause of death after tissue procurement.

Generic contraindications

- Active systemic infection, such as sepsis or viraemia, meningitis, endocarditis
  If the aetiology of an active infection cannot be established, the donor is not a suitable candidate for donation.

  Donors with systemic infection, which is not controlled at the time of donation (including bacterial diseases, systemic viral, fungal or parasitic infections, or significant local infection in the tissues and cells to be donated), should be excluded.

  Donors with bacterial septicaemia may be evaluated and considered for eye donation.
  - Haematological malignancy (presence or previous history)

Risk of transmission of diseases caused by prions

- Patients diagnosed with Creutzfeldt-Jakob disease, or variant Creutzfeldt-Jakob disease, or having a family history of non-iatrogenic prion risk;
- Any suspicion of prion-associated disease (CJD, vCJD), such as rapid progressive dementia;
- Diagnosis of dementia without a confirmed primary cause unless prion-associated disease has been ruled out by microscopic examination. If dementia has a primary cause, like dementia of vascular origin, donation can be accepted;
- Degenerative or demyelinating disease or a disorder of unknown aetiology involving the central nervous system;
- Recipients of hormones derived from the human pituitary gland, such as growth hormones;
- Recipients of grafts of cornea, sclera and dura mater, and persons that have undergone undocumented neurosurgery where dura mater may have been used;
- recent history of vaccination with a live attenuated virus
- transplantation with organs
- transplantation with xenografts
- intoxications (such as cyanide, lead, mercury, gold)
- If ingestion or exposure to a toxic substance caused death, the quality of some tissue/cell types can be affected and may cause harm to recipients if those tissues/cells are used for transplantation
- if it is known that the potential donor was excluded or deferred from donating blood by a blood collection establishment, and the specific reason for deferral cannot be discovered, the donor may be considered ineligible for tissue donation

**Behavioural risks**

- intravenous or intranasal drug use;
- history of tattoos, body piercing or acupuncture in the last 4 months when it is not known that only sterile instruments or procedures were used;
- if the patient has overdosed on non-prescribed drugs, this can increase the risk of an infectious disease due to high risk behaviour associated with the use of recreational drugs. However, this risk should be evaluated on a case-by-case basis and can be mitigated by collecting reliable information.
- sexually-transmitted disease – increased risk if recent occurrence, especially for genital ulcerative disease (syphilis, gonorrhoea, herpes);

**Exposure events (travel, residency or occupation)**

- high-risk sexual behaviour in the past 12 months, having sex: with a male who had sex with another male, in exchange for money or drugs, with a person from a high-risk region for endemic disease (HIV-1 Group O, HTLV-I), with an intravenous or intranasal drug user, with a recipient of certain human clotting factor concentrates, with someone who has tested positive for HIV, HBV or HCV, or with a person with clinically active symptoms, involving frequent changes of sexual partners;
- incarceration (prison or a juvenile correctional facility)
- residence with someone who has HBV or clinically active, symptomatic HCV in the past 12 months

**Exposure events (travel, residency or occupation)**

- chronically transfused with blood or blood products (concern is raised if the administration of blood or blood products occurred many years ago, before adequate disease screening tests became available);
- occupational or other exposure to a toxic substance in amounts sufficient to affect tissues/cells and affect transplantation outcome (e.g. ethylene glycol)

**New and emerging diseases**

- MERS,
- dengue fever,
- yellow fever,
- malaria,
- Chagas’ disease,
- tuberculosis,
- plague,
- Chikungunya virus,
- West Nile virus (WNV),
- Q fever,
- antibiotic-resistant diseases,
- variant Creutzfeldt-Jakob disease (vCJD)
- HIV-1 Group O.
Tissue specific criteria

Musculoskeletal tissues

- age:
  - bone tissue: from 15 years;
  - tendons, ligaments, aponeuroses: 15-65 years;
  - osteochondral grafts, cartilage, menisci: 15-45 years;
  - cartilage for chondrocyte culture: 15-55 years.
- diffuse connective tissue autoimmune diseases
- metabolic bone diseases (osteoporosis, osteopetrosis, Paget’s disease, etc.);
- donor location has been exposed to radiation
- history of local osteo-arthritis;
- evidence of local trauma (e.g. open fracture).

