Original Article
https://doi.org/10.17816/2311-2905-1725

CC BY

Microbiological Profile of the Implantation Zone under Different Mechanical Compression of Percutaneous Implants

Maksim V. Stogov [1], Andrey A. Emanov [1], Natalia V. Godovykh [1], Evgenyi N. Ovchinnikov [1], Natalia V. Tushina [1], Viktor P. Kuznetsov [1, 2]

[1] *National Ilizarov Medical Research Centre for Traumatology and Ortopaedics, Kurgan, Russia*

[2] *Ural Federal University named after the first President of Russia B.N. Yeltsin, Ekaterinburg, Russia*

Background. Infection of percutaneous implants in patients with limb amputation is the most common complication. ***This study aimed*** to evaluate the microbiological contamination of the implantation zone depending on the implant mechanical compression under the conditions of the additional external fixation.

Methods. The study was performed on 36 male rabbits. The tibia of all the rabbits was sawn at the border of the upper and middle parts. The medullary canal was reamed and a percutaneous implant was placed in the tibial stump. The segment and the implant were fixed with an Ilizarov apparatus. An additional compression device was installed in 30 animals. We used 5 compression modes, accordingly, 6 experimental groups were formed, 6 animals in each: group 1 — without compression, group 2 — compression on the implant with force of 0.053 N/mm^2, group 3 — compression on the implant with force of 0.105 N/mm^2, group 4 — compression on the implant with force of 0.158 N/mm^2, group 5 — compression on the implant with force of 0.211 N/mm^2, group 6 — compression on the implant with force of 0.263 N/mm^2. The restraint was removed 6 weeks after implantation for a total follow-up of 26 weeks. The microflora of the place where the implant enters the skin (the implant / skin interface) was investigated, the level of blood leukocytes and the level of C-reactive protein in blood serum were determined.

Results. On days 9-10 after implantation, significant differences in the microbial landscape were found at the site of the exit of the metal implant in animals of different groups. The largest number of strains was found in animals of groups 1, 5 and 6, the smallest in groups 2 and 3. The most frequently detected strains: *S. saprophyticus* and *Enterococcus* spp. It was found that the greatest statistically significant increase in the level of CRP in the blood serum was observed in animals of group 6. The level of leukocytes in animals of all groups did not change statistically significantly relative to preoperative values. Animals with better osseointegration (groups 2 and 3 — no cases of implant loss) showed a minimal number of growing strains.

Conclusions. The microbiological profile of the implantation zone of percutaneous implants changes depending on the amount of mechanical compression. The optimal mode is 0.053-0.105 N/mm^2.

Keywords: prosthetics, osseointegration, implant, microflora, compression, Ilizarov apparatus.

Cite as: Stogov M.V., Emanov A.A., Godovykh E.N., Ovchinnikov E.N., Tushina N.V., Kuznetsov V.P. [Microbiological Profile of the Implantation Zone under Different Mechanical Compression of Percutaneous Implants]. *Travmatologiya i ortopediya Rossii* [Traumatology and Orthopedics of Russia]. 2022;28(2):38-47. (In Russian). https://doi.org/10.17816/2311-2905-1725.

✉ *Maksim V. Stogov*; e-mail: stogo_off@list.ru

Submitted: 13.01.2022. Accepted: 10.03.2022. Published Online: 30.03.2022.

© Stogov M.V., Emanov A.A., Godovykh E.N., Ovchinnikov E.N., Tushina N.V., Kuznetsov V.P., 2022

Научная статья
УДК 616.71-089.84:616.5-74-079
https://doi.org/10.17816/2311-2905-1725

CC BY

Микробиологический профиль зоны имплантации в условиях различной механической компрессии чрескожных имплантатов

М.В. Стогов [1], А.А. Еманов [1], Н.В. Годовых [1], Е.Н. Овчинников [1], Н.В. Тушина [1], В.П. Кузнецов [1, 2]

[1] *ФГБУ «Национальный медицинский исследовательский центр травматологии и ортопедии им. акад. Г.А. Илизарова» Минздрава России, г. Курган, Россия*

[2] *ФГАОУ ВО «Уральский федеральный университет им. первого Президента России Б.Н. Ельцина» Минобрнауки России, г. Екатеринбург, Россия*

Актуальность. Инфицирование чрескожных имплантатов у пациентов с ампутациями конечностей является наиболее частым осложнением. ***Цель исследования*** — оценка микробиологического обсеменения зоны имплантации в зависимости от механической компрессии имплантата в условиях его дополнительной внешней фиксации.

