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Analysis of the application of different bone grafting procedures in patients with intra-articular fractures

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From 2008 to 2014, 298 patients with traumatic cancellous bone were treated in N.V. Sklifosovsky Research Institute for Emergency Medicine of Moscow Healthcare Department. The bone graft substitutes used were an autograft from the iliac crest, allogenic grafts (non-demineralized cancellous bone, collagen type 1 sponge with bone chips, combined perforated non-demineralized cancellous bone with collagen type 1 sponge).

Long-term outcomes after allografting did not differ from the outcomes of the treatment with the iliac crest autograft. The allograft of non-demineralized cancellous bone has a long-lasting mechanical strength, which in combination with osteosynthesis provides a stable fixation, and early weight bearing. Despite a lower mechanical strength, the collagen type 1 sponge with bone chips has a more pronounced osteoconductive effect. We have created and applied a combined perforated non-demineralized cancellous bone with collagen type 1 sponge that has good mechanical properties and a pronounced osteoconductive effect.

Keywords: traumatic cancellous bone defect, intra-articular fracture, allograft.

Rationale

Current developments of innovative technologies throughout the world have led to an increased number of high-energy injuries associated with sustained bone defects. Traumatic cancellous bone defects often occur as a result of pressure (compression) of one joint surface to another. Cancellous bone, besides being broken, is also wrinkled, forming a defect. The cause of traumatic diaphyseal defects lies in the loss of diaphyseal segments in open trauma [1]. The most popular technique of bone repair after a partial diaphyseal bone loss is the distraction osteogenesis, a bone transport technique by Ilizarov, or an initial shortening of the limb in the area of defect with following bone lengthening using the Ilizarov technique [2].

Despite the disadvantages (a time- and resource-consuming treatment, risks of pyo-septic inflammation at the sites of passing transosseous elements, inconvenience associated with wearing the device, the risk of malunion at the site of joining fragments), this approach makes it possible to repair diaphyseal defects of large extent [3].

The management of large diaphyseal defects using diverse bone grafts gives unsatisfactory results much more likely, than the method of distraction osteogenesis, due to a traumatic surgery, the risk of infection, graft fracture or lysis [4].

To restore the cancellous bone in metaepiphyseal zone defects, various plastic materials have been used, their choice being rather wide nowadays. Traditionally, an autologous bone graft remains a preferable standard for bone grafting. It has some advantages over other reconstructive techniques: no risk of immunological rejection, a pronounced

osteoreparative potential owing to retained bone marrow cells present in cancellous bone in large quantities. The graft has mechanical strength and all necessary properties of a plastic material, namely osteoconductive and osteoinductive properties. Its drawback is associated with the need for additional surgery for harvesting an autologous bone graft (the pain induced beyond the main intervention site, an increased intraoperative time and a higher risk of infection) [5].

Even a "standard" autograft is rearranged during a long period (from several months to several years). In larger defects, graft resorption processes may prevail over the process of bone formation resulting in a structurally defective bone formation [6].

Bone allografts are made of cadaver material, i.e. their use does not imply additional trauma to the patient. Such allografts are used for filling the bone defect and stimulating the bone formation [7]. Allogenic grafts can be frozen or lyophilized, demineralized, or non-demineralized. The non-demineralized allografts are believed to possess osteoconductive properties only, and the partially demineralized grafts also have osteoinductive properties. The grafts are usually pre-shaped in the most convenient form of blocks, wedges, chips, or crumbs. When using allogenic bone grafts, the risk of infecting a patient is minimized as the allografts are subjected to sterilization by fast electrons or gamma radiation. The risk of immunological allograft rejection is very small because the manufacturing technology involves a careful removal of blood cells and bone marrow bearing the gene products of the major histocompatibility complex [8-10].

There are a number of osteoplastic materials used both as a pure substance, and as a composite. These include biologically inert bioceramics (alumina, carbon-base bioceramics, non-porous hydroxyapatite),

biodegradable bioceramics (porous hydroxyapatite, tricalcium phosphate, calcium carbonate), bioceramics with a biologically active surface (bioglass) [8, 11].