- iatrogenic or degenerative tears or lesions detected during procurement of cartilage, meniscus, tendons and osteoarticular grafts contraindicate the use of the tissue.

Heart valves

- age: 18 months to 65 years.
- cardiac valvulopathy of the aortic and pulmonary valves, with moderate to severe incompetence (the vessels can still be acceptable);
- aortic dissection (detachment of the intima and adventitia);
- direct (open) and massive traumas in the procurement area;
- Marfan’s syndrome;
- bacterial or fungal endocarditis;
- chronic alcoholism with myocardial dilatation;
- pneumonia in previous days without evidence of effective treatment;
- previous cardio-surgical interventions on the tissue to be procured.

Eye tissue

- age: no upper donor age limit
- malignancies: retinoblastoma, haematological malignancies or malignant tumour of the anterior segment or the fundus of the eye.
- localised infection (bacterial, viral, parasitic or mycotic), including past ocular herpes infection.
- donors colonised with multidrug-resistant bacteria should always be excluded.
- ocular inflammation (including known ocular involvement by systemic disease, e.g. sarcoidosis, rheumatoid arthritis);
- corneal disorders including keratococcus, keratoglobus and dystrophy;
- corneal opacity, scarring, pterygium or other superficial disorders of the conjunctiva or corneal surface that involve the central optical area of the corneal button.
- prior intraocular or anterior segment surgery
  - prior surgery that would prejudice graft outcome;
  - receipt of a corneal, sclera or limbal allograft;
  - refractive corneal surgical procedures, e.g. radial keratotomy, lamellar inserts, laser refractive surgery (photo-refractive keratectomy keratomileusis).

Amniotic membrane

- significant local bacterial, viral, parasitic or mycotic infection of the genital tract, especially amniotic infection syndrome;
- gestational diabetes of the donor;
- (known) malformation of the unborn/newborn;
- premature rupturing of membranes;
- endometritis;

Initial screening of donor

- Procurement from deceased donors ➞ brain death ➞ cardiac arrest
  - hospitals: NHBD – Heart-Beating Donors
  - forensic, mortuary: NHBD – Non-Heart-Beating Donors
- Procurement from living donors
  - hospitals
  - hospitals: amputation material (e.g. femoral head)
  - obstetric: amnion
Article 36
1. Handling of cells, tissues and organs consisting in:
   1) recovery of cells, tissues and organs from living donors – may be performed exclusively in health care institutions;
   2) recovery of organs for transplantation from cadavers – may be performed exclusively in health care institutions;
   3) recovery of cells and tissues from cadavers - may be performed in health care institutions, forensic medicine departments, anatomical pathology departments of medical academies and universities that have a medical faculty, medical research and development units and funeral parlors having a dissection room;
   4) storage of organs - may be performed exclusively in health care institutions carrying out transplantations;
   5) transplantation - may be performed exclusively in health care institutions.
1a. The activities, referred to in para. 1 items 1, 4 and 5 may be performed by entities having the permission granted by the minister competent for health.

Article 21
It is allowed to recover cells, tissues or organs with the aim of transplanting them from organs or parts of organs, which were removed for reasons that differ from a recovery of cells, tissues or organs, after obtaining a consent to their use from the donor or a legal representative of the donor.

Cord blood collection

HSC collection

Reconstruction of the body after procurement!

Serological donor screening

- obligatory blood testing:
  - anti HIV 1 & 2
  - HBsAg, anti HBe
  - anti HCV
  - syphilis (TPHA)

- additional tests to be consider
  - anti CMV (IgM and IgG)
  - anti Toxo (IgM and IgG)
  - anti HTLV-1

- NAT tests, eg. PCR (genetic testing)
Personnel
- In sufficient number and be qualified for the tasks they perform.
- Competency of the personnel must be evaluated at appropriate intervals specified in the quality system.
- Clear, documented and up-to-date job descriptions.
- Provided with initial/basic training, updated training as required when procedures change or scientific knowledge develops and adequate opportunities for relevant professional development.
- The training programme must ensure and document that each individual:
  (a) has demonstrated competence in the performance of their designated tasks;
  (b) has an adequate knowledge and understanding of the scientific/technical processes and principles relevant to their designated tasks;
  (c) understands the organisational framework, quality system and health and safety rules of the establishment in which they work; and
  (d) is adequately informed of the broader ethical, legal and regulatory context of their work.

