Genij Ortopedii. 2021. Vol. 27, no. 6. P. 795-799.

Original article

DOI 10.18019/1028-4427-2021-27-6-795-799

Assessment of knee osteoarthritis risk following canine tibial prosthetics (pilot experimental morphological study)

T.A. Stupina[1✉], A.A. Emanov[1], V.P. Kuznetsov[1,2], E.N. Ovchinnikov[1]

[1] National Ilizarov Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation
[2] Ural Federal University named after the First President of Russia B.N. Yeltsin, Yekaterinburg, Russian Federation

Corresponding author: Tatiana A. Stupina, StupinaSTA@mail.ru;

Abstract

Objective was to obtain preliminary data on the knee osteoarthritis risk following canine tibial prosthetics using one-stage osseointegration, external fixation and compression loading. **Material and methods** The study was carried out on 5 intact (control group) and 3 experimental (experimental group) animals aged 1.8 ± 0.5 years with a body weight of 19 ± 1.2 kg. Osteotomy was performed at the boundary of the upper and middle third of tibia and a PressFit type construct implanted. A special device was used for bone fixation and compression loading of F_N = 20 N. Paraffin sections of the articular cartilage and the underlying subchondral bone were used for histomorphometric examination. **Results** The zonal structure of the articular cartilage and cytoarchitectonics were shown to be maintained in all experimental animals with insignificant destructive changes in the form of impaired homogeneity of the intercellular substance in the upper third of the surface zone. There was a two-fold decrease in the thickness of the calcified cartilage and a 1.9-fold decrease in the thickness of the subchondral bone. The volumetric density of trabeculae in the subchondral bone decreased to 22.31 ± 5.41 % in experimental animals and to 46.94 ± 1.94 % in controls. Complete absence of calcified cartilage and the subchondral bone were observed in one case with vessels and bone marrow pannus invading the noncalcified cartilage. **Conclusion** Structural changes in the contact zone of the articular cartilage and the subchondral bone seen in the knee following experimental canine tibial prosthetics indicated the risk of developing knee osteoarthritis.

Keywords: experiment, shin prosthetics, knee joint, articular cartilage, subchondral bone, histomorphometry

For citation: Stupina T.A., Emanov A.A., Kuznetsov V.P., Ovchinnikov E.N. Assessment of knee osteoarthritis risk following canine tibial prosthetics (pilot experimental morphological study). *Genij Ortopedii*, 2021, vol. 27, no 6, pp. 795-799. https://doi.org/10.18019/1028-4427-2021-27-6-795-799.

INTRODUCTION

Changes in mechanical loading lead to pathological processes in the articular cartilage and subchondral bone that can be problemic after a traumatic and unilateral lower limb amputation with reduced loads on the involved limb and increased loads on the intact limb [1, 2, 3]. The prevalence of contralateral knee osteoarthritis (OA) is significantly greater in individuals with lower limb amputation than in a control group without amputation [4, 5]. Patients with limb below the knee amputation are at high risk of flexion contractures of the knee joint. Prolonged restriction of movements in the joint leads to decreased cartilage mass [6, 7, 8]. OA can progress to a stage when joint replacement surgery may be the only option to relieve pain and improve function and quality of life. However, joint replacement may not be an option for patients with lower limb amputation.

The subchondral bone plays a crucial role in the pathogenesis of OA [9, 10, 11]. The mechanical stress is known to affect subchondral contours and shape [12, 13, 14]. Anatomical and functional manifestations of the amputated limb in adult patients include contractures and deforming arthrosis in the above joints [15]. In the early stages of prosthetic use [16], young individuals with transtibial amputation display few biomechanical risk factors for knee OA development. Fundamental studies of articulation components are important for the functional restoration of the amputated limbs and development of functional rehabilitation techniques for the patients. There is a significant increase in osseointegration surgeries offered to restore functions of amputated lower limbs [17, 18, 19, 20]. There have been no studies to date reporting the effect of an osseointegrated prosthetic on the knee joint during functional recovery of an amputated lower limb.

