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Method of manufacturing a lyophilized allograft bone

V.B. Khvatov, A.V. Svishchev, A.Yu. Vaza, N.V. Borovkova, A.S. Mironov, D.Yu. Pokhitonov, Yu.V. Andreev

N.V. Sklifosovsky Research Institute for Emergency Medicine of Moscow Healthcare Department, Moscow, Russia

Contact: Dmitriy Yu. Pokhitonov, madbrat@list.ru

The article describes a lyophilized sterile allograft made of bone tissue, and its implementation as a medical device in the practice of reconstructive surgery.

Possessing an osteoconductive effect, the graft helps to restore the structure of a damaged bone, and accelerates the regeneration of bone defects. According to the RF Government Regulation dated 27.12.2012 No. 1416, the product has to pass the authorization procedure including the conduct of specific technical, toxicological, and clinical trials to be certified by Roszdravnadzor for clinical use.

Keywords: allograft bone, certification, technical regulations.

Rationale

With the development of technological progress, the number of patients undergoing high-energy trauma, has been growing steadily. The number of patients with open and comminuted fractures, fractures associated with bone defects has increased. Imperfect methods of the bone defect reconstruction result in a significant growth of non-union and pseudarthrosis cases that account for 6-51.8% in the treatment of long tubular bone

fractures, for example. [1] One of the options to treat such patients is filling the traumatic bone defect or the defect after the bone fragment resection with a demineralized or non-demineralized cancellous bone allograft [2]. Such grafts possess the mechanical strength to provide a basis for the reposition of small osseous fragments, after which they serve as a support for a long time period. According to the RF Government Regulation dated 27.12.2012 No. 1416, prior to being widely implemented in clinical practice, bone allografts have to pass the authorization procedure, including the conduct of specific technical, toxicological, and clinical trials in order to be certified by Roszdravnadzor for clinical use [3].

The study objective was to substantiate the method of manufacturing a medical device "A bone allograft, lyophilized, sterile (hereinafter referred to as "the graft") with its further implementation in clinical practice.

Material and Methods

1. Characteristics of bone allografts, types and sizes

For large-scale production of bone allografts, we have developed technical specifications, TU 9398003019670812015, that regulate technological requirements to the product, the product environmental safety, the product acceptance procedure, testing for the compliance with the product specification requirements, transportation, storage, and disposal conditions, quality assurance activities.

In accordance with the Russian Federal Law dated 21.11.2011 No.323-FZ and the RF Public Healthcare Ministry Order dated 25.05.2007 No.357/40, a transplant shall be made of allogenous material free from HIV, hepatitis B and C viruses, syphilis (in conformity with the RF Public Healthcare Ministry Order dated 07.09.2000 No.336).

The graft represents a homogeneous osseous tissue without visible bone defects, cracks, and splits, a bone powder being granular, of taupe color, without impurities.

There are two kinds of commercially manufactured grafts: demineralized grafts of three types, shown in Table. 1, and non-demineralized grafts of 4 types, shown in Table. 2, with regard to different application areas (Fig. 1).

Table 1. Technical specifications of demineralized grafts.

Type / No.	Type of bone graft	Size, No.	Length, mm	Width, mm	Thickness,mm	Weight, g
1	Cancellous bone graft	1	10.0±2.0	10.0±2.0	10.0±2.0	1.5±0.5
2	Cortical bone graft	1	50.0±10.0	20.0±5.0	3.0±0.5	1.0±0.5
3	Bone chips	1 2 3 4	1.5±0.5[2] 0.63±0.06 0.16±0.02[2] 0.063±0.006[2]	-	-	1.0 ± 0.1[1]

[1] A total mass of a single item.

[2] The diameter of individual powder particles.

Table 2. Technical specifications of non-demineralized grafts

Type / No.	Type of bone graft	Size, No.	Length, mm	Width, mm	Thickness, mm	Weight, g
1	Cancellous bone graft	1	20.0 ± 3.0	20.0 ± 3.0	20.0 ± 3.0	3.1 ± 0.4
		2	20.0 ± 2.0	20.0 ± 3.0	10.0 ± 1.5	2.2 ± 0.3
		3	10.0 ± 2.0	10.0 ± 2.0	10.0 ± 2.0	1.5 ± 0.5
2	Cancellous-cortical bone graft	1	100.0±10.0	70.0±10.0	8.0 ± 3.0	13.0 ± 3.0
		2	70.0 ± 10.0	70.0 ± 10.0	8.0 ± 3.0	11.0 ± 3.0
		3	35.0 ± 5.0	25.0 ± 3.0	15.0 ± 3.0	5.4 ± 0.6
		4	40.0 ± 5.0	30.0 ± 5.0	10.0 ± 1.0	7.5 ± 1.0
3	Cortical bone graft	1	100.0±10.0	5.0 ± 1.0	5.0 ± 1.0	7.0 ± 2.0
		2	100.0±10.0	15.0 ± 2.0	3.0 ± 0.5	8.4 ± 0.8
4	Bone chips	1	1.5 ± 0.5[2]	-	-	1.0±0.1[1]
		2	0.63±0.06[2]			1.0±0.1[1]
		3	0.16±0.02[2]			1.0±0.1[1]
		4	0.063±0.006[2]			1.0±0.1[1]

[1] A total mass of a single item.