Third parties
- Procurement organizations
- Testing of donor/material
- Powerplant
- Transport services
- Suppliers: e.g. liquid nitrogen, technical gases, plastic, clothes, water
- Medical waste disposal
- Equipment service/validation
- Facilities qualification

Administration
Director
Responsible for the daily administration, supervision of technical staff and the development

Responsible person
- Practitioner or diploma, certificate or other evidence of formal qualifications in the field of medical or biological sciences awarded on completion of a university course of study or a course recognised as equivalent by the Member State
- At least two years' practical experience in the relevant fields.
- Responsible for:
  (a) ensuring that human tissues and cells intended for human applications in the establishment for which that person is responsible are procured, tested, processed, stored and distributed in accordance with law,
  (b) providing information to the competent authority or authorities,
  (c) implementing the requirements of inspection, reporting, notification of SAE and SAR, quality assurance, tissue banking activities from selection evaluation and procurement to tissue graft distribution and documentation.

Quality manager
- Responsible for ensuring that the prepared grafts are reliable for clinical use.

Organisation
- Organisational structure and operational procedures appropriate to the activities
- Access to a nominated medical registered practitioner to advise on and oversee the establishment’s medical activities
- Documented quality management system applied to the activities
- Risks inherent in the use and handling of biological material are identified and minimised, consistent with maintaining adequate quality and safety for the intended purpose of the tissues and cells. The risks include those relating in particular to the procedures, environment, staff health status specific to the tissue establishment.

Equipment
- Equipment and material must be designed and maintained to suit its intended purpose and must minimise any hazard to recipients and/or staff.
- Critical equipment and technical devices must be identified and validated, regularly inspected and preventively maintained in accordance with the manufacturers’ instructions. Where equipment or materials affect critical processing or storage parameters (e.g. temperature, pressure, particle counts, microbial contamination levels), they must be identified and must be the subject of appropriate monitoring, alerts, alarms and corrective action, as required, to detect malfunctions and defects and to ensure that the critical parameters are maintained within acceptable limits at all times. All equipment with a critical measuring function must be calibrated against a traceable standard if available.
- New and repaired equipment must be tested when installed and must be validated before use. Test results must be documented.

Agreements between tissue establishments and third parties. Third party agreements must specify the terms of the relationship and responsibilities as well as the protocols to be followed to meet the required performance specification.

Documented system in place, supervised by the responsible person, for ratifying that tissues and/or cells meet appropriate specifications for safety and quality for release and for their distribution

Traceability of data and material concerning the quality and safety of cells and tissues

Documented system in place that ensures the identification of every unit of tissue or cells at all stages of the activities
Lab determinations

Tissue establishment laboratories should be suitably designed so that there is adequate space for receiving, processing, packaging/labeling, storage, etc., to: minimize contaminants; assure orderly handling procedures and prevent mixups.

Lab determinations

When tissue bank activities include processing of tissues and cells, this shall take place in an environment with specified air quality and cleanliness in order to minimise the risk of contamination, particularly cross-contamination between samples.

The effectiveness of these measures shall be validated and monitored.

Lab determinations

Floors, walls and ceilings of non porous smooth surfaces,
Temperature control,
Air filtered environment (HEPA),
Documented system for environmental monitoring temperature, air supply conditions, particle number, CFU),
Documented system for cleaning and disinfection, (rooms, equipment and instruments),
Documented system for gowning and laundry,
Adequate space for staff, processing and storage,
Access limited to authorised personnel.

Lab determinations

Critical parameters (e.g. temperature, humidity, potential contamination) must be controlled, monitored, and recorded to demonstrate compliance with the specified storage conditions.

Physically separate areas should be provided for the storage of tissues and cells prior to release / quarantine, and for released and for rejected tissues and cells. A separate area should be allocated in both quarantine and released storage locations for certain tissues and cells collected in compliance with special criteria (e.g., Autologous or Directed Donations and known infected materials).

The areas in which cells/tissues are stored shall be accessible only to authorized persons.