The objective was to obtain preliminary data on the knee osteoarthritis risk following canine tibial prosthetics using one-stage osseointegration, external fixation and compression loading.

MATERIAL AND METHODS

The study was performed using eight mongrel dogs including 5 intact control and 3 experimental animals aged 1.8 ± 0.5 years with a body weight of 19 ± 1.2 kg. Surgical intervention was produced under general anesthesia. Tibial osteotomy was performed at the boundary of the upper and middle third with fibula removed at the same level. The channel was drilled to a diameter of 7 mm and a 7.5 mm PressFit-like implant (RF Patent No. 194912) [21] made of Ti6Al4V alloy powder by laser sintering using EOSINT M280 system was hammered in with short blows. Soft tissues were excised at the hock joint. A hole was made in skin flap to allow the outer implant portion exit, a stump simulated and soft tissues sutured in layers.

A special device (RF Patent No. 185647) [21] made of the Ilizarov components was used to fix the implant and provide compression to the bone (Fig. 1a). Compression of $F_и$ = 20 N was based on previous studies of implant osseointegration in the femur of rabbits [22] and recalculated based on the similarity of masses for the first 42 postoperative days followed by prosthesis placed for a period of 4 months. The PressFit-like implant (Fig. 1b) had an entry step part A with cutting teeth B with the diameter increasing to the ring of the head B. The cutting part of the tooth provided the bone hole size required for the calibrated part and the primary axial implant fixation. A prosthesis was attached to the implant with crossing wires fixed to the fixation and compression device that was dismantled after 6 weeks. The animals were sacrificed after 6 months of the experiment.

The manipulations were produced in accordance with the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, SP 2.2.1.3218-14 “Sanitary and epidemiological requirements for the design, equipment and maintenance of experimental biological clinics (vivariums)”, GOST 33215-2014 Guidelines for the maintenance and care of laboratory animals. Rules for equipment of premises and organization of procedures, GOST 33217-2014 Guidelines for the maintenance and care of laboratory animals. Rules for the maintenance and care of laboratory carnivores. The study received a favourable opinion from the relevant research ethics committee (Abstract of minutes № 2 (57) dtd 17.05.18).

The knee joint was exposed for pathomorphological examination and the articular cartilage and the underlying subchondral bone excised off the femoral condyles with a scalpel. Bone-cartilage blocks were fixed in formalin, decalcified in a mixture of formic and hydrochloric acids, dehydrated in alcohols, embedded in paraffin and paraffin sections were stained with hematoxylin and eosin and Masson’s trichrome stain. Light microscopic examination, digitization and morphometry were performed using an AxioScope. A1 microscope and AxioCam digital camera and Zenblue software (Carl Zeiss Microimagingg GmbH, Germany). The thickness (microns, M ± m) of uncalcified ($h_{uncal.cr}$), calcified ($h_{cal.cr}$) cartilage, subchondral bone graft ($h_{subch.b.pl}$) was measured. The volumetric density of bone trabeculae in the subchondral zone was calculated (%, M ± m). Data analysis was performed with descriptive statistics. The nonparametric Wilcoxon test was used with differences being considerable at $p < 0.05$ (AtteStat program, version 9.3.1.).

