The influence of bone substitute material on mechanical properties of trabecular bone in augmentation of intra-articular impression fractures. Experimental study

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The purpose was to evaluate the effect of bone substitute materials on mechanical properties of trabecular bone adjacent to the joint.

**Material and methods** A total of 21 female chinchilla rabbits weighing 3-3.5 kg was used for the experimental study. A bilateral impression fracture was simulated in the medial tibial condyle and surgically augmented with one of the bone substitution materials: beta-tricalcium phosphate, xenoplastic material and carbon nanostructures. The animals were sacrificed at 6, 12 and 24 weeks postsurgery. Uniaxial compression test was performed to determine mechanical properties of the tibial fragments. Bone microstructure was evaluated with scanning electron microscopy. Statistical data analysis was performed with nonparametric tests.

**Results** Beta-tricalcium phosphate augmentation of the bone interface led to slow resorption accompanied by formation of adequate high-grade bone tissue with mechanical properties gradually increasing with greater observation time that indicated to the bone substitute integrated well with the host bone of the impression bone defect. Xenoplastic augmentation resulted in rapid resorption accompanied by formation of immature bone with mechanical properties declining at 6 to 12 weeks of observation. Carbon nanostructure augmentation of the bone interface caused perifocal bone resorption and absence of osteointegration with mechanical properties declining at 12 to 25 weeks of observation.

**Keywords:** trabecular bone, bone substitution material, mechanical properties, augmentation, intra-articular impression fracture

**INTRODUCTION**

New materials and technologies used for the functional organ replacement associated with the growing elderly population and increasing life expectancy are important prerequisites to hasten the transition to personalized medicine, high-tech healthcare and health protection technologies as approved by Decree of the President of the Russian Federation dated 01.12.2016 No. 642 “About the strategy of scientific technology development of the Russian Federation” [1].

Restoration of congruent articulating surfaces and prevention of secondary bone displacement for the prophylaxis of posttraumatic osteoarthritis is one of major principles of surgical repair of intra-articular impression fractures. Different osteotropic materials are used for the treatment of bone defect to achieve the result. Clinical trials indicate to the high effectiveness of current bone substitution materials used to augment bone defect with the outcomes being comparable with those achieved with autologous grafting and showing lower rate of posttraumatic complications, at the donor site, in particular [2, 5, 4, 5, 6].

Interrelationship of implant and host bed is a complicated process linked with vital bone functions at the defect site, substitute-bone contact area and compatibility of the bone substitute material with surrounding tissues by physicochemical, biological and mechanical properties. Observation of bioequivalence conditions helps to counter balance the problem of implant failure due to immune reaction, fatigue fracture or osteoresorption at implant-bone interface. Mechanical properties of the bone substitute material must be equivalent to those of native tissue to avoid lesion of the surrounding tissue. An experimental model of subchondral trabecular bone tissue is of interest to evaluate the behavior at the site of implant augmentation [7, 8, 9].

The purpose was to evaluate the effect of bone substitute materials on mechanical properties of trabecular bone adjacent to the joint.
MATERIAL AND METHODS

Design of the study was approved by local Ethics Committee of the Ural State Medical University MZ RF (Record Nº 6 dtd 16.12.2016).

Mechanical properties of bone substitute materials including synthetic beta-tricalcium phosphate (bTCP, manufactured by Science & Bio Materials, France), Osteomatrix xenoplastic material (manufactured by Connectbiopharm, Russia) and carbon nanostructures (CNS, manufactured by Nanotechmedplus, Russia) used for bone defect reconstruction were studied at the strength and fracture mechanics laboratory named after the First President of Russia B.N.Elnsin. Cylindrical samples with the diameter of 6 mm and the height of 6 mm were produced with crown diamond drill and abrasive paper. The samples underwent monoaxial compression at the rate of 0.5 mm/min using a testing machine Shimadzu AG-X 50kN (Japan).

A total of 21 female chinchilla rabbits aged 6 months weighing 3–3.5 kg was used for the experimental study held in vivarium of the Ural State Medical University Minzdrava Rossii. All manipulations with animals were conducted under the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes [10].