Материал и методы. Исследование выполнено на 36 самцах кроликов. Всем животным осуществляли распил большеберцовой кости на границе верхней и средней третей. Затем рассверливали костномозговой канал и устанавливали чрескожный имплантат в культю большеберцовой кости. Сегмент и имплантат фиксировали аппаратом Илизарова. Тридцати животным дополнительно устанавливали компрессионное устройство. Использовали 5 режимов компрессии, соответственно этому было сформировано 6 экспериментальных групп по 6 животных в каждой: группа 1 — без компрессии; группа 2 — компрессия на имплантат силой 0,053 Н/мм^2; группа 3 — компрессия на имплантат силой 0,105 Н/мм^2; группа 4 — компрессия на имплантат силой 0,158 Н/мм^2; группа 5 — компрессия на имплантат силой 0,211 Н/мм^2; группа 6 — компрессия на имплантат силой 0,263 Н/мм^2. Удерживающее устройство демонтировали через 6 нед. после имплантации, общий период наблюдения составил 26 нед. Исследовали микрофлору места вхождения имплантата в кожу (интерфейс имплантат/кожа), определяли уровень лейкоцитов в крови и уровень С-реактивного белка в сыворотке крови.

Результаты. На 9–10-е сут. после имплантации в месте выхода металлического имплантата у животных разных групп обнаруживались существенные отличия микробного пейзажа. Наибольшее количество штаммов обнаружено у животных групп 1, 5 и 6; наименьшее — в группах 2 и 3. Наиболее часто обнаруживаемые штаммы — *S. saprophyticus* и *Enterococcus* spp. Наибольшее статистически значимое повышение уровня С-реактивного белка в сыворотке крови отмечалось у животных группы 6. Уровень лейкоцитов у животных всех групп статистически значимо не изменялся относительно дооперационных значений. У животных с лучшей остеоинтеграцией (в группах 2 и 3 не было случаев выпадения имплантатов) наблюдалось минимальное число растущих штаммов.

Заключение. Микробиологический профиль зоны имплантации в условиях различной механической компрессии чрескожных имплантатов изменяется в зависимости от величины нагрузок. Применение нагрузок в пределах 0,053–0,105 Н/мм^2 лучше сказывается на приживаемости имплантатов и обсемененности зоны имплантации, чем отсутствие компрессии.

Ключевые слова: протезирование, остеоинтеграция, имплантат, микробиологическое обсеменение, компрессия, аппарат Илизарова.

Стогов М.В., Еманов А.А., Годовых Н.В., Овчинников Е.Н., Тушина Н.В., Кузнецов В.П. Микробиологический профиль зоны имплантации в условиях различной механической компрессии чрескожных имплантатов. *Травматология и ортопедия России*. 2022;28(2):38-47. https://doi.org/10.17816/2311-2905-1725.

Стогов Максим Валерьевич; e-mail: stogo_off@list.ru

Рукопись получена: 13.01.2022. Рукопись одобрена: 10.03.2022. Статья опубликована онлайн: 30.03.2022.

© Стогов М.В., Еманов А.А., Годовых Н.В., Овчинников Е.Н., Тушина Н.В., Кузнецов В.П., 2022

BACKGROUND

Osseointegration technology is recently widely used in clinical practice in patients with limb amputation, when a percutaneous implant integrated into the bone provides a direct mechanical connection between the bone and the external prosthesis [1]. The clinical application of this technology is increasing [2, 3, 4, 5]. In this case, the most frequent complications are implant instability and infection [6,7, 8].

Many authors regard a comprehensive solution to these problems mainly as an improvement of the characteristics of the implant primarily in surface modification to improve its biocompatibility and provide antibacterial characteristics [9, 10, 11, 12]. A certain solution to these problems can be an improvement of the implantation procedure, particularly by the transition from a two-stage technology that is currently the most recognized [13] to a one-stage one that has recently started to be developed [14]. In this field, we have developed a one-stage implantation technology with additional fixation of the implant having an external fixation device and the ability to perform compression (Utility Model Patent No. 185647, Invention Patent No. 2631631).

This study aimed to evaluate the microbiological contamination of the implantation zone depending on the implant mechanical compression under the conditions of the additional external fixation.

METHODS

The experiment was performed on 36 male chinchilla rabbits aged 6–11 months, with an average weight of 3.6 ± 0.4 kg. Animals were received from a nursery. They were conventional animals according to their microbiological status.

The study was performed in accordance with GOST R ISO 10993-1-2011, GOST 33215-2014, and GOST 33216-2014.