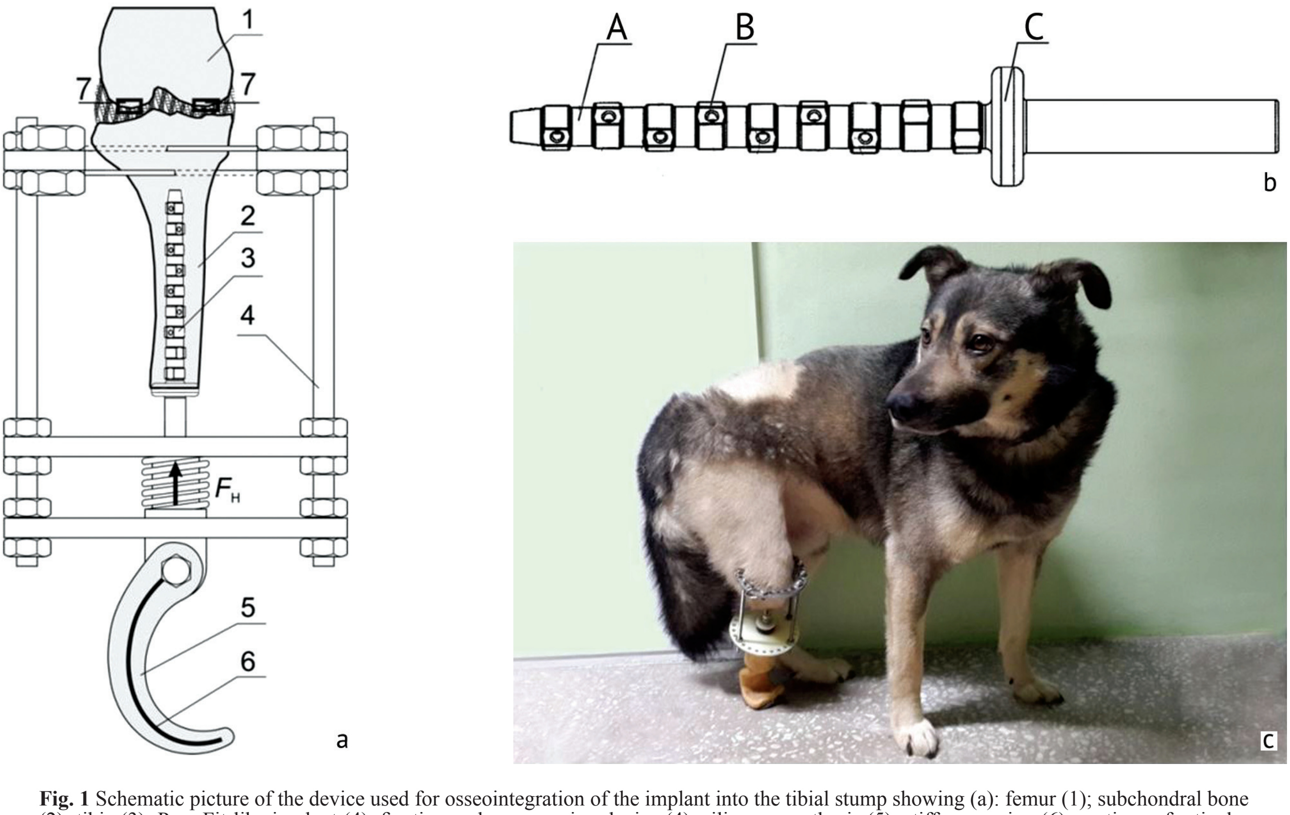

Fig. 1 Schematic picture of the device used for osseointegration of the implant into the tibial stump showing (a): femur (1); subchondral bone (2); tibia (3); PressFit-like implant (4); fixation and compression device (4); silicone prosthesis (5); stiffness spring (6), portions of articular cartilage and the underlying subchondral bone explored (7); implant design (b) and general view of the experimental animal (c)

RESULTS

The animals could bear some weight postoperatively with the limb fixed using fixation and compression device and experience gradual functional recovery of the operated limb after removal of the device and use of the prosthesis. Lameness of the supporting limb was noted after four months of the experiment.