Biodegradable osteoplastic materials of calcium phosphate ceramics have been the most widely used ones. The main advantage of calcium phosphate materials is their ability to form a strong bond with the bone. Possessing a mechanical strength, the porous hydroxyapatite blocks are not completely rearranged and may stay secure in bones for years and decades. Hydroxyapatite blocks can be used as spacers in the corrective osteotomy [8, 12].

Hydroxyapatite in combination with collagen in pellets has been increasingly used as a substitute material for traumatic bone defects [13]. *Kollaplan* is the most widely known native-manufactured product [8, 14]. In this form, the osteoconductive effect is more pronounced and the drug can be completely resorbed. A more pronounced osteoconductive property is inherent in allogenic or xenogenic hydroxyapatite (inorganic bone) rather than in the synthetic one. [8].

Tricalcium phosphate is produced as a pure substance or in combination with hydroxyapatite. Using pure tricalcium phosphate for the replacement of major defects is impractical because its resorption comes earlier than the new bone has formed. Porous calcium phosphates may be used as carriers of drugs and biologically active substances [8, 15].

A number of manufactured composite plastic materials are based on the calcium phosphate. Calcium phosphate osseous cements can be easily moulded, quickly solidify with a slight increase in temperature, and then are replaced by a bone [15].

Thus, a large amount of materials used for cancellous bone repair suggests that an optimal method is still lacked. And the distraction osteogenesis technique, despite all its drawbacks, still remains the most appropriate for the treatment of patients with diaphyseal defects.

Study objective: To investigate the results of using various bone grafts to justify the need for the development and production of a new combined non-demineralized cancellous bone allograft with type 1 collagen.

Material and Methods

The study protocol was approved by the Biomedical Ethics Committee of the N.V.Sklifosovsky Research Institute for Emergency Medicine (Minutes № 6-13 of December 16, 2013).

One of the tasks of the Laboratory for Blood Transfusion, Tissue Preservation and Artificial Nutrition in the N.V.Sklifosovsky Research Institute is to develop allogenic plastic materials to be used as bone defect substitutes also stimulating osteogenesis, if damaged. Allografts are prepared in advance to simplify their intraoperative use. Currently fabricated allografts are the following:

- non-demineralized (cancellous, cortical, or cancellous-cortical) bone,
- demineralized (cancellous, cortical) bone,
- bone chips of different diameters,
- type 1 collagen sponge with bone chips, an allograft originally developed by the N.V.Sklifosovsky Research Institute for Emergency Medicine (RF Patent number 2364360 of 20.08.2009),

- combined perforated non-demineralized cancellous bone graft with type 1 collagen sponge developed by the N.V.Sklifosovsky Research Institute for Emergency Medicine (RF Patent number 2524618 of 07.27.2014).

Allogenic bone grafts were procured in accordance with the regulations effective on the territory of the Russian Federation. All grafts met the technical requirements and were manufactured in conformity with technological specification.

The tissues were harvested from patients who died suddenly of an acute cardiovascular failure, brain death as a result of cranial trauma or stroke, in the initial 24 hours after their death.

In order to ensure the biological safety, the harvested bone fragments were quarantined until the forensic autopsy data and the results of blood tests for the presence of blood-transmissible infections had been obtained. For quarantine purpose, the bone fragments were rinsed with 20% glycerol in the Locke-Ringer's solution and then were placed in a low-temperature refrigerating unit at a temperature of -40° C.

After the biological safety had been confirmed, the bones were mechanically purified from the surrounding soft tissues, sawed, yielding cortical cancellous bone fragments of different sizes, shapes, or bone powder.

Then, blood cells and bone marrow cells were removed, for which purpose the material was immersed in a 3% hydrogen peroxide solution and placed in a vacuum chamber. After thorough washing with isotonic sodium chloride solution, the bone fragments were kept in a hydrogen peroxide solution, dehydrated in 95% alcohol for 1 day, and defatted with a solution

representing the mixture of 95% alcohol and ether in a ratio of 1:1 (for 48 hours), and then with ether (for 24 hours). Passive drying of allogenic bone grafts was performed in a fume hood at +37° C (Fig. 1, 2).

Fig. 1. Cutting the bone fragments.

Fig. 2. Formed grafts prior to further processing

While fabricating demineralized bone grafts, the defatted bone preforms were gauzed dry and immersed in a solution of 2N hydrochloric acid. The optimal time for demineralization made 1-2 days. Demineralized bone fragments were retrieved from the process solution, washed with a 3% sodium thiosulfate solution and then in an isotonic sodium chloride solution.

