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Influence of different methods of bone processing on bone mechanical properties

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Abstract

Introduction Bone graft is the best option to repair postsurgical bone defects. The biomaterial is highly adaptive, structurally dynamic, metabolically active and characterized by high strength. Standard preparation of grafts for implantation includes cleaning followed by deep freezing and sterilization. However, methods used for processing bone material and reagents can change the biomechanical properties of the bone. **The purpose** was to explore the effect of chemical purification and subsequent lyophilization on the mechanical strength of bone grafts in comparison with native fresh frozen bone. **Material and methods** Metaepiphyseal sections of a single level of one tibia of a single cattle were used to rule out the influence of the variable density of native bone obtained from different donors. The bone was cut into blocks with a hand saw. Three groups of samples formed depending on the processing method included freshly frozen native bone, bone purified by combined chemical and physical methods and bone purified by the same technique followed by lyophilization. Mechanical properties were measured by axial compression mode using a 1958U-10-1 strength machine. Statistical data analysis was performed using the Kolmogorov-Smirnov (K-S) test and the Lilliefors correction with statistical significance of differences assessed with one-way analysis of variance (One-Way ANOVA). **Results** The cross-sectional area of hand-made blocks was comparable. No decrease in bone strength below the baseline was recorded regardless of the method of bone processing. Purified bone blocks demonstrated maximum strength characteristics prior to lyophilization. The sample strength decreased after lyophilization and was higher as compared to freshly frozen native bone. No statistically significant differences in the maximum force applied and the cross-sectional area were recorded between groups of samples. Modulus of elasticity and relative deformation had statistically significant differences in the groups ($p < 0.05$). **Conclusion** Modern methods of bone processing were shown to maintain biomechanical properties of the bone and can be used in the form of bone blocks or chips and as a structural graft.

Keywords: biomechanical strength, bone bank, allograft, graft, processing method

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INTRODUCTION

There are 2.2 million reconstructive operations performed annually worldwide with use of bone allografts and an estimated market value of $2.4 billion (as of 2016) [1-3]. The global bone grafts and substitutes market is expected to reach USD 4.3 billion by 2028 according to a new study by Polaris Market Research. The allografts sub-segment is estimated to dominate the market in 2020, growing at a compound annual growth rate of 4.8 % from 2021 to 2028 [4]. Autologous transplantation with a predictable result is the best option for the reconstruction of bone defects [5, 6]. The technology requires highly trained operating surgeon and surgical team, longer anesthesia and leads to an increased cost of the treatment [7]. The use of allobone is one of the solutions with available bone bank supplying donor bone material.

Allografts are used to restore bone tissue and ensure sufficient stability for fracture repair [8] or total and revision joint arthroplasty [9] with the annual growing number [10]. A variety of allografts used included chips, cubes and structural bone allografts depending on the size and configuration of the defect [11]. Short-term and long-term clinical and radiological outcomes showed promising results with allografts used for revision procedures of the acetabulum and the femur [12. 13]. Bone graft is highly adaptive, structurally dynamic, metabolically active and superior to all other biomaterials in terms of strength [14]. Bone is continuously modified which leads to the formation, maintenance or degradation of bone mass due to a complex process of cellular regulation, coordination of osteoblasts and osteoclasts (bone matrix resorption) [15]. The lower cost in comparison with bioceramics and individual designs are the advantages of allografts.

The mechanical integrity and characteristics of the used bone under various loading conditions directly depend on its mechanical properties including strength, which can be affected by the method of bone processing, storage, cleaning and sterilization conditions [16-18]. Despite the reports of improved strength bone characteristics after delipidization and removal of formed elements [19, 20], subsequent sterilization using gamma radiation demonstrates a negative effect on the strength characteristics of the material. Lansdown et al. reviewed 18 studies evaluating the effect of irradiation on the strength of allobone-based osteoplastic material

and described negative biomechanical effects of moderate radiation doses and highlighted the ambiguous results regarding the effects of radiation at lower doses (< 2 mrad) [21]. A chemical sterilization technique using peracetic acid and supercritical CO_2 was reported to have a negative effect on the strength of grafts [22]. Bui et al. explored the effect of supercritical CO_2 and gamma irradiation (2-2.8 mrad) on the biomechanical properties of meniscus allografts and found that both methods caused greater stiffness and decreased elasticity compared to native samples [23].