The zonal structure of articular cartilage and cytoarchitectonics were preserved in all experimental animals (Fig. 2a). Collagen fibers were visualized in the upper layers of the superficial zone (Fig. 2b) that indicated a decrease in glycosaminoglycans in the matrix providing homogeneity of the intercellular substance. Few chondrocytes with signs of destruction were seen in the cartilage everywhere. Singular isogenic groups were mostly observed in the deep zone. Continuous basophilic line separating uncalcified and calcified cartilage was well visualized in most cases. Calcified cartilage appeared to have different thickness and was thin. There were areas with uncalcified cartilage and the basophilic line bordering the subchondral bone (Fig. 2c). The subchondral bone appeared to be irregular everywhere and thin or completely absent in some areas. The calcified cartilage in these areas was in contact with the bone marrow. Bone trabeculae were rarefied in the subchondral spongy bone, irregularly oriented with no parallel arrangement, and fat bone marrow occupied intertrabecullar spaces (Fig. 2a).

One of the three observations showed areas with no cartilage calcification and subchondral bone plate. Vessels and bone marrow pannus penetrated into the uncalcified cartilage from the subchondral bone (Fig. 2d). No signs of inflammation, inflammatory infiltration were detected. A significant decrease in all parameters was recorded (Table 1) with the most reducion observed in the thickness of calcified cartilage (by 2 times) and the thickness of the subchondral bone plate (by 1.9 times). The volumetric density of trabeculae decreased ($p = 0.0026$) in the subchondral bone to 22.31 ± 5.41 % as compared to the controls measuring 46.94 ± 1.94 %.