The animals (N = 21; 100.0 %) were subdivided into two groups: index (N = 18; 85.7 %) and control (N = 3; 14.3 %). Control group of animals (N = 3, 14.3 %) was intact in relation to operative methods of synthetic augmentation. Rabbits of index group were subdivided into three subgroups with regard to the type of substitute bone material used. Synthetic bTCP was augmented in subgroup I (N = 6; 33.4 %); Osteomatrix xenoplastic material was used in subgroup II (N = 6; 33.4 %) and xenoplastic CNS was employed in subgroup III (N = 6; 33.4 %). A bilateral impression fracture was simulated in the medial tibial condyle (MTC). The animals were anesthetized, operative field washed with antiseptics, and MTC was approached by sharp dissection of the soft tissues in the medial tibia. Soft tissues were pulled apart to provide approach to the bone and periosteum, and periosteum shifted in the distal direction with bone scraper. The insertion of the conjoined medial knee tendons into the anteromedial proximal tibia, also referred to as superficial pes anserinus was an anatomical landmark for further manipulations. Diamond metal cut-off wheel (d = 10 mm, thickness = 1.5 mm) was used for quadrilateral bone saw-cut of 7 x 8 mm at the MTC in front of the above anatomical formation at the depth of the cortical bone outside the cavity of the knee joint to extract the rectangular bone. With the rectangular tibial metaepiphysis removed the proximal condylar fragment was distally dispositioned into the bone defect in a dynamic powered manner with hammer and impactor to ensure incongruent articular surface simulating intra-articular impression fracture. Then the impacted portion was elevated onto the defect site and press fit osteoplastic material augmentation was produced depending on the subgroup (RF patent application № 2017136912).

The animals were sacrificed at 6, 12 and 24 weeks postsurgery.

Tibial fragments were mechanically tested at the strength and fracture mechanics laboratory (UrFU) to identify strength properties and effect of bone substitute materials on mechanical characteristics of the bone after augmentation. Proximal tibial fragment (PTF) was fixed in a special hob of Shimadzu AG-X 50kN testing machine (Japan) with a predrilled hole of larger diameter than that of the bone cross section to simulate uniaxial compression at the rate of 1 mm/ min (Fig. 1).

Maximal loading (Fmax) that the PTF was capable to sustain without damage and elastic deformity at maximal loading were measured with mechanical testing. The sample compression process was presented as a curve showing correlation between the strain and magnitude of the deformity.

The Jeol JSM-6390LV scanning electron microscope was used to evaluate topology and microstructure of the trabecular bone surface adjacent to the joint at the specialized laboratory FGBUN IGG UrO RAN. Technique of preparation of the bone sample surface was devised and introduced into practice to study the microstructure with scanning electron microscope for element approval (RF patent application № 2017138725). The invention was aimed at simplification of the technique, reduction of time and expenses to prepare bone samples for scanning electron microscope examination. The structure of sample surface was evaluated before and after uniaxial compression tests.
Kruskal-Wallis statistical test was used to determine cumulative differences in mechanical properties of subchondral bone in the experimental groups. For calculations, a significance level of < 0.05 was adopted. Statistical analysis was performed using the tools of Stata MP software, Microsoft Excel 2010 and Stata MP (version MP 13.0 SN 3471502014).

RESULTS

Mechanical properties of PTF in rabbits with augmented bone substitute materials were assessed separately for unbiased estimation. The mechanical characteristics of bone substitute materials were determined through uniaxial compression test. Mechanical parameters are presented in Table 1.

Deformation behavior of CNS and bTCP was identical to that of brittle materials and xenoplastic materials showed similar characteristics of elastic plastic solid bodies. Deformation curves of bone substitute materials and human trabecular bone are presented in Figure 2.

Results of mechanical tests of tibial fragments with implanted augment in experimental animals are presented in Table 2.

Diagrams of deformation behavior of PRF samples with different types of bone substitute materials are presented in Figure 3.

### Table 1

<table>
<thead>
<tr>
<th>Mechanical parameter *</th>
<th>bTCP**</th>
<th>CNS***</th>
<th>Xenobone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young’s modulus [GPa]</td>
<td>5.87 ± 1.87</td>
<td>6.56 ± 1.17</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td>Ultimate strength [MPa]</td>
<td>28.01 ± 7.18</td>
<td>113.7 ± 14.6</td>
<td>2.85 ± 1.17</td>
</tr>
<tr>
<td>Elastic strain [%]</td>
<td>0.76 ± 0.07</td>
<td>2.61 ± 0.69</td>
<td>2.84 ± 1.16</td>
</tr>
</tbody>
</table>

* p = 0.012 as identified by Kruskal-Wallis test; bTCP** – b-tricalcium phosphate; CNS*** – carbon nanostructures.