Thereafter, the grafts were dried in the freeze drier. The drying mode was adjusted so that the final degree of graft moisture content was within 1-6%.

While making a combined bone allograft of non-demineralized cancellous bone and type 1 collagen sponge, the bone fragments, after passive drying, were impregnated with a collagen gel. Through holes were drilled in the grafts to reduce the mass of non-demineralized bone. The grafts were impregnated with collagen solution using a syringe and then dried in the freeze drying chamber (Fig. 3).

Fig. 3. Combined perforated graft of non-demineralized cancellous bone and type 1 collagen sponge

To fabricate type 1 collagen sponge with bone chips, the collagen gel was filled with bone chips of 160 to 1000 microns in particle size, in a ratio of 1:5, and gently stirred; the resulting mixture was poured into Petri dishes and dried in a freeze drier.

The ready bone grafts were packed and sterilized using gamma-radiation at dose of 2.5 Mrad. The sterility was tested by bacteriological cultures of 3-4 specimens of bone grafts from each batch. The fabricated bone allografts can be stored at a room temperature for 2 years.

In the period from 2008 to 2014, 298 patients underwent surgery for a traumatic epiphyseal bone defect replacement in N.V.Sklifosovsky Research Institute for Emergency Medicine.

The main objective in the treatment of intra-articular fractures was to restore the congruence of the articular surfaces. During surgery, the depressed articular surface was returned to its anatomical position. The bone allograft was secured into the defect that had formed between the crushed epiphyseal bone and the articular surface. External fixation of fracture was made using a bone metal framework.

The fractures localized in the head and surgical neck of the humerus in 9 patients (3.0%), in the distal humerus metaepiphysis in 3 (1.0%), in the distal radius metaepiphysis in 5 (1.7%), in posterior parts of the acetabulum in 8 (2.7%), in femoral condyle in 21 (7.0%), in the proximal tibial metaepiphysis in 218 (73.1%), in the distal tibia metaepiphysis in 18 (6, 0%), and in the heel bone in 16 injured (5.4%).

The iliac crest bone autografts of were used in 14 cases, namely for filling a cancellous bone defect in 9 cases and osteochondral defects in 5 cases.

Non-demineralized cancellous bone allografts were used for filling bone defects in 254 cases, including 16 cases where associated cartilaginous defects were filled with collagen type 1 sponge with bone chips.

In one case of the traumatic destruction of the tibia and tibial outer condyle cartilage, an osteochondral graft was used.

Type 1 collagen sponge with bone chips was used for filling bone defects in 18 cases.

In 11 cases, we used a combined perforated non-demineralized cancellous bone graft with allogenic type 1 collagen sponge.

After surgery, the operated joints in all the patients were X-rayed in two standard views to assess position of fragments, the fixator, and the ratio of articular surfaces. X-ray was repeated at 6 weeks after surgery to assess the congruence of the articular surfaces, the degree of union, and to estimate the limb weight bearing. The X-ray was performed again at 6 months and at 1 year of follow-up to assess the recovery of bone structure, and to evaluate the functional outcome in the same time period.

A good outcome was defined as no pain in the operated joint, no limb deformity, and the range of motion limited to no more than 10°.

The outcome was defined as satisfactory in the cases of moderate pain appearing after physical exertion, the limb deformity of no more than 10°, and the range of motion limited to no more than 30°.

The outcome was considered poor in case the pain appeared irrespective of the physical exercise, the limb deformity made more than 10°, the range of motion was limited to over 30°.

Computed tomography (CT) of the operated joint was performed at a long-term postoperative follow-up to compare the rearrangement quality between various plastic materials.

Results and discussion

Bone allograft application was associated with deep suppuration developed in 3 cases (1.1%), with an outcome in chronic osteomyelitis in one case (0.4%).

There were no cases of nonunion. At 6-12 month follow-up, all the patients had X-ray signs of cancellous bone recovery at the site of defect. However, a CT scan revealed an incomplete recovery of bone structure. Cysts of varying sizes being present indirectly indicated a lack of the

allograft osteoconductive effect. These signs were more pronounced after applying a non-demineralized cancellous bone allograft than after the use of allogenic type 1 collagen sponge with bone chips. The incomplete recovery of the cancellous bone structure did not show any clinical manifestations (Fig. 4, 5).