The purpose of the study was to explore the effect of purification technology and subsequent lyophilization on the mechanical strength of fresh frozen cancellous bone.

MATERIAL AND METHODS

A single section of the tibial metaepiphysis of a cattle specimen was used in the study to rule out an effect of variability in the density of native bone obtained from different donors. At the first stage, spongy bone was separated from compact bone and marked with a grid to obtain the largest number of samples from a single cut (Fig. 1). Samples were taken evenly from the central and peripheral parts of the cancellous bone cut for each of the groups. Then the bone was sawn with a hand saw into bars measuring 7 ± 1.5 × 7 ± 1.27 × 10 mm according to the marking.

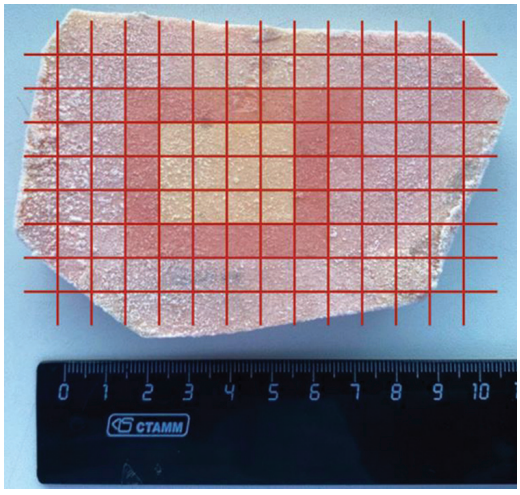

Fig. 1 Cross-section of the tibial metaepiphysis of the cattle with the cortical layer removed, fresh frozen (the zones from which samples were taken for research are highlighted in color)

Three groups of samples formed included:

(1) fresh frozen bone (n = 8), the test was carried out with the grafts thawed at a room temperature of 20 ± 2 degrees for 3 hours in an airtight container;

(2) bone (n = 13), chemically cleaned using the experimental technology developed at the R.R. Vreden NMRC TO). The technique included sequential processing using chemical (alcohols and ethers solutions) and physical methods (shaking, mixing, hydrodynamic jet).

(3) bone (n = 11) treated similarly to group 2 and additionally lyophilized for 40 hours in a HETO PowerDry PL3000 sublimation unit (Denmark).

The material used in groups 2 and 3 was prepared according to the original method we developed (patent RU 2722266 C1). Electron micrographs were obtained with the Carl Zeiss Supra-55 scanning electron microscope (Germany) to control the bone quality after cleaning. Measurement of the mechanical properties of bone samples was carried out within 3 hours from the manufacture at room temperature in the uniaxial compression mode using the 1958U-10-1 tensile testing machine (Russia) at a test speed of 1 mm/min. Bone blocks were installed vertically between the clamping planes of the mechanical testing machine. The modulus of elasticity E, compressive strength σ_p, ultimate strain before failure ε_p were determined based on the measurements.

Statistical analysis was performed using the Statistica software package. Data were presented as mean ± error of the mean (M ± m). The Kolmogorov-Smirnov (K-S) test and the Lilliefors correction (Lilliefors), were used to assess the normality of the distribution of values and the one-way analysis of variance (One-Way ANOVA) was employed to identify statistically significant differences. P values less than 0.05 were considered statistically significant.

RESULTS

The cross-sectional area of hand-made blocks was comparable and averaged to 51.45 ± 1.2 mm^2. Figure 2 shows the results of scanning microscopy after bone cleaning and lyophilization. Absence of formed cellular elements and a small number of microcracks in the mineral-collagen matrix could be seen (Fig. 2). Regardless of the method of bone processing, No decrease in strength below that of the native bone was recorded (Table 1).