Lab determinations

Grade A: The local zone for high risk operations, e.g. cell cultures, making aseptic connections. Normally such operations are protected by a laminar unidirectional air flow work station (Laminar Flow cabinets). Laminar Unidirectional air flow systems shall provide a homogeneous air speed in a range of 0.36 – 0.54 m/s at the working position in open clean room applications flow at a defined working plane. The air velocity shall be specified and measured 150-300 mm from the filter face and close to the working plane. In open cleanroom applications the air flow velocity would typically be 0.45 m/s +/-20% (guidance value) 150-300 mm from the filter face.

Lab determinations

Grade B: this is the background environment for grade A zones.

Grade C and D: Clean areas for carrying out less critical stages of processing or when final sterilization of a product is applied.
### Cleanroom

#### Lab determinations

<table>
<thead>
<tr>
<th>Class</th>
<th>Standby</th>
<th>Operating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max. number of particles/μm in size no. logarithm:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5μm</td>
<td>5μm</td>
</tr>
<tr>
<td>A</td>
<td>3 500</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>3 500</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>350 009</td>
<td>2 000</td>
</tr>
<tr>
<td>D</td>
<td>3 500 009</td>
<td>20 000</td>
</tr>
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</table>

#### Lab determinations

<table>
<thead>
<tr>
<th>Class</th>
<th>Limits for microbiological contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>air sample</td>
</tr>
<tr>
<td></td>
<td>cfu/m³</td>
</tr>
<tr>
<td>A</td>
<td>&lt;1</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
</tr>
<tr>
<td>D</td>
<td>200</td>
</tr>
</tbody>
</table>

### Lab determinations

#### Cleanroom

**Class Name**

<table>
<thead>
<tr>
<th>Class Name</th>
<th>Air Flow</th>
<th>Volume Units</th>
<th>Volume Units</th>
<th>Volume Units</th>
<th>Volume Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>300</td>
<td>100</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>B</td>
<td>100</td>
<td>50</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>200</td>
<td>100</td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**MEASURED PARTICLE SIZE (MICROMETERS):**

<table>
<thead>
<tr>
<th>CLASS</th>
<th>≤0.5</th>
<th>0.5-1</th>
<th>1-5</th>
<th>5-10</th>
<th>≥10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>35</td>
<td>75</td>
<td>10</td>
<td>NA</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>370</td>
<td>100</td>
<td>10</td>
<td>NA</td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>70</td>
</tr>
</tbody>
</table>

### Facilities

**A less stringent environment than specified in point 3 may be acceptable where:**

(a) a validated microbial inactivation or validated terminal sterilisation process is applied;

(b) or, where it is demonstrated that exposure in a Grade A environment has a detrimental effect on the required properties of the tissue or cell concerned;

(c) or, where it is demonstrated that the mode and route of application of the tissue or cell to the recipient implies a significantly lower risk of transmitting bacterial or fungal infection to the recipient than with cell and tissue transplantation;

(d) or, where it is not technically possible to carry out the required process in a Grade A environment (for example, due to requirements for specific equipment in the processing area that is not fully compatible with Grade A).

### Facilities

When these activities include processing of tissues and cells while exposed to the environment, this must take place in an environment with specified air quality and cleanliness in order to minimise the risk of contamination, including cross-contamination between donations. The effectiveness of these measures must be validated and monitored.

Unless otherwise specified, where tissues or cells are exposed to the environment during processing, without a subsequent microbial inactivation process, an air quality with particle counts and microbial colony counts equivalent to those of Grade A as defined in the current European Guide to Good Manufacturing Practice (GMP), Annex 1 and Directive 2003/94/EC is required with a background environment appropriate for the processing of the tissue/cell concerned but at least equivalent to GMP Grade D in terms of particles and microbial counts.

### Facilities

**Tissue and cell banking vs Good Manufacturing Practice**
Characteristics such as temperature and relative humidity depend on the product and nature of the operations carried out. The parameter settings should be such not to interfere with the defined cleanliness standard.

For temperature and relative humidity, the general accepted guidance values are 18 ± 2°C and 40% to 60%, respectively.

When selecting the environmental temperature and relative humidity limits the requirements for the product, process, operative comfort and area Grade shall be taken into account.

Typical operating ranges would be 17-21°C and 35 to 55% relative humidity (guidance values).

Outdoor clothing should not be brought into changing rooms leading to grade B and C rooms.