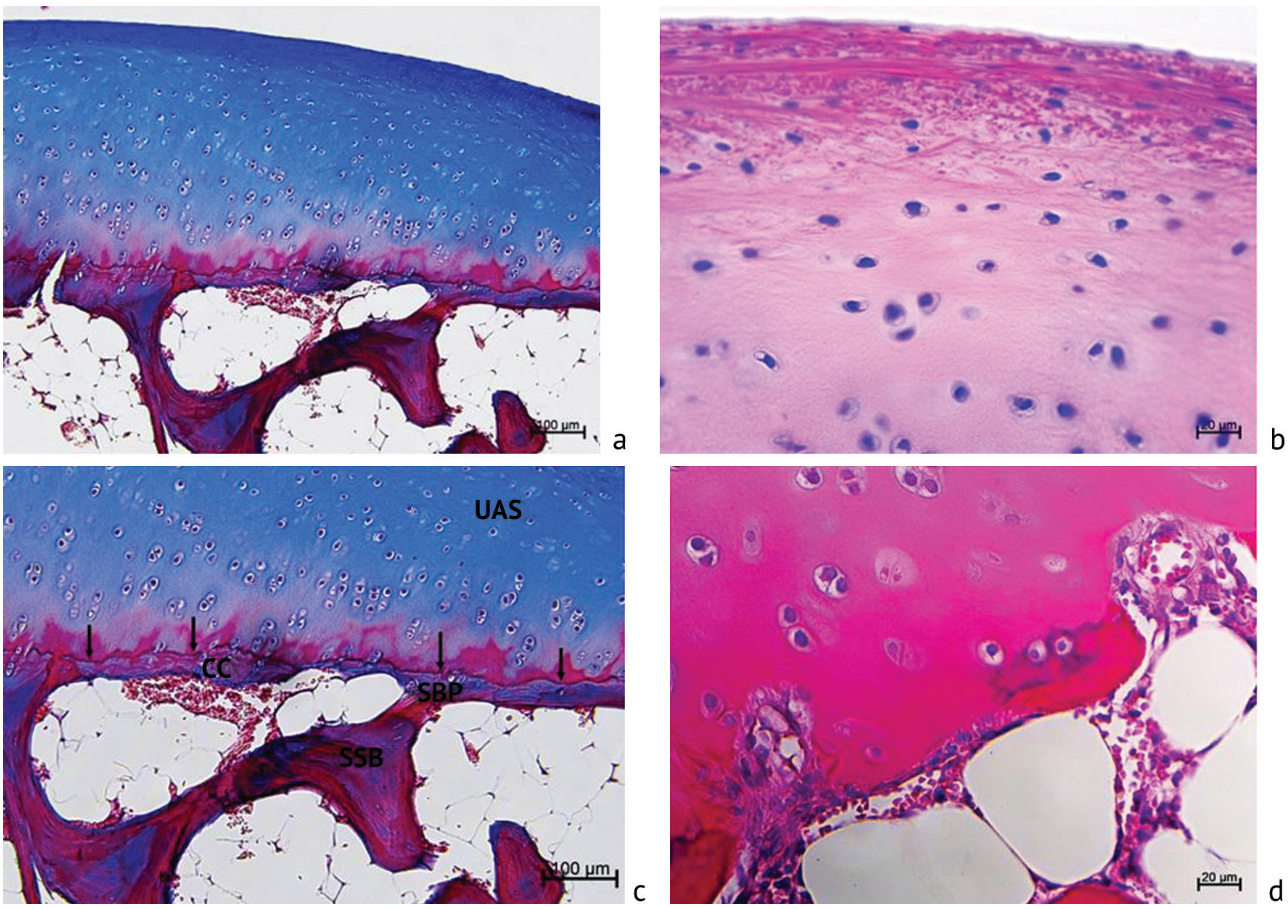

Fig. 2 Typical structure of articular cartilage of the femoral condyle. The experiment lasted for 180 days: (a) general appearance; (b) collagen fibers exposed in the superficial zone of cartilage; (c) the contact zone of uncalcified articular cartilage (UAC) and subchondral bone with the central area with no calcified cartilage (CC), thin subchondral bone plate (SBP), impaired architectonics of the subchondral spongy bone (SSB); (d) the basophilic line not visualized, vessels penetrating into the deep zone of uncalcified cartilage. Stained with Masson's trichrome (a, c), H&E (b, d). Magnification 100× (a), 400× (b, c, d)

Table 1

Thickness of the articular cartilage and subchondral bone in experimental and control animals (mcm, M ± m)

Description	Experimental group	Control group
$h_{uncal.cr}$	382.46 ± 13.65 (p = 0.00657)	475.53 ± 4.27
$h_{cal.cr}$	40.72 ± 3.84 (p = 0.00499)	87.89 ± 3.37
$h_{subch.b.pl}$	52.68 ± 10.04 (p = 0.00507)	102.42 ± 2.81

DISCUSSION

A light microscopical examination of histological preparations showed preserved zonal structure and cytoarchitectonics of the cartilage with the articular surface being not fibrous. Histomorphometric analysis revealed structural changes in the contact area of the articular cartilage and the subchondral bone. Articular cartilage and subchondral bone are dynamic structures that are responsible for carrying the load and complementing each other in the functioning unit. Structural changes in each of the zones will affect the properties and functions of the bone-cartilage articulation [11, 23]. The subchondral bone maintains important cushioning, supporting functions and nutrition for the articular cartilage. The subchondral bone entity includes two separate objects: a subchondral bone plate and a subchondral spongy bone [12, 24].

Experimental findings showed a pronounced thinning of the calcified cartilage zone, the subchondral bone plate, impaired architectonics and rarefied trabeculae in the subchondral spongy bone, a 2.1-fold decrease in the volumetric density of trabeculae. Vessels and bone marrow pannus were shown to penetrate into uncalcified cartilage in one case. Structural changes in the subchondral bone affected the mechanical strength, resorption dominated in the bone remodeling subsequently leading to an impaired continuity of the basophilic line, with vessels penetrating into the uncalcified cartilage from the subchondral bone [10, 11]. Vascular components penetrating into calcified cartilage at the sites of microcracks and the cracks of the bone-cartilage articulation initiated destruction and calcification of uncalcified cartilage [25].

Anatomical and functional features of post-amputation lower limb stumps include contractures and deforming arthrosis in the above joints in adult patients [26]. The findings of this study showed that structural changes in the subchondral bone plate and the subchondral spongy bone can be the initiating factors for destruction of the articular cartilage. The study was of a pilot nature. Although the limitation of the study was a relatively small sample (three experimental dogs) the tendency to thinning of the calcified cartilage and the subchondral bone was consistent with the data reported on the role of the subchondral bone in the pathogenesis of destruction of articular cartilage. [27, 28, 29, 30].