Despite the absence of statistically significant differences, chemical cleaning of the samples was found to increase the ultimate strength of bone blocks by 2.3 times. Subsequent lyophilization reduced the parameter and exceeded the tensile strength in group 1 by 1.8 times. There were no statistically significant differences the maximum force and cross-sectional area between the groups. Measurements of the modulus of elasticity and relative deformation between the groups showed statistically significant differences ($p < 0.05$). These parameters were significantly higher in groups 2 and 3 as compared to group 1 ($p < 0.05$) (Fig. 3, 4).

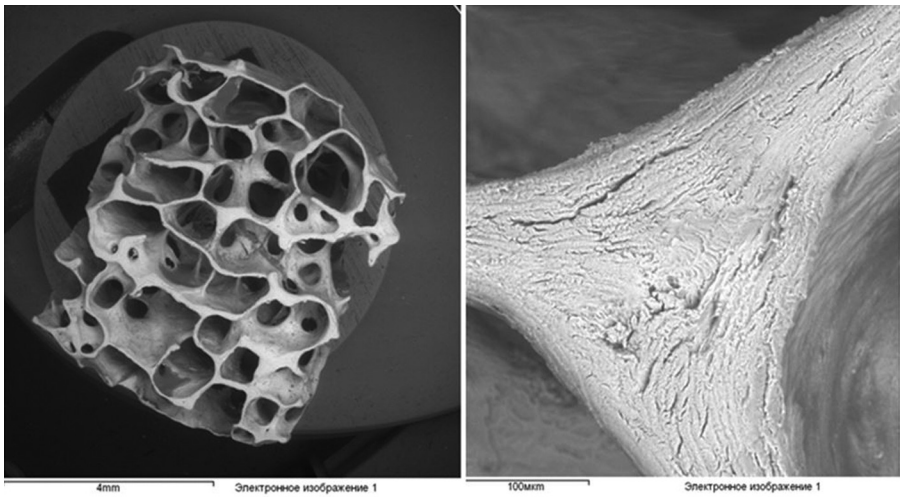

Fig. 2 Microphoto of bone tissue after cleaning and lyophilization (scanning electron microscopy)

Table 1

Sample measurements and measurements of strength characteristics in groups

Description	Group 1	Group 2	Group 3	p
Width, mm	7.7 ± 0.6	7.6 ± 0.4	7.4 ± 0.4	0.927
Thickness, mm	7.1 ± 0.4	6.9 ± 0.3	7.0 ± 0.4	0.914
Cross-sectional area, mm^2	52.9 ± 2.0	50.8 ± 1.3	50.7 ± 2.5	0.723
Maximal force, N	452.2 ± 83.1	1022.3 ± 181.8	848.8 ± 246.3	0.164
Tensile strength, MPa	8.8 ± 1.9	20.3 ± 3.6	16.0 ± 4.2	0.116
Elastic modulus, MPa	139.5 ± 34	345.5 ± 28	193.6 ± 41.4	0.009
Relative strain at failure, %	35.4 ± 6.3	10.7 ± 2.7	32.2 ± 7.7	0.007