For every worker in a Grade A/B area, clean sterile (sterilised or adequately sanitised) protective garments should be provided at each work session, or at least once a day if monitoring results justify it. Gloves should be regularly disinfected during operations.

Masks and gloves should be changed at least at every working session.

Equipment and non-disposable supplies that come into contact with tissues or cells should be constructed so surfaces do not alter the safety or quality of the biological material. Equipment should be designed, manufactured and qualified for appropriate cleaning and should be sterilised or decontaminated after each use.

A separate set of clean, sterile instruments should be used for each donor.

There should be SOPs for monitoring, inspection, maintenance, calibration and cleaning procedures for all equipment.

Refrigerators, freezers and other equipment required to maintain a specific temperature, should be inspected on a regularly scheduled basis.

Appropriate certification and maintenance records should be maintained for instruments and equipment.

Solvent contact with the bare skin should be avoided, as most solvents will remove the natural skin oils and cause excessive skin flaking.

The use of paper or fabric towels is not recommended — washrooms should have electrically powered, warm-air dryers.

Approved pliers, tweezers or lint-free gloves must be used to handle manufacturing materials, components, or finished devices.

No cosmetics of any kind are to be applied or removed in the clean area. Skin lotions or lanolin-base soaps in the restrooms for employees to use to guard against flaking due to dry skin.

Non-laminar Airflow Clean rooms (C and D Class)

Garments shall be pocket-less, lintfree coveralls, with snug fitting fasteners at the neck, wrist, and ankles.

Lintfree caps must be worn and must completely cover the hair and head except for the eyes, nose, mouth, and chin.

Shoes shall be cleaned and covered with a nonshedding boottype cover or changed to approved clean room footwear. If special footwear is provided, it shall not be worn outside the clean room and dressing room.

Janitorial services shall be performed only by adequately trained and supervised personnel, each of whom must be properly garbed.

All equipment to be brought into the clean room shall be qualified for clean room use and first be thoroughly cleaned. Use only equipment that will minimize the generation of contaminants.

Traffic into and within the clean room shall be restricted to authorized and properly garbed personnel, and unnecessary movements by these personnel shall be minimized.
Lab determinations

Laminar Airflow Clean Rooms (B and A Class)

- Garments may vary with the operation being performed, but the minimum garment shall be a pocket-less, lint-free smock which extends to at least 15 inches below the work surface. The collar and cuffs shall have fasteners.

- Head covering shall be worn, and shall completely cover the hair. If the operation requires the wearer to lean over the work, or move into the airstream between the filter bank and the work piece, the front, sides, and rear neck areas of the head shall also be covered.

- A face mask may be needed if an operator has a cold, or if the nose and mouth must be brought very close to the work piece for work on miniature components or devices.

Type of tissue processing

- sterile
- clean

*with subsequent sterilization/decontamination

Main preservation procedures

Processing of tissue grafts

- freezing at -72°C
- washing in 0.9% NaCl
- lyophilization
- sealing in plastic envelopes
- radiation sterilization with a dose of 35 kGy
- validation of radiation sterilization procedure

Cleanroom concept

- increasing pressure
- increasing cleanliness

Cleanroom concept pressure

- +45 MPa
- +30 MPa
- +15 MPa
- 40 times/hour
- 20 times/hour
- 15 times/hour

Cleanroom concept communication

- A
- B
- C
- D

- X
Cleanroom concept communication

Limited access
Appropriate governing

Only D class
D&C class
D, C, B&A class

barrier
anti-electrostatic
anti-dust

Adequate disinfection
Validated sanitation

Adequate transfer of personnel and material

Appropriate wall, ceiling, floors and bench finishes

Consequences of aseptic processing

Safety

Adequate transfer of personnel and material

Adequate disinfection
Validated sanitation

Appropriate governing

Limited access

barrier
anti-electrostatic
anti-dust
### Alternative power supply

### Technical floor/ storage area

#### Measurements

<table>
<thead>
<tr>
<th>Test</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Air particle count</td>
<td>every 6 months</td>
</tr>
<tr>
<td>2 Integrity of HEPA filters</td>
<td>once a year</td>
</tr>
<tr>
<td>3 Air exchange</td>
<td>every 6 months</td>
</tr>
<tr>
<td>4 Pressure cascade</td>
<td>daily</td>
</tr>
<tr>
<td>5 Temperature and humidity</td>
<td>daily</td>
</tr>
</tbody>
</table>