CONCLUSION

Structural changes in the contact zone of the articular cartilage and the subchondral bone seen in the knee following experimental canine tibial prosthetics indicated the risk of developing knee osteoarthritis. The preliminary data obtained on structural changes in the contact zone of articular cartilage and subchondral bone are important for planning investigation on a larger sample of animals.

REFERENCES

1. Farrokhi S., Mazzone B., Yoder A., Grant K., Wyatt M. A Narrative Review of the Prevalence and Risk Factors Associated With Development of Knee Osteoarthritis After Traumatic Unilateral Lower Limb Amputation. *Mil. Med*., 2016, vol. 181, no. S4, pp. 38-44. DOI: 10.7205/MILMED-D-15-00510.
2. Smirnova L.M. Biomekhanicheskie pokazateli peregruzki sokhrannoi konechnosti u patsientov s amputatsiei goleni, bedra ili vychleneniem v tazobedrennom sustave [Biomechanical indicators of intact limb overload in transtibial and transfemural amputees and patients with disarticulation in the hip joint]. *Genij Ortopedii*, 2018, vol. 24, no. 1, pp. 50-56. DOI: 10.18019/1028-4427-2018-24-1-50-56.
3. Orekhov G., Robinson A.M., Hazelwood S.J., Klisch S.M. Knee joint biomechanics in transtibial amputees in gait, cycling, and elliptical training. *PLoS One*, 2019, vol. 14, no. 12, pp. e0226060. DOI: 10.1371/journal.pone.0226060.
4. Melzer I., Yekutiel M., Sukenik S. Comparative study of osteoarthritis of the contralateral knee joint of male amputees who do and do not play volleyball. *J. Rheumatol*., 2001, vol. 28, no. 1, pp. 169-172.
5. Pröbsting E., Blumentritt S., Kannenberg A. Veränderungen am Bewegungsapparat als Folge von Amputationen an der unteren Extremität [Changes in the Locomotor System as a Consequence of Amputation of a Lower Limb]. *Z. Orthop. Unfall*., 2017, vol. 155, no. 1, pp. 77-91. (in German) DOI: 10.1055/s-0042-112821.
6. Neogi T. The epidemiology and impact of pain in osteoarthritis. *Osteoarthritis Cartilage*, 2013, vol. 21, no. 9, pp. 1145-1153. DOI:10.1016/j.joca.2013.03.018.
7. Kim S.B., Ko C.Y., Son J., Kang S., Ryu J., Mun M. Relief of knee flexion contracture and gait improvement following adaptive training for an assist device in a transtibial amputee: A case study. *J. Back Musculoskelet. Rehabil*., 2017, vol. 30, no. 2, pp. 371-381. DOI: 10.3233/BMR-160736.
8. Ghazali M.F., Abd Razak N.A., Abu Osman N.A., Gholizadeh H. Awareness, potential factors, and post-amputation care of stump flexion contractures among transtibial amputees. *Turk. J. Phys. Med. Rehabil*., 2018, vol. 64, no. 3, pp. 268-276. DOI: 10.5606/tftrd.2018.1668.
9. Makushin V.D., Stupina T.A. K voprosu ob aktivizatsii protsessov, reguliruiushchikh vosstanovlenie struktury sustavnogo khriashcha (Obzor literatury i sobstvennye dannye) [To the problem of activating the processes regulating articular cartilage structure recovery (Review of literature and our own data)]. *Genij Ortopedii*, 2014, no. 1, pp. 82-88. (in Russian)