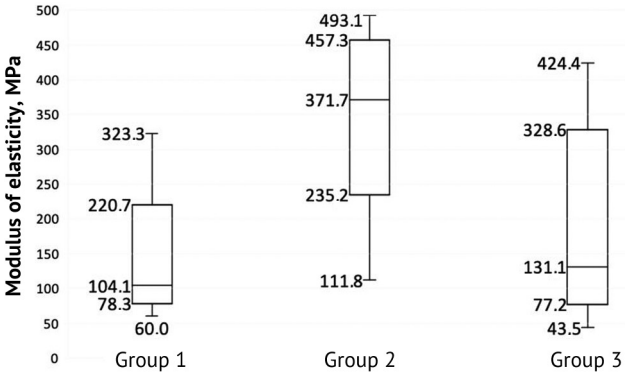

Fig. 3 Measurements of the modulus of elasticity for each of the groups

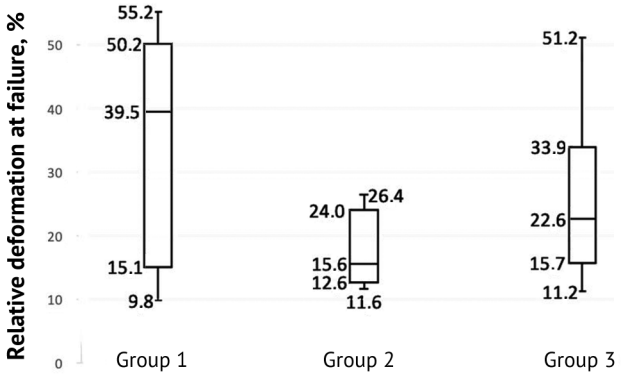

Fig. 4 Measurements of the index of relative deformation at failure for each of the groups

DISCUSSION

Screening of bone allograft donors and examination of the material for infections remain the "gold standard" in the preparation of bone tissue for subsequent transplantation. Despite the precautions taken, cases of infected recipients can be recorded. Safety of bone material can be improved with improved methods of cleaning, processing and storage. The risk of infection during transplantation can be reduced by treating allografts with methods that do not adversely affect the properties of the allograft and ensure complete cleansing of the bone material [24]. Freeze-drying facilitates graft properties remaining unchanged for 5 years. The method involves deep freezing of bone tissue with subsequent heating of the material under low pressure to a residual moisture content of less than 5 % [25]. However, the effect of the processing method on bone strength is ambiguous. Matter et al. and Kang et al. reported no effect of cryopreservation before drying and multiple repeated freezing on bone strength [26, 27]. Some authors reported decreased bone strength during lyophilization [28, 29]. R.R. Pelker reported no effect with the method on the properties

of bone tissue [29]. The cancellous bone was shown to degrade to a much lesser extent during drying compared to cortical bone [30]. Our series showed lower strength of the samples additionally lyophilized as compared to samples chemically cleaned, and they were superior to fresh frozen bone blocks in terms of the parameters tested. The result obtained was most likely due to damaged collagen fibers and the appearance of bone microcracks because of drying (Fig. 4, 5) [31].

P. Garnero et al. reported the effect of collagen bonds on bone strength, regardless of the content of minerals, that may decrease during chemical purification and subsequent sublimation [32]. The strength of bone tissue is known to be affected by chemical reagents used for the processing and the method of subsequent sterilization. Hydrogen peroxide is often used to clean the bone. It has been established that this reagent is able to change the structure of proteins by affecting the peptide chains and can increase the porosity of hard tissues without affecting collagen fibers in an acidic environment. H.K. Chang et al. demonstrated a significant decrease in the strength of dentin in an experiment with a 30 % hydrogen peroxide solution [33]. The combination of physical and chemical methods of cleaning grafts helped to avoid the use of aggressive reagents and led to decreased strength of the grafts. A greater strength of grafts at intermediate stages could be caused by the alkaline environment formed at one of the stages of bone cleansing creating conditions for collagen hydrolysis [34-36]. The increased percentage of minerals resulted in increased bone strength and decreased relative deformation index. Although the bone became stronger during chemical treatment and more brittle in comparison with fresh-frozen samples, subsequent lyophilization led to the restoration of the parameter. The results obtained demonstrated the preservation and increase in the strength of the grafts after processing and lyophilization with decreased graft plasticity.

CONCLUSION

In the ever-evolving orthopaedic surgery, the availability of safe allografts is a priority in the treatment of many musculoskeletal pathologies and injuries. The study showed that the experimental method of chemical cleaning and subsequent lyophilization of bone blocks did not lead to decreased bone strength. Thus, the bone substitute material was found to be promising for further research and can potentially be used as a structural and supporting graft. It is essential to explore the effect of various sterilization methods (physical or chemical) on the strength of the osteoplastic material.

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