In all rabbits, the tibia was cut at the interface of the upper and middle thirds using a Gigli saw in the operating room. Then, the medullary canal was reamed to 4.0 or 4.5 mm, and the implant (RF Patent No. 152558) with a diameter of 4.5 or 5.0 mm (depending on the diameter of the medullary canal), respectively, was screwed into the tibial stump (Fig. 1). Soft tissues were sutured in layers. An incision was made in the skin flap to remove the outer part of the implant, and a stump was formed. Then, the Ilizarov apparatus was mounted. For this purpose, wires were passed at an angle of 90° through the proximal tibia and the distal part of the abutment, which comprised a thrust platform. Then, a compression device (Patent No. 2631631) was installed on the bone and prosthesis (30 rabbits). Five compression modes were used.

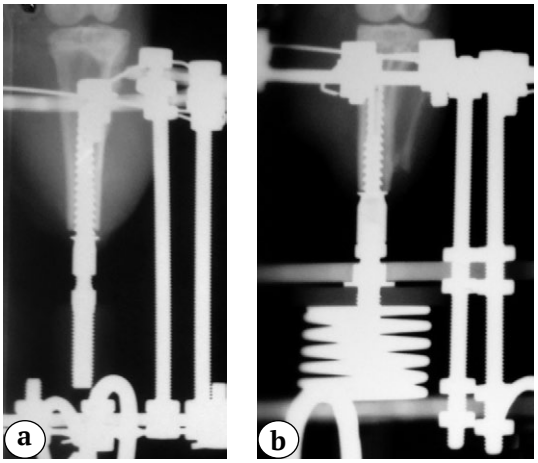

Fig. 1. Postoperative X-rays. Tibial implant:
a — without compression device (group 1);
b — with compression device

In total, six experimental groups were formed, with six rabbits in each group: group 1 without compression, group 2 with compression on the implant with a force of 0.053 N/mm^2, group 3 with compression on the implant with a force of 0.105 N/mm^2, group 4 with compression on the implant with a force of 0.158 N/mm^2, group 5 with compression on the implant with a force of 0.211 N/mm^2, and group 6 with compression on the implant with a force of 0.263 N/mm^2. Before the surgery, the animals were randomized into groups.

Postoperative follow-up and maintenance of animals

The retainer was removed 6 weeks after implantation. The total follow-up period was 26 weeks. In the first 3 days, all animals received antibiotics (Enroxil 5 mg/kg); additionally, in the first 5 days after the surgery, antiseptic treatment was performed through the hole in the implant with 3 ml of 1% chlorhexidine solution. The wound

was treated with 0.05% chlorhexidine solution for 10 days. The exit sites of the retainer wire were treated with a 3% hydrogen peroxide solution for 10–14 days.

During the study, the animals were kept in a specialized vivarium of the research center. The rabbits were kept in cages, with one animal in each cage. The cages were equipped with containers for food and water. The flooring was sawdust of coniferous trees. Wet cleaning of the cages was performed daily. Food was given once a day, and drinking water was given without restrictions. Before the experiment, the animals were quarantined for 21 days.

Planned euthanasia of animals was performed 26 weeks after implantation by introducing repeatedly excess doses of barbiturates. If the implant fell out, the animals were sacrificed unscheduled, immediately after the fallout detection.

Evaluation of results

The implant survival rate was assessed by the absence of its loss at the final follow-up period, i.e., at week 26 after implantation. X-ray control was performed at weeks 3, 6, 9, 12, 15, 18, 21, and 26 of implantation. After removing the compression device, a daily clinical test was performed to assess the implant mobility.

Laboratory studies included bacteriological examination of the site of the implant entry into the skin (implant/skin interface), counts of leukocytes in the blood, and blood serum level of C-reactive protein (CRP) at the time of the experiment.

Samples for microbiological examination were collected from wounds intraoperatively in compliance with asepsis rules and on days 9–10 after device implantation, from the site of the implant entering the skin. The samples selected were immediately delivered to the laboratory. To isolate aerobic and facultative anaerobic bacteria, inoculation was performed on the nutrient media of nutrient agar containing 5% blood, yolk-salt agar, Levin medium, and Sabouraud's medium. The inoculations were incubated at 37°C for 24–48 h. To determine the degree of contamination, the inoculation was divided into sectors. After incubation, the number of colonies of each type in sectors was counted, and the result was expressed in terms of the decimal logarithm of the size of the grown colonies (CFU/ml). Generic and species identification of isolated bacterial cultures was performed by the traditional method by studying their tinctorial, cultural, and biochemical properties. The antibiotic susceptibility of the isolated strains was determined by the disk diffusion method on the Muller–Hinton broth. The tested drugs were chosen according to clinical guidelines*. The tested drugs included cefoxitin, gentamicin, clindamycin, erythromycin, ciprofloxacin, and vancomycin for gram-positive microorganisms; ampicillin, amoxicillin/claulanate, ceftazidime, ceftriaxone, meropenem, ciprofloxacin, and gentamicin for *Enterobacteriaceae*; and cefepime, imipenem, meropenem, ciprofloxacin, amikacin, gentamicin, and ceftazidime for nonfermenting gram-negative bacteria.