10. Stupina T.A., Stepanov M.A., Teplen'kii M.P. Role of subchondral bone in the restoration of articular cartilage. *Bull. Exp. Biol. Med.*, 2015, vol. 158, no. 6, pp. 820-823. DOI: 10.1007/s10517-015-2870-4.
11. Li G., Yin J., Gao J., Cheng T.S., Pavlos N.J., Zhang C., Zheng M.H. Subchondral bone in osteoarthritis: insight into risk factors and microstructural changes. *Arthritis Res. Ther.*, 2013, vol. 15, no. 6, pp. 223. DOI: 10.1186/ar4405.
12. Goldring S.R. Alterations in periarticular bone and cross talk between subchondral bone and articular cartilage in osteoarthritis. *Ther. Adv. Musculoskelet. Dis.*, 2012, vol. 4, no. 4, pp. 249-258. DOI: 10.1177/1759720X12437353.
13. Pan J., Wang B., Li W., Zhou X., Scherr T., Yang Y., Price C., Wang L. Elevated cross-talk between subchondral bone and cartilage in osteoarthritic joints. *Bone*, 2012, vol. 51, no. 2, pp. 212-217. DOI: 10.1016/j.bone.2011.11.030.
14. Yu D., Xu J., Liu F., Wang X., Mao Y., Zhu Z. Subchondral bone changes and the impacts on joint pain and articular cartilage degeneration in osteoarthritis. *Clin. Exp. Rheumatol.*, 2016, vol. 34, no. 5, pp. 929-934.
15. Susliaev V.G., Shcherbina K.K., Smirnova L.M., Zamilatskii Iu.I., Koltsov A.A., Sokurov A.V., Ermolenko T.V. Ranniaia protezno-ortopedicheskaia pomoshch kak osnova meditsinskoi reabilitatsii detei s vrozhdennymi i amputatsionnymi defektami nizhnikh konechnostei [Early prosthetic and orthopedic care as the basis for medical rehabilitation of children with congenital and amputation defects of the lower extremities]. *Genij Ortopedii*, 2020, vol. 26, no. 2, pp. 198-205. DOI: 10.18019/1028-4427-2020-26-2-198-205.
16. Russel Esposito E., Wilken J.M. Biomechanical risk factors for knee osteoarthritis when using passive and powered ankle-foot prostheses. *Clin. Biomech.* (Bristol, Avon), 2014, vol. 29, no. 10, pp. 1186-1192. DOI: 10.1016/j.clinbiomech.2014.09.005.
17. Haque R., Al Jawazneh S., Hoellwarth J., Akhtar M.A., Doshi K., Tan Y.C., Lu W.Y., Roberts C., Al Muderis M. Osseointegrated reconstruction and rehabilitation of transtibial amputees: the Osseointegration Group of Australia surgical technique and protocol for a prospective cohort study. *BMJ Open*, 2020, vol. 10, no. 10, pp. e038346. DOI: 10.1136/bmjopen-2020-038346.
18. Taylor C.E., Zhang Y., Qiu Y., Henninger H.B., Foreman K.B., Bachus K.N. Estimated forces and moments experienced by osseointegrated endoprostheses for lower extremity amputees. *Gait Posture*, 2020, vol. 80, pp. 49-55. DOI: 10.1016/j.gaitpost.2020.05.018.
19. Li Y., Brånemark R. Osseointegrated prostheses for rehabilitation following amputation: The pioneering Swedish model. *Unfallchirurg*, 2017, vol. 120, no. 4, pp. 285-292. DOI: 10.1007/s00113-017-0331-4.
20. Overmann A.L., Aparicio C., Richards J.T., Mutreja I., Fischer N.G., Wade S.M., Potter B.K., Davis T.A., Bechtold J.E., Forsberg J.A., Dey D. Orthopaedic osseointegration: Implantology and future directions. *J. Orthop. Res.*, 2020, vol. 38, no. 7, pp. 1445-1454. DOI: 10.1002/jor.24576.