![Deformation curves of bone substitute materials and human trabecular bone. Abbreviations: 1 – CNS; 2 – bTCP; 3 – xenoplastic material; 4 – human trabecular bone adjacent to the joint](image)

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The photos in Figure 1 show a PTF sample of a rabbit with implanted CNS (a) prior to uniaxial compression and (b) after uniaxial compression with an arrow showing impression injury and fracture line at the posterior tibial plateau.
Mechanical properties of PTF in rabbits

Table 2

<table>
<thead>
<tr>
<th>Mechanical parameter</th>
<th>Type of osteoplastic material</th>
<th>Ultimate load [N]</th>
<th>Elastic deformation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bTCP**</td>
<td>CNS***</td>
<td>Xenobone</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>1446 ± 12.2</td>
<td>1.42 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>1487 ± 6.4</td>
<td>1.77 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>25 weeks</td>
<td>1246 ± 6.28</td>
<td>1.51 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>1410.02 ± 5.21</td>
<td>2.54 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>1118 ± 7.56</td>
<td>1.54 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>25 weeks</td>
<td>1104 ± 6.36</td>
<td>1.45 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>1417.33 ± 8.58</td>
<td>2.32 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>889 ± 9.55</td>
<td>2.94 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>25 weeks</td>
<td>1624 ± 7.23</td>
<td>2.14 ± 0.22</td>
</tr>
</tbody>
</table>

* - p = 0.0174 for ultimate loading and p = 0.0412 for elastic deformation including all timepoints (Kruskal-Wallis test); bTCP** – b-tricalcium phosphate; CNS*** – carbon nanostructures; control group**** – intact tibia with no surgical augmentation performed

Mechanical properties of PTF under ultimate loading showed no significant changes at 6 weeks of impression defect augmented with bTCP and CNS in comparison with controls. Ultimate loading decreased by 1.13 times for PTF samples augmented with xenoplast material in comparison with controls. Mechanical strength and elasticity was shown to decrease for bTCP at 12 weeks of augmentation as compared to control group (ultimate loading decreased by 1.32 times and elastic deformation reduced by 1.5 times; mechanical strength and elasticity appeared to decrease for CNS as compared to control group (ultimate loading decreased by 1.48 times and elastic deformation reduced by 1.18 times. Mechanical strength and elasticity was shown to decrease in PTF sample augmented with xenoplast material as compared to control group (ultimate loading decreased by 1.30 times and elastic deformation reduced by 1.51 times).

Mechanical strength completely recovered in PTF samples at 25 weeks of bTCP augmentation and exceeded reference control parameters (ultimate loading increased by 1.25 times and elastic deformation enhanced by 2.02 times); further decrease in major physico-strength parameters was seen in PTF samples with CNS (ultimate loading decreased by 1.62 times and elastic deformation reduced by 1.31 times).

Morphological images of PTF samples at 6-weeks follow-up are presented in Figure 4.

Figure 4 shows restored trabecular structure of intertrabecular space at the site of bTCP augmentation, clear appearance of the forming cortical bone and evident neoosteogenesis in the central part of the simulated bone defect at 6-week follow-up. CNS sample demonstrated some cavities at the implant-bone interface, delineated cortical plate of sufficient thickness at the augment site and adjacent cortical bone. However, the augmented implant did not fit closely against the host bed that was likely be caused by connective tissue layer formed at the implant-bone interface. Xenoplast sample showed active signs of bone formation with bone trabeculae growing in from maternal bone at the augmentation site of cortical bone, and the trabecular space restored due to intense resorption of xenograft and formation of bone trabeculae at the site of osteoplastic reaction.

Morphological images of PTF samples at 25-week follow-up are presented in Figure 5.
Figure 5 shows the augmented bTCP at the site of implantation and adjacent areas as singular isles being replaced with perifocal mass of new bone tissue at 25-week follow-up. A dense network of bone trabeculae and bone marrow components was seen in inter trabecular space at the metaphyseal side. CNS sample demonstrated trabecular and cortical components with clearly delineated interface, thick and hypertrophied bone stock, peri-implant cavities indicating to the presence of perifocal connective tissue capsule. Xenoplastic samples showed elements of mature bone tissue containing no components of the latter with grown inter trabecular network of bone trabeculae and stromal components of bone marrow at the center.

Evaluation of deformation behavior showed slight changes in elastic deformity with persistent ultimate loading in PTF samples with implanted bTCP at 6 weeks as compared to controls, and decrease in both ultimate load and elastic deformity at 12 weeks of augmentation that could be ascribed to proper loading and PTF remodeling. Increase in major mechanical properties was observed at 25 weeks of augmentation with proper loading realized and bone remodeling accomplished indicating to the bone substitute material completely integrated with the impression bone defect. Decrease in both parameters of mechanical strength was noted in xenoplastic samples at 6 and 12 weeks of augmentation due to resorption of the augment replaced with new trabecular bone. Slight increase in the main physico-strength parameters was seen at 25 weeks of augmentation with proper loading realized and bone remodeling accomplished indicating to the accomplishment in remodeling of impression bone defect. Decrease in both parameters of mechanical strength was observed in CNS samples at 12 and 25 weeks of augmentation due the absence of osteointegration at the bone-material interface and eventual resorption of perifocal trabecular bone.