Leukocyte counts were determined on a ProCyte Dx automatic hematological analyzer (IDEXX Lab., Netherlands). CRP concentration was determined on a Hitachi/BM 902 automatic biochemical analyzer (F. Hoffmann-La Roche Ltd., Italy) using reagent kits from Vital Diagnostic (Russia).

Statistical analysis

The results of quantitative analyses are presented as a median and 1-3 quartiles (Me; Q1–Q3). The normality of the samples was determined using the Shapiro-Wilk test. The statistical assessment of the significance of differences among parameters during the experiment with preoperative values was performed using the Wilcoxon W-test. The significance of intergroup differences was assessed using the nonparametric Kruskal-Wallis test. The minimum significance level (p) was equal to 0.05. Statistical analysis was performed using the AtteStat 13.1 add-in for Excel spreadsheets.

RESULTS

In this study, a single growth of microorganisms in wound samples was taken intraoperatively (Table 1). In four animals of groups 1, 4, 5, and 6, single bacterial cells were found in the samples, which were representatives of the normal microflora of the skin of animals, belonging to *Staphylococcus epidermidis* (n = 2) and *Enterococcus* spp. (n = 2). Microbial content for these strains was less than 10^3 CFU/ml.

* Clinical guidelines. Determination of the sensitivity of microorganisms to antimicrobial drugs. Version 2021-01:225.

On days 9–10 after implantation, at the metal implant exit site, animals of different groups showed significant differences in the microbial landscape (Table 2). The largest number of strains was detected in groups 1, 5, and 6, whereas the smallest number was registered in groups 2 and 3. The most common strains were *Staphylococcus saprophyticus* and *Enterococcus* spp. When analyzing the antibiograms of bacteria isolated from the wounds of experimental animals, the predominance of several resistant isolates of gram-positive microorganisms was established. In group 6 on days 9–10 after implantation, the microbial landscape was the most specific compared with other groups. Four strains were not detected in animals of other groups, namely, *Staphylococcus warneri, Staphylococcus haemolyticus, Enterobacter* spp., and *Acinetobacter* spp. This pattern indicated that high values of compression, as well as its absence, were associated with an increase in the infection of the implant exit zone.

Table 1

Species composition of bacteria isolated intraoperatively from animal wounds

Range of isolated bacteria	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Staphylococcus epidermidis (+)	–	–	–	<103	–	<103
Enterococcus spp. (+)	<103	–	–	–	<103	–
Total Number of strains Number of animals	1 1	0 0	0 0	1 1	1 1	1 1

(+) Gram-positive bacteria.

Table 2

Species composition of bacteria isolated from animal wounds on days 9–10 after implantation

Range of isolated bacteria	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Staphylococcus aureus (+)	4 (10^5)*	–	–	–	2 (10^5)	1 (10^6)
Staphylococcus saprophyticus (+)	3 (10^5)	1(10^5)	2(10^5)	–	1 (10^5)	1 (10^6)
Staphylococcus epidermidis (+)	2** (10^5)	–	–	–	2** (10^5)	1** (10^6)
Staphylococcus warneri (+)	–	–	–	–	–	4(10^4)
Staphylococcus haemolyticus (+)	–	–	–	–	–	1(10^6)
Streptococcus spp. (+)	1 (10^4)	–	–	–	1 (10^4)	–
Corynebacterium spp. (+)	1 (10^5)	–	–	–	1 (10^5)	–
Enterococcus spp. (+)	2 (10^5)	–	–	2 (10^4)	1 (10^5)	2 (10^6)
Enterobacter spp. (–)	–	–	–	–	–	1 (10^6)
Enterobacter cloacae (–)	1 (10^6)	–	–	1 (10^5)	1 (10^6)	–
Acinetobacter spp. (-–)	–	–	–	–	–	1 (10^6)
Pseudomonas aeruginosa (–)	–	–	–	1 (106)	–	1 (10^5)
Proteus mirabillis (–)	1 (10^4)	–	–	–	1 (10^4)	–
Citrobacter spp. (–)	1 (10^6)	–	–	–	1 (10^6)	–
Escherichia coli (–)	1 (10^7)	1 (10^4)	–	–	1 (10^7)	–
Total Number of strains Number of animals Loss of implants Purulent inflammation of the tissues around the implant	10 4 1 1	2 1 0 0	1 2 0 0	3 2 1 0	10 4 2 1	9 5 4 1