21. Kuznetsov V.P., Gubin A.V., Gorgots V.G., Anikeev A.V., Borzunov D.Iu., Emanov A.A. *Ustroistvo dlia osteointegratsii implantata v kost kulti nizhnei konechnosti* [The device for osseointegration of the implant into the bone of the lower limb stump]. Patent RF no. 185647, A 61 F 2/78, A 61 F 2/28, 2018. (in Russian)
22. Emanov A.A., Gorbach E.N., Stogov M.V., Kuznetsov V.P., Diachkov A.N. Vyzhivaemost chreskozhnykh implantatov v usloviiakh razlichnoi mekhanicheskoi nagruzki na kost [Survival of percutaneous implants under various mechanical loading to the bone]. *Genij Ortopedii*, 2018, vol. 24, no. 4, pp. 500-506. DOI: 10.18019/1028-4427-2018-24-4-500-506.
23. Goldring S., Goldring M. Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage-bone crosstalk. *Nat. Rev. Rheumatol.*, 2016, vol. 12, no. 11, pp. 632-644. DOI: 10.1038/nrrheum.2016.148.
24. Castañeda S., Roman-Blas J.A., Largo R., Herrero-Beaumont G. Subchondral bone as a key target for osteoarthritis treatment. *Biochem. Pharmacol.*, 2012, vol. 83, no. 3, pp. 315-323. DOI: 10.1016/j.bcp.2011.09.018.
25. Imhof H., Sulzbacher I., Grampp S., Czerny C., Youssefzadeh S., Kainberger F. Subchondral bone and cartilage disease: a rediscovered functional unit. *Invest. Radiol.*, 2000, vol. 35, no. 10, pp. 581-588. DOI: 10.1097/00004424-200010000-00004.
26. Susliaev V.G., Shcherbina K.K., Smirnova L.M., Sokurov A.V., Ermolenko T.V. Meditsinskaia tekhnologiia rannego vosstanovleniia sposobnosti k samostoiatelnomu peredvizheniiu posle amputatsii nizhnei konechnosti [Medical technology for early restoration of the ability to move independently after amputation of the lower limb]. *Vestnik Rossiiskoi Voenno-Meditsinskoi Akademii*, 2019, vol. 66, no. 2, pp. 101-109. (in Russian)
27. Pavlova V.N., Pavlov G.G., Shostak N.A., Slutskii L.I. *Sustav: morfologiia, klinika, diagnostika, lechenie* [Joint: morphology, clinical picture, diagnostics, treatment]. M., 2011, 552 p. (in Russian)
28. Alekseeva L.I., Zaitseva E.M. Rol subkhondralnoi kosti pri osteoartroze [The role of the subchondral bone in osteoarthrosis]. *Nauchno-Prakticheskaia Revmatologiia*, 2009, vol. 47, no. 4, pp. 41-48. (in Russian) DOI: 10.14412/1995-4484-2009-1149.
29. Goldring M.B., Goldring S.R. Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. *Ann. NY Acad. Sci.*, 2010, vol. 1192, pp. 230-237. DOI: 10.1111/j.1749-6632.2009.05240.x.
30. Findlay D.M., Atkins G.J. Osteoblast-chondrocyte interactions in osteoarthritis. *Curr. Osteoporos. Rep.*, 2014, vol. 12, no. 1, pp. 127-134. DOI: 10.1007/s11914-014-0192-5.

The article was submitted 17.12.2020; approved after reviewing 25.03.2021; accepted for publication 19.10.2021.

Information about the authors:

1. Tatiana A. Stupina – Doctor of Biological Sciences, StupinaSTA@mail.ru;
2. Andrei A. Emanov – Candidate of Veterinary Sciences, a_emanov@list.ru;
3. Viktor P. Kuznetsov – Doctor of Technical Sciences;
4. Evgenij N. Ovchinnikov – Candidate of Biological Sciences, omu00@list.ru.

Conflict of interest: *none.*

The work was supported by the Ministry of Health of the Russian Federation according to the program developed within the research project of the National Ilizarov Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russia for 2018-2020, state registration number AAAA A18-118011190120-1. D.R. 11.01.2018.