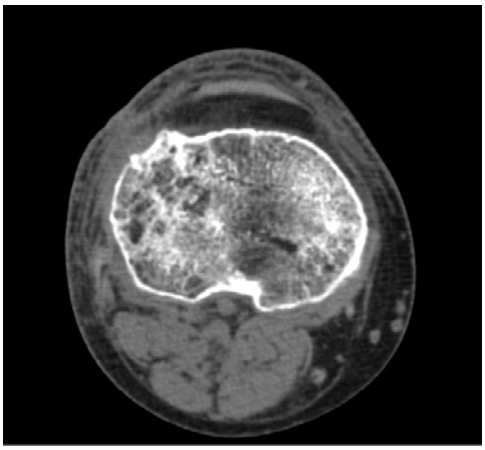

Fig. 4. Recovery of cancellous bone structure after the repair with type 1 collagen sponge with bone chips (spiral computed tomography).

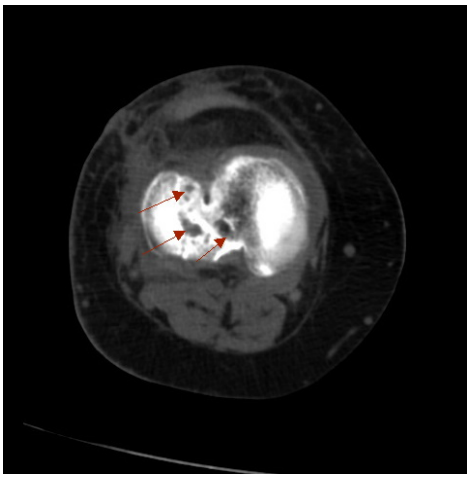

Fig. 5. Recovery of cancellous bone structure after the repair with non-demineralized cancellous bone graft (spiral computed tomography). Large cysts (*arrows*) are visualized

A long-lasting mechanical strength of non-demineralized cancellous bone graft was considered its main advantage over type 1 collagen sponge having a more pronounced osteoconductive and osteostimulation properties. This mechanical strength in couple with the osteosynthesis provided a stable fixation, and early weight bearing.

To combine the mechanical strength of non-demineralized cancellous bone graft with a pronounced osteoconductive effect of allogenic type 1 collagen, we have developed and implemented a combined perforated graft from non-demineralized cancellous bone and a collagen sponge. This graft has been used for the recent 8 months. The follow-up of patients for this period of time has demonstrated its efficacy.

The outcomes after using the iliac crest autografts to fill the defects of cancellous bone did not differ from those after using bone allografts.

The freeze-dried osteo-cartilaginous allografts used for filling the osteo-chondral defect of the tibial outer condyle were subjected to lysis rather than rearrangement. It resulted in the development of a severe valgus deformity of the knee that was progressing with the graft resorption. Such grafts were not used any further for repair of osteo-chondral defects, and the defect was filled with an autograft of the iliac crest so that a smooth flat periosteum-covered part would replace the cartilage surface. No cases of graft lysis have been reported with this type of repair, satisfactory functional results have been obtained. Usually, in such cases, the patient is not bothered by the pain, there is a slightly limited flexion ability observed: from 10° to 30°. A control arthroscopy in the long-term postoperative period demonstrated the recovered articular surface area being covered with a scar tissue.

The functional outcomes were either good or satisfactory in all the patients in whom the cartilage defects were filled with allogenic type 1 collagen sponge. In 2 cases, a control arthroscopy was performed in the late postoperative period and revealed the articular surface at the repair site being covered with a smooth matte whitish tissue.

Long-term outcomes were studied in 62 patients with follow-up period of 2 to 7 years.

The analysis of obtained data demonstrated good functional results achieved in 72% of cases, satisfactory results in 23.8%, and poor outcomes in 4.2% of patients.

Conclusion

Most commonly, traumatic cancellous bone defect filling is required for the injured with fractures of the proximal tibial metaepiphysis (73%).