* Here and below: 4 is the number of animals with the strain detected; 105 the average value of bacterial contamination for this strain;
** presence of methicillin-resistant *Staphylococcus* epidermidis (MRSE) strains; (+), (–) gram-positive and gram-negative bacteria, respectively.

The determination of antibiotic sensitivity showed that some strains of *Staphylococcus* spp. were resistant to the action of β-lactam drugs. Specifically, our study revealed methicillin-resistant *S. epidermidis* resistant to cefoxitin and, consequently, to all antibiotics belonging to the β-lactam group (groups 1, 5, and 6). Ciprofloxacin and clindamycin had pronounced activity against staphylococci. Strains of *Enterococcus* spp. were sensitive to gentamicin and ciprofloxacin. Vancomycin-resistant enterococci were not detected. Ceftriaxone and gentamicin showed maximum activity in relation to representatives of the *Enterobacteriaceae* family. Ciprofloxacin was the most effective drug for non-fermenting gram-negative bacteria.

In two animals of group 5 and four animals of group 6, signs of the implant instability (loosening) were noted immediately after the removal of the retainer, and on days 3–4, the implant fell out. In one animal each of groups 1 and group 4, signs of instability were recorded on days 8–9 after the retainer was removed; in these cases, implants fell out on days 13–14 after the retainer removal.

We separately analyzed the microbiocenosis of animal wounds after the implant loss (eight cases in all groups). The microbial landscape of samples taken from these animals intraoperatively was similar to other experimental groups. Gram-negative microorganisms *Proteus mirabilis, Enterobacter cloacae, Citrobacter* spp., and *Escherichia coli* were detected in the species composition after the implant loss, and microbial content was 10^6 CFU/ml. Purulent discharge during implant loss was not detected.

The largest significant increase in the blood serum level of CRP was revealed in animals of group 6 (Table 3). For other groups, no obvious relationship was found between the magnitude of compression with an increase in CRP level. This finding probably indicates the absence of development of systemic infection in groups 1–5 because the leukocyte count in all groups did not change significantly relative to preoperative values.

Nevertheless, acute purulent inflammation of the soft tissues around the implant was detected in one animal each of groups 1, 5, and 6 on days 12–16 after implantation. Purulent inflammation was stopped by antibiotic therapy for 7–10 days (cefazolin 0.05 g/kg). In addition, in six rabbits (2 from group 1 and one each from groups 2, 4, 5, and 6), inflammation of the soft tissues around the wires of the external fixation device was noted, which disappeared following treatment with antiseptic agents.

Table 3

Dynamics of C-reactive protein (mg/L) in the blood serum of rabbits during the experiment, Me (Q1–Q3)

Term, weeks	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
0	0 (0–2)	0 (0–1)	0 (0–1)	0 (0–4)	0 (0–3)	0 (0–2)
1	15* (9–20)	7* (4–11)	13* (6–22)	20* (17–30)	12* (5–18)	33* (22–38)
2	21* (10–28)	11* (8–22)	10* (4–17)	12* (5–16)	12* (9–14)	36* (23–44)
6	19(9–24)	2 (0–17)	2 (0–3)	18* (9–23)	9* (7–19)	8* (6–10)
20	7* (5–11)	7* (4–15)	10* (6–19)	10* (5–24)	11* (7–14)	8* (7–12)
26	7* (4–10)	11* (7–30)	4* (2–21)	18* (6–27)	8* (7–10)	5* (4–6)

* Significantly different from preoperative (term 0) values at $p < 0.05$;
bold type indicates significant differences between the groups ($p < 0.05$).

DISCUSSION

The study demonstrated varying degrees of growth of microbial flora around the percutaneous implant in all experimental groups. These data are quite consistent with clinical cases when the growth of microbial flora around percutaneous implants, despite antimicrobial measures, is observed in more than half of the patients [8, 15].