#### Tissue bank monitoring system

- ventilation
- temperature and humidity
- pressure
- HEPA filters
- freezer alarms
- chemical waste materials

#### Temperature and humidity monitoring

- Ventilation system monitoring
Pressure monitoring

HEPA filters monitoring

Refrigerators and freezers monitoring

Chemical waste materials monitoring

Allogeniczna mrożona kość zbita
- belka dł. 10 cm
Sterylizowana Radiacyjnie (35 kGy)
SERIA 102320/01.001
Data przygotowania: 16.01.2003
Termin ważności: (-70°C - 5 lat) lub (-20°C - 3 m-ce)

Allogeniczna mrożona kość gąbczasta
- gruz 30 cm³
Sterylizowana Radiacyjnie (35 kGy)
SERIA 102320/02.001
Data przygotowania: 16.01.2003
Termin ważności: (-70°C - 5 lat) lub (-20°C - 3 m-ce)
Zakład Transplantacji
I Centralny Bank Kąsek Akademii Medycznej w Warszawie
ul. Chałubińskiego 5, 02-004 Warszawa,
tel./fax: (22) 621 75 43, tel. (22) 696 13 36
http://ib.amwaw.edu.pl/transp/

Allogeniczna mrożona kość gąbczasta - plaster
Sterylizowana Radiacyjnie (35 kGy)
Data przygotowania: 16.01.2003
Termin ważności: (70°C - 5 lat) lub (20°C - 3 m-ce)

Allogeniczna mrożona kość udowej głowa kości udowej
Sterylizowana Radiacyjnie (35 kGy)
Data przygotowania: 16.01.2003
Termin ważności: (70°C - 5 lat) lub (20°C - 3 m-ce)

Allogeniczna mrożona nasada dalsza kości udowej
Sterylizowana Radiacyjnie (35 kGy)
Data przygotowania: 16.01.2003
Termin ważności: (70°C - 5 lat) lub (20°C - 3 m-ce)

Allogeniczna mrożona nasada bliższa kości udowej
Sterylizowana Radiacyjnie (35 kGy)
Data przygotowania: 16.01.2003
Termin ważności: (70°C - 5 lat) lub (20°C - 3 m-ce)

Allogeniczna mrożona kość odwapniona - granulat 0,2 g
Sterylizowana Radiacyjnie (35 kGy)
Data przygotowania: 16.01.2003
Termin ważności: (70°C - 5 lat) lub (20°C - 3 m-ce)

Allogeniczna świeża chrząstka żebrowa konserwowana w 0,9%NaCl
Sterylizowana Radiacyjnie (35 kGy)
Data przygotowania: 16.01.2003
Termin ważności: (70°C - 5 lat) lub (20°C - 3 m-ce)
HISTORY OF RADIATION-Sterilization of Tissue Grafts in Poland

1963 - 1966 in channels of switched off nuclear reactor in cadmium screened aluminum containers in order to absorb remaining neutrons as present in a nuclear fuel; in the same containers ionization chambers were placed to measure the absorbed dose of ionizing radiation (Institute of Nuclear Research at Swierk/Warsaw)

1967 - till now with gamma rays in an irradiation chamber loaded with 20,000 Ci of 60Co (Institute of Applied Radiation Chemistry of the Polytechnic School in Lodz)

1973 - till now linear electron accelerator LAE-13/9 with energy of 10 MeV and beam power of 6 kW (Institute of Nuclear Chemistry and Technology in Warsaw)

1963 - 1997 sterilization dose: 33 kGy

1997 - till now sterilization dose: 35 kGy

XENOGRAFT

PORCINE SKIN

BOVINE FASCIAE

PORCINE COLLAGEN DERIVED SPONGES AND FILMS

PORCINE COLLAGEN GLUE

DIRECT AND INDIRECT EFFECT OF IRRADIATION ON COLLAGEN - A MAJOR CONSTITUENT OF CONNECTIVE TISSUE GRAFTS

Methods

1. The effect of various methods of preservation with subsequent radiation-sterilization on the osteoinductive properties of bone grafts;

2. The effect of preservation procedures with subsequent radiation sterilization on the mechanical properties of bone grafts;

3. The effect of various methods of preservation with subsequent radiation-sterilization on the solubility in vitro of graft collagen;

4. The effect of various methods of preservation with subsequent radiation-sterilization on the susceptibility of in vitro enzyme action;

5. The effect of various methods of preservation with subsequent radiation-sterilization on the graft collagen cross-linking.
Material and methods

Femurs for evaluation of osteoinductive and mechanical properties of bone grafts were obtained from 20 week-old WAG male rats.