[2] The diameter of individual powder particles.

Fig. 1. Liophylized bone allografts.

The graft shall be sterile; the sterilization is achieved by gamma irradiation in the dose of 15-25 kGy, according to National Certification System GOST ISO 11137-2, and GOST R 503 252 011.

Each graft shall be housed in individual plastic packaging of polypropylene, GOST 2699686, the bone fragments are to be packed in an additional polypropylene bag, or in a polypropylene tube for a bone powder. The package should be hermetically vacuum-sealed.

The graft in the transport packaging shall be resistant to mechanical factors during transportation according to GOST R 50444.

The average shelf life of a graft shall be 60 months from the date of sterilization. Limiting state criteria are defined as the altered product appearance, loss of sterile integrity, and individual packaging damage.

2. Methods of graft manufacturing

Bone grafts are obtained from bone fragments harvested from cadaver donors.

Bone harvesting is undertaken from those suddenly died (Specific donors) in the initial 12 postmortem hours in the absence of a refrigerator unit for a cadaver storage, and within up to 24 postmortem hours in conditions of a refrigerator corpse storage (at an optimum temperature +2-4° C). The medical examiner's permission for bone harvesting shall be documented in the form of the Donor Sheet (cadaver).

The preliminary whole blood sampling from the specific donor is undertaken for Stage I screening that includes: rapid tests for HIV, viral hepatitis types B and C, syphilis (the RF Public Healthcare Ministry Order dated 07.09.2000 No. 336), and for bilirubin.

Bone harvesting is undertaken in the operating room following the requirements of asepsis and antisepsis. After harvesting, the bone fragments shall be wrapped in a sterile material and placed in a low-temperature refrigeration unit for the quarantine. Further processing of the bone material is undertaken after obtaining the donor blood test results (Stage II screening: enzyme immunoassay tests for antibodies to human immunodeficiency virus HIV [anti-HIV], for the surface antigen of hepatitis B virus [HBsAg], and for the antibodies to hepatitis C virus [anti-HCV antibodies] in human serum, serum tests for syphilis using cardiolipin and treponemal antigens, in accordance with the RF Public Healthcare Ministry Order dated 07.09.2000 No. 336), and a medicolegal autopsy report.

After the medical examiner's statement has been obtained and in the absence of contraindications to bone harvesting, the bones shall be mechanically purified from soft tissue, and cut into blocks, using the electromechanical saw, in accordance with the technical specifications. The resulting bone chips and powder are collected separately in a cloth for further manufacturing of bone chips.

The obtained bone fragments and bone chips shall be defatted. The grafts shall be packed and the packages sealed hermetically, labeled and sterilized by gamma irradiation at a dose of 15-20 kGy. Prior to being released, the grafts shall be tested for sterility and toxicity according to established protocols. The obtained test results are documented in the log, and only then the grafts can be used in clinic.

3. Production of demineralized bone grafts

Years of research experience have shown that demineralized bone grafts possess high osteoinductive properties, low antigenicity, and are well

tolerated by a graft recipient. There are several methods of manufacturing demineralized grafts [4-8].

We have developed and used an original technique to demineralize bone grafts. The core of the technique is to purify the obtained bone blocks off soft tissues, to subject them to mechanical processing that includes their cutting and a complete removal of cancellous bone, possibly together with myeloid and fatty bone marrow. Superficial cuts or through holes are inflicted on the bone at sites with high density, especially at the verge of bones, for a better penetration of the acid into the bone. The demineralization is performed by immersing the bone blocks in hydrochloric acid solution for a period not exceeding maximum 4-5 days, the optimal period makes 2-3 days. The process solutions should be changed daily, their stirring accelerates the leaching of salts from the harvested grafts.

After the first 24 hours, the bone blocks are subjected to mechanical processing where the superficial layer damaged at previous bone cutting is peeled off by a scalpel along the bone-saw lines. In all cases, the demineralization is performed at a temperature of +5-7° C [9].

The freeze-drying is one of the preservation techniques that provides a dehydration that can range from 1 to 10% (Fig. 2). The obtained grafts are hermetically packed, labeled, sterilized by gamma irradiation at a dose of 15-20 kGy. Prior to being released, the grafts shall be tested for sterility and toxicity according to established protocols. After the results of bacteriological examinations and toxicology tests have been obtained, the grafts are logged and released for clinical use.

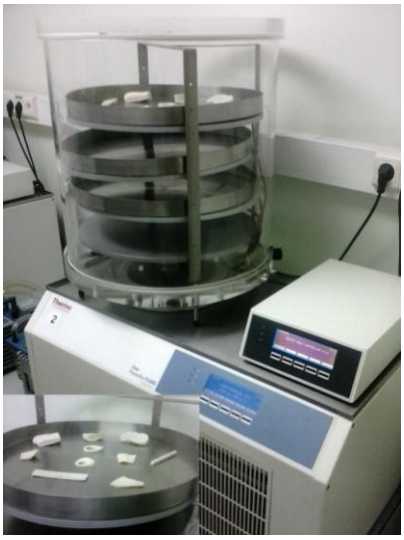

Fig. 2. Bone graft preservation using the method of freeze-drying.