The species composition of the microorganisms detected in the implantation site indicates that the landscape was formed due to the growth of opportunistic microflora, which is also noted in the clinical presentation after surgery [16]. Although the increase in the number of bacteria on the skin near the implant is not equivalent to the clinical manifestation of infection (in our case, an increase in contamination was found in 18 of 30 animals, while a purulent-inflammatory process developed in 3 animals), the high frequency of microbial colonization, providing a high bacterial load, can potentially provoke the development of not only a superficial infectious process but also deep infection [17]. The latter is also contributed by the formation of bacterial biofilms on the implant surface, which ensures the dissemination of pathogens into the soft tissues and the bone [18, 19, 20].

In our study, we did not observe substantial signs of a systemic reaction in experimental animals, associated with the release of bacteria into the blood, as evidenced by a relatively low level of CRP; a significant increase in this marker indicates the presence of bacteria in the blood [21]. All processes were localized in the vicinity near the contact zone, and the absence of cases of deep infection in animals supports the fact that the implant was not a source/gateway for the penetration of microorganisms from outside. Indeed, in clinical practice, deep infection, including osteomyelitis, develops rarely in patients with percutaneous implants [22, 23].

Based on the comparison results of these data, we can conclude that the presence or absence of implant compression is not associated with the development of deep infection. However, the association of the implant mechanical compression with the growth of microbiological contamination at the implant–skin interface is obvious. Specifically, our results indicate that both the absence and presence of implant compression in the range of 0.158–0.211 N/mm^2 were accompanied by a significant increase in the contact zone contamination. The minimal compressive loads studied in the range of 0.053–0.105 N/mm^2 were accompanied by minimal contamination.

If the causes of changes in the contamination of the implantation zone and on the implant surface are described and confirmed in the literature [24], then the relationship we detected between the implant compression value and the contamination of the implant–skin interface has not been previously described.

This phenomenon can be explained by the concept described by A. G. Gristina [25]. According to this concept, during implantation into living tissues, competition between bacteria and tissue cells for adhesion occurs on the implant surface. Moreover, if osteoblasts are the first to colonize the surface of the product, then implant integration occurs; if tissue cells cannot displace bacterial colonies, implant integration decreased and infection developed. Subsequently, the applicability of this concept was confirmed by several works. Specifically, experimental models confirmed that early osseointegration of the implant into the tissue prevents the attachment of bacteria and, consequently, the formation of biofilms [25, 26, 27, 28]. This concept is complemented by the possibility of direct interaction between osteoblasts and microbial flora [29, 30]. Consequently, the adhesion process between osteoblasts and microbial flora is competitive in nature, which determines not only further osseointegration but also the possibility of implant infection.

This concept can explain our results as well. No implant loss occurred in animals with better osseointegration (groups 2 and 3), and the minimum amount of growing strains at the implant–skin interface was noted. The positive effects of compression in stimulating osteogenesis are described in detail in the literature [31, 32, 33]. These data suggest that the minimal compression of percutaneous implants under the conditions of the experimental model studied stimulates the differentiation of osteoblasts, which creates a competitive advantage for them in the implant surface adhesion. This not only promotes better device integration but also prevents the formation of biofilms and a significant increase in microbiological contamination in the implantation site.

The implantation technology, which includes additional fixation of the implant with an external fixation device, also implies the presence of a new adverse response, namely, an inflammatory reaction near the retainer wires. This is the most common reaction in the application of the Ilizarov apparatus, and the methods of its relief are described and are not difficult [34].

CONCLUSIONS

Thus, the results of this study demonstrated that the microbiological profile of the implantation site under conditions of various mechanical compressions of percutaneous implants changes depending on the magnitude of the loads. The optimal modes of mechanical compression of the percutaneous implants under additional fixation can be identified. The discovery of a relationship between the implant survival rate and the growth of microbiological contamination is related to the fact that loads ranging from 0.053 to 0.105 N/mm^2 have better effects on the implant survival rate and implantation site contamination than the absence of compression. The latter finding suggests that percutaneous implant integration is more effective in the presence of a certain level of compression.

DISCLAIMERS

Author contribution

Stogov M.V. — research concept and design; writing the text of an article.

Emanov A.A. — research concept and design; collection, analysis or interpretation of data.

Godovykh N.V. — collection, analysis and interpretation of data; editing the text.

Ovchinnikov E.N. — research concept and design; collection, analysis and interpretation of data.

Tushina N.V. — collection, analysis and interpretation of data; editing the text.

Kuznetsov V.P. — research concept and design.

All authors have read and approved the final version of the manuscript of the article. All authors agree to bear responsibility for all aspects of the study to ensure proper consideration and resolution of all possible issues related to the correctness and reliability of any part of the work.