- lyophilized irradiated at room temperature with doses 25, 35, 50 or 100 kGy (γ-source)
- deep-frozen irradiated at -72°C with doses 25, 35, 50 or 100 kGy (10MeV electron beam accelerator)
- fresh irradiated at room temperature with doses 25, 35, 50 or 100 kGy (γ-source)
- non-irradiated controls

Scheme of transplantation of bone matrices into muscles of rat abdominal wall

X-ray examination at 6 wks after transplantation of bone matrices into rat abdominal wall muscles

Computerized morphometric analysis of bone matrices at 6 wks after transplantation into rat abdominal wall muscles

Mechanical properties (maximal force - N) of rat femurs

Material and methods

- rat compact bone (35 year-old)
- human compact bone (14 week-old)
- human rib cartilage (20 year-old)
- calf Achilles tendon (6 week-old)

fresh and lyophilized irradiated at room temperature with doses 25, 35, 50 or 100 kGy (γ-source)
Evaluation of in vitro solubility of collagen of tissue samples

Pulverized tissue samples were extracted with:

(a) 0.5N NaCl (pH 7.0) at 4°C for 48 hrs to determine neutral soluble collagen (NSC)

and the residues subsequently with:

(b) citric buffer (pH 3.6) at 4°C for 48 hrs to determine acid soluble collagen (ASC)

Material and methods

The amount of Pro-OH in extracts was measured and calculated as:

\[
\text{mg Pro-OH} / \text{g dry tissue mass}
\]

Total soluble collagen (TSC) was calculated as:

\[
\text{TSC} = \text{NSC} + \text{ASC}
\]
Effect of ionizing radiation on collagen

- **Direct** (dry state-lyophilized)
  - hydrogen peroxide ($\text{H}_2\text{O} \rightarrow \cdot\text{OH}$)
  - polypeptide chain scission
  - mechanical properties
  - solubility in vitro
  - susceptibility to enzyme action
  - resorption rate in vivo

- **Indirect** (wet state: $\text{H}_2\text{O} \rightarrow \cdot\text{OH}$)
  - inter- and intramolecular crosslinking

Healing of bone grafts

- Creeping substitution
  - type of bone allograft (compact, cancellous)
  - method of conservation (lyophilization, freezing)
  - conditions of sterilization (dose, temperature)

Healing of bone allografts (1)

- chemotaxis of makro- and mikrofages
- resorption of graft (BMP)
- differentiation and proliferation of osteogenic progenitor cells of periostium

Healing of bone allografts (2)

- resorption of graft (BMP)
- creation newly formed bone bridges

Healing of bone allografts (3)

- resorption of graft (BMP)
- newly formed bone remodeling

Results of treatment

- Bone allografts, lyophilized, radiation sterilized
- 1010 cases
- 37% very good
- 54% good
- 9% unsatisfactory
Results of treatment
Bone allografts, frozen, radiation sterilized

83% 1125 cases
very good
good
satisfactory
unsatisfactory

Coding system

Regulates:
- gene therapy,
- somatic cell therapy,
- tissue engineering products.

Tissue engineered product
- contains or consists of engineered cells or tissues, and
- is presented as having properties for, or is used in or administered to human beings with a view to regenerating, repairing or replacing a human tissue.
- contain cells or tissues of human or animal origin, or both;
- the cells or tissues may be viable or non-viable;
- contain additional substances, such as cellular products, biomolecules, biomaterials, chemical substances, scaffolds or matrices.

Engineered cells or tissues
- the cells or tissues subject to substantial manipulation
- cutting, grinding, shaping, sterilization, killing in anesthetic or antiseptical solution, freezing, cryopreservation, refreezing.
Engineered cells or tissues

- the cells or tissues are not intended to be used for the same essential function or functions in the recipient as in the donor.

Amniotic membrane casus!!!
Thank you for your attention

Questions?