4. Production of demineralized bone powder

The technique implies clearing the obtained specimens off the bone marrow and periosteum, their washing with water, defatting with ethanol, and further drying for 24 hours; then, by using a compact mill, they are ground into a powder that is sieved to 70-420 micron size. The powdered mass is demineralized in fractions of 20 g with hydrochloric acid (for 1 hour), rinsed in ethanol using a magnetic stirrer for degreasing. Finally, the powder is rinsed again in a mixture of ethanol and ether and left for the final drying that lasts for a day. The bone powder is packaged, labeled, and sterilized by gamma irradiation at a dose of 15-20 kGy. After obtaining the results of bacteriological examinations and toxicology tests, the grafts are logged and are available in clinic.

5. Internal quality assurance

Internal quality assurance procedure for bone allografts comprises 4 stages:

Stage I: before the bone processing. The cadaver material is checked for the compliance with legal requirements. The biomaterial is considered eligible for manufacturing grafts after obtaining the test results (for HIV, RW, HBsAg, HCV, according to the RF Public Healthcare Ministry Order dated 07.09.2000 No. 336, total bilirubin, the test results for toxicity and bacterial blood cultures from the corpse), and a medical examiner's statement (on the absence of the following: cancer, infection, autoimmune diseases, liver cirrhosis, peptic ulcers, acute or exacerbated chronic inflammatory diseases at any body site or system).

Phase II: after bone graft manufacturing. The graft is evaluated for residual moisture content. The moisture content (X) is assessed as the following ratio: the weight of the water removed from the material (WW) to the retained weight of the material after drying (WD), i.e. X=WW/WD. Residual moisture of bone grafts should range from 1 to 10%. Bone grafts are subjected to tensile stress in a comparative testing of mechanical properties (tensile strength testing). When checking aqueous extract pH, this parameter should range within 6.5-7.5.

Phase III: after packaging. Checks of the package integrity shall be made. Cortical and cancellous bone grafts should be vacuum-wrapped in double plastic bags. Bone chips and powder are placed in plastic tubes and then double-wrapped by a plastic vacuum pack. The package shall be checked for integrity and no free air. Also the package appearance is assessed for matching the size, labeling, and the absence of seal defects.

Stage IV: after sterilization. After having been sterilized by gamma-irradiation, the finished products shall be subjected to bacteriological screening. Four random samples from each batch shall be sent for

bacteriology culture. The product is considered sterile provided there is no colony growth on nutrient media.

Each production cycle is carried out in accordance with the "checklist" that shows all the stages of the production, indicating the date, time, name, and the signature of the responsible physician.

In case of finding a defect, the appropriate entry should be made in the log of *Internal Quality Assurance for products (bone grafts)* and the batch should be discarded and disposed in accordance with the relevant Sanitary and Epidemiological Surveillance procedure.

6. Authorization procedure

As defined by the RF Government Regulation dated 27.12.2012 No. 1416, a medical product or device, prior to be used in clinical practice, should pass the authorization procedure including the conduct of specific technical, toxicological tests, and clinical trials to be certified by Roszdravnadzor for clinical use. The sequence of authorization procedure stages is governed by the Administrative Regulations of the Federal Service for Surveillance in Healthcare (Roszdravnadzor) in terms of providing the State Service of Registration of Medical Devices (approved by the RF Healthcare Ministry Order dated October 14, 2013 No. 737n). At the first stage, the product shall be subjected to toxicological studies and technical tests to be assessed for its compliance with specifications, as well as for its quality, efficacy, and safety. After the results of the tests conducted by an expert institution approved by Roszdravnadzor of the Russian Federation have been obtained, the conclusion shall be made with regard to feasibility of conducting the clinical trials of a medical device.

Results

The obtained graft samples after sterilization by gamma irradiation were submitted for preliminary toxicological studies and technical tests to the Laboratory of the Federal Research and Clinical Centre for Physico-Chemical Medicine of the Federal Biomedical Agency (FMBA), in accordance with the Administrative Regulations. The tests were conducted according to the series of standards GOST R ISO 10993, GOST R 51148-97, and GOST R 52770-2007.

Toxicological studies have been successfully completed, and the biological safety of developed grafts, their sterility and pyrogen-free nature have been confirmed and documented by certificates No.10404.011, and No.10403.011 dated December 6, 2011.

Technical tests have been successfully completed, and the manufactured product compliance with the parameters stated in the specifications, have been confirmed by certificate No. 12.846 PIT/2012 dated June 26, 2012

Thus, the product has passed the toxicological and technical tests that confirm the proper quality of manufactured freeze-dried bone allografts.

Conclusion

We have applied a proper manufacturing process to obtain bone allografts that meet the technical requirements and can be used in clinical practice.

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