Filling the epiphyseal defects with allogenic bone grafts yielded good functional results in 72% of cases. Long-term functional results were similar to the outcomes of treatment with the iliac crest autografts.

The best option for filling large osteo-chondral defects has been the autograft of the iliac crest secured into the defect so that a smooth flat periosteum-covered side would replace the cartilage surface.

Allogenic type 1 collagen sponge with bone chips provides a more pronounced osteoconductive effect than a non-demineralized cancellous bone, thus producing a more complete restoration of the epiphyseal structure at the site of the defect.

The allograft of a non-demineralized cancellous bone has a long-lasting mechanical strength that in combination with the osteosynthesis provides a stable fixation, and early weight bearing.

The originally developed combined perforated allograft of cancellous bone with type 1 collagen sponge combines the mechanical strength of the non-demineralized bone graft and the pronounced osteoconductive effect of the sponge of allogenic type 1 collagen. The initial clinical use of this allograft gives promising results and suggests its wide prospects.

References

1. Chaddha M., Gulati D., Singh A.P., et al. Management of massive posttraumatic bone defects in the lower limb with the Ilizarov technique. *Acta Orthop. Belg*. 2010; 76 (6): 811–820.

2. Bobrov G.D., Gold S., Zinar D. Ten year experience with use of Ilizarov bone transport for tibial defects. *Bull Hosp. Jt. Dis.* 2003; 61 (3–4): 101–107.

3. Green S.A., Jackson J.M., Wall D.M., et al. Management of segmental defects by the Ilizarov intercalary bone transport method. *Clin. Orthop. Relat. Res.* 1992; 280: 136–142.

4. Green S.A. Skeletal defects: a comparison of bone grafting and bone transport for segmental defects. *Clin. Orthop. Relat. Res.* 1994; 301: 111–117.

5. Nazirkar G., Singh S., Dole V., Nikam A. Effortless effort in bone regeneration: a review. *J. Int. Oral. Health.* 2014; 6 (3): 120–124.

6. Fellah B.H., Gauthier O., Weiss P., et al. Osteogenicity of biphasic calcium phosphate ceramics and bone autograft in a goat model. *Biomaterials.* 2008; 29 (9): 1177–1188.

7. Zwingenberger S., Nich C., Valladares R.D., et al. Recommendations and considerations for the use of biologics in orthopedic surgery. *BioDrugs.* 2012; 26 (4): 245–256.

8. Pankratov A.S., Lekishvili M.V., Kopetskiy I.S. Kostnaya plastika v stomatologii i chelyustno-litsevoy khirurgii [Bone grafting in dentistry and maxillofacial surgery]. In: ed. Pankratov A.S. *Osteoplasticheskie materialy: ruk-vo dlya vrachey* [Osteoplastic materials: a guide for doctors]. Moscow: BINOM Publ., 2011. 272 p. (In Russian).

9. Aponte-Tinao L.A., Ritacco L.E., Albergo J.I., et al. The principles and applications of fresh frozen allografts to bone and joint reconstruction. *Orthop. Clin. North Am.* 2014; 45 (2): 257–269.

10. Arner J.W., Santrock R.D. A historical review of common bone graft materials in foot and ankle surgery. *Foot Ankle Spec.* 2014; 7 (2): 143–151.

11. Lee E.J., Kasper F.K., Mikos A.G. Biomaterials for tissue engineering. *Ann. Biomed. Eng.* 2014; 42 (2): 323–337.

12. Lash N.J., Feller J.A., Batty L.M., et al. Bone grafts and bone substitutes for opening-wedge osteotomies of the knee: A systematic review. *Arthroscopy.* 2015; 31 (4): 720–730.

13. Parikh S.N. Bone graft substitutes: past, present, future. *J. Postgrad. Med.* 2002; 48 (2): 142–148.

14. Kesyan G.A., Berchenko G.N., Urazgil'deev R.Z., et al. Opyt primeneniya Kollapana v travmatologii i ortopedii [Experience with Collapan in traumatology and orthopedics]. *Poliklinika.* 2012; 4 (2): 50–51. (In Russian).

15. Polo-Corrales L., Latorre-Esteves M., Ramirez-Vick J.E. Scaffold design for bone regeneration. *J. Nanosci. Nanotechnol.* 2014; 14 (1): 15–56.