Funding source. State budgetary funding.

Competing interests. The authors declare that they have no competing interests.

Ethics approval. Approval from the local ethics committee was obtained before the start of the study. The study was conducted in compliance with the principles of humane treatment of laboratory animals in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experiments and other Scientific Purposes and Directive 2010/63/EU of the European Parliament and the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes.

Consent for publication. Not required.

REFERENCES

1. Zaid M.B., O'Donnell R.J., Potter B.K., Forsberg J.A. Orthopaedic osseointegration: state of the art. *J Am Acad Orthop Surg.* 2019;27(22):e977-985. doi: 10.5435/JAAOS-D-19-00016.
2. Koriukov A.A., Gubin A.V., Kuznetsov V.P., Borzunov D.Iu., Antipov A.V., Ovchinnikov E.N. et al. [Possibilities of improving the function and esthetic appearance of finger stumps using the method of osseointegration]. *Genij Ortopedii.* 2016;(4):22-28. (In Russian). doi: 10.18019/1028-4427-2016-4-22-28.
3. Branemark R., Berlin O., Hagberg K., Bergh P., Gunterberg B., Rydevik B. A novel osseointegrated percutaneous prosthetic system for the treatment of patients with transfemoral amputation: a prospective study of 51 patients. *Bone Joint J.* 2014;96-B(1):106-113. doi: 10.1302/0301-620X.96B1.31905.
4. Hoyt B.W., Walsh S.A., Forsberg J.A. Osseointegrated prostheses for the rehabilitation of amputees (OPRA): results and clinical perspective. *Expert Rev Med Devices.* 2020;17(1):17-25. doi: 10.1080/17434440.2020.1704623.
5. Reif T.J., Khabyeh-Hasbani N., Jaime K.M., Sheridan G.A., Otterburn D.M., Rozbruch S.R. Early experience with femoral and tibial bone-anchored osseointegration prostheses. *JBJS Open Access.* 2021;6(3):e21.00072. doi: 10.2106/JBJS.OA.21.00072.
6. Diaz Balzani L., Ciuffreda M., Vadalà G., Di Pino G., Papalia R., Denaro V. Osseointegration for lower and upper-limb amputation a systematic review of clinical outcomes and complications. *J Biol Regul Homeost Agents.* 2020;34(4 Suppl. 3):315-326.
7. Hebert J.S., Rehani M., Stiegelmar R. Osseointegration for lower-limb amputation: a systematic review of clinical outcomes. *JBJS Rev.* 2017;5(10):e10. doi: 10.2106/JBJS.RVW.17.00037.
8. Ontario Health (Quality). Osseointegrated prosthetic implants for people with lower-limb amputation: a health technology assessment. *Ont Health Technol Assess Ser.* 2019;19(7):1-126. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6939984/.