Scanning electron microscopy allowed verification of neoosteogenesis stages starting from separate mineralization foci at the areas of capillary growth to the formation of complicated porous structure. Post compression test slices were used to observe the process of defect formation at implantation site on micro-level. Our findings suggested that xenoplastic material and bTCP underwent resorption and dissociation in biological medium being gradually replaced by immature bone tissue. Xenograft showed greater rate of bioresorption than bTCP, and CNS demonstrated no integration with the surrounding bone interface.
DISCUSSION

The low mechanical strength poses limitation to many materials with good osteoconductive properties in their use [11, 12]. According to foreign authors, synthetic bone substitutes based on mainly hydroxyapatite calcium phosphates that mimic the structure and composition of mineral bone matrix have osteoinductive and osteoconductive capacity but do not provide mechanical support that limits their use. Mechanical properties of the material can be improved by incorporating silicon oxide, manganese oxide, organic polymers and others [13–17]. For instance, 5 and 10 wt % Nylon N6 nanofiber membranes mixed with matrix of hydroxyapatite was shown to enhance the Young’s modulus from 9.8 MPa to 19.2 MPa and 35.7 MPa, correspondingly [18]. V. H. Ingole et al. (2017) measured mechanical properties of material based on hydroxyapatite and an average mechanical strength and elastic modulus were found 46.6 MPa and 2824 MPa [19]. There are also extensive reports on composites based on carbon fiber that are capable to enhance mechanical strength and compatibility with biological tissues and are being used in a variety of applications in medicine [20, 21].

Mechanical characteristics of trabecular bone and an adequate choice of bone substitute material are important to achieve and maintain stable implant that can be compromized in decreased bone mineral density. S. Salmasi et al. (2016) reported compressive strength of trabecular bone of 2–12 MPa and Young’s modulus of trabecular bone of 0.5–0.005 GPa [22]. G. Wang и соавт. (2014) found that an implant introduced into the proximal and distal tibial epimetaphysis of goats resulted in accumulated perifocal microdamages due to sporadic distribution of stress at the bone-implant interface and accelerated bone remodeling and decrease in calcium and phosphorus. Measurements of mechanical properties with nanoindentation showed decrease in bone hardness and elastic modulus that led to changes in the bone structure and density at peri-implant area [23].

Changes in mechanical properties suggest the capability of an implant to replace patient’s own bone early at fracture consolidation phase causing no microdamage accumulation at the contact with host bed, the rate of resorption of bone substitute material as well as the capacity of the new trabecular bone to withstand an external loading. Decreased height of the sample to several mm, depressed portion of tibial plateau and cuneiform fractures of epimetaphysis were visualized after the test.

A group of researcher with the guidance of L.Yu Naumenko (2014) made an experiment of osteomatrix being implanted in the femur of a rat and observed no toxicity of composite xenomaterial in the animal and found its positive effect on bone reparation. None of the operated animals developed an implant failure or a local infection that suggested a good biocompatibility of the material [24]. J. Lorenz, K. Eichler et al. (2016) explored the effectiveness of xenogeneic Bio-Oss® material used to repair bone defects in former tumor patients and found good clinical outcomes with the implant well integrated into bone tissue and bone density increased [25]. An interesting study was performed by C. Stacchi et al. (2017) who compared clinical results of maxillary sinus augmentation using hydroxyapatite and xenobone in 28 patients. Both materials led to the formation of vital bone tissue. No significant differences in mechanical strength of the materials were observed.

CONCLUSION

1. Beta-tricalcium phosphate augmentation of the bone interface led to slow resorption accompanied by formation of adequate high-grade bone tissue with mechanical properties gradually increasing with greater observation time that indicated to the bone substitute integrated well with the host bone of the impression bone defect.

2. Xenoplastic augmentation resulted in rapid resorption accompanied by formation of immature bone with mechanical properties declining at 6 to 12 weeks of observation and recovering at 25 weeks being identical to those in the control group.

3. Carbon nanostructure augmentation of the bone interface caused perifocal bone resorption and absence of osteointegration with mechanical properties declining at 12 to 25 weeks of observation.

The authors declare no conflict of interests.
REFERENCES


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