9. Calabrese G., Franco D., Petralia S., Monforte F., Condorelli G.G., Squarzoni S. et al. Dual-functional nano-functionalized titanium scaffolds to inhibit bacterial growth and enhance osteointegration. *Nanomaterials (Basel)*. 2021;11(10):2634. doi: 10.3390/nano11102634.
10. Fischer N.G., Chen X., Astleford-Hopper K., He J., Mullikin A.F., Mansky K.C. et al. Antimicrobial and enzyme-responsive multi-peptide surfaces for bone-anchored devices. *Mater Sci Eng C Mater Biol Appl*. 2021;125:112108. doi: 10.1016/j.msec.2021.112108.
11. Song Y.W., Paeng K.W., Kim M.J., Cha J.K., Jung U.W., Jung R.E. et al. Secondary stability achieved in dental implants with a calcium-coated sandblasted, large-grit, acid-etched (SLA) surface and a chemically modified SLA surface placed without mechanical engagement: A preclinical study. *Clin Oral Implants Res*. 2021;32(12):1474-1483. doi: 10.1111/clr.13848.
12. Wang X., Ning B., Pei X. Tantalum and its derivatives in orthopedic and dental implants: osteogenesis and antibacterial properties. *Colloids Surf B Biointerfaces*. 2021;208:112055. doi: 10.1016/j.colsurfb.2021.112055.
13. Li Y., Branemark R. Osseointegrated prostheses for rehabilitation following amputation : the pioneering Swedish model. *Unfallchirurg*. 2017;120(4):285-292. doi: 10.1007/s00113-017-0331-4.
14. Thesleff A., Branemark R., Hakansson B., Ortiz-Catalan M. Biomechanical characterisation of bone-anchored implant systems for amputation limb prostheses: a systematic review. *Ann Biomed Eng*. 2018;46(3):377-391. doi: 10.1007/s10439-017-1976-4.
15. Branemark R.P., Hagberg K., Kulbacka-Ortiz K., Berlin O., Rydevik B. Osseointegrated percutaneous prosthetic system for the treatment of patients with transfemoral amputation: a prospective five-year follow-up of patient-reported outcomes and complications. *J Am Acad Orthop Surg*. 2019;27(16):e743-e751. doi: 10.5435/JAAOS-D-17-00621.
16. Meric G., Mageiros L., Pensar J., Laabei M., Yahara K., Pascoe B. et al. Disease-associated genotypes of the commensal skin bacterium Staphylococcus epidermidis. *Nature Communications*. 2018;9(1):5034. doi: 10.1038/s41467-018-07368-7.
17. Zaborowska M., Tillander J., Branemark R., Hagberg L., Thomsen P., Trobos M. Biofilm formation and antimicrobial susceptibility of staphylococci and enterococci from osteomyelitis associated with percutaneous orthopaedic implants. *J Biomed Mater Res Part B*. 2017;105(8):2630-2640. doi: 10.1002/jbm.b.33803.
18. Tillander J., Hagberg K., Berlin O., Hagberg L., Branemark R. Osteomyelitis risk in patients with transfemoral amputations treated with osseointegration prostheses. *Clin Orthop Relat Res*. 2017;475(12):3100-3108. doi: 10.1007/s11999-017-5507-2.
19. Egert M., Simmering R., Riedel C.U. The association of the skin microbiota with health, immunity, and disease. *Clin Pharmacol Ther*. 2017;102(1):62-69. doi: 10.1002/cpt.698.
20. Dantas T., Padrao J., da Silva M.R., Pinto P., Madeira S. et al. Bacteria co-culture adhesion on different texturized zirconia surfaces. *J Mech Behav Biomed Mater*. 2021;123:104786. doi: 10.1016/j.jmbbm.2021.104786.
21. Pääkkönen M., Kallio M.J., Kallio P.E., Peltola H. C-reactive protein versus erythrocyte sedimentation rate, white blood cell count and alkaline phosphatase in diagnosing bacteraemia in bone and joint infections. *J Paediatr Child Health*. 2013;49(3):E189-192. doi: 10.1111/jpc.12122.
22. Gayuk V.D., Kliushin N.M., Burnashov S.I. [Pin site soft tissue infection and osteomyelitis: literature review]. *Genij Ortopedii*. 2019;25(3):407-412. (In Russian). doi: 10.18019/1028-4427-2019-25-3-407-412.
23. Overmann A.L., Aparicio C., Richards J.T., Mutreja I., Fischer N.G., Wade S.M. et al. Orthopaedic osseointegration: Implantology and future directions. *J Orthop Res*. 2020;38(7):1445-1454. doi: 10.1002/jor.24576.
24. Lenneras M., Tsikandylakis G., Trobos M., Omar O., Vazirisani F., Palmquist A. et al. The clinical, radiological, microbiological, and molecular profile of the skin-penetration site of transfemoral amputees treated with bone-anchored prostheses. *J Biomed Mater Res A*. 2017;105(2):578-589. doi: 10.1002/jbm.a.35935.
25. Gristina A.G. Biomaterial-centered infection: microbial adhesion versus tissue integration. *Science*. 1987;237(4822):1588-1595. doi: 10.1126/science.3629258.
26. Pilz M., Staats K., Tobudic S., Assadian O., Presterl E., Windhager R. et al. Zirconium nitride coating reduced staphylococcus epidermidis biofilm formation on orthopaedic implant surfaces: an in vitro study. *Clin Orthop Relat Res*. 2019;477(2):461-466. doi: 10.1097/CORR.0000000000000568.
27. Rochford E.T., Subbiahdoss G., Moriarty T.F., Poulsson A.H., van der Mei H.C., Busscher H.J. et al. An in vitro investigation of bacteria-osteoblast competition on oxygen plasma-modified PEEK. *J Biomed Mater Res A*. 2014;102(12):4427-4434. doi: 10.1002/jbm.a.35130.
28. Subbiahdoss G., Kuijer R., Busscher H., van der Mei H. Mammalian cell growth versus biofilm formation on biomaterial surfaces in an in vitro post-operative contamination model. *Microbiology*. 2010;156(Pt 10):3073-3078. doi: 10.1099/mic.0.040378-0.
29. Campoccia D., Testoni F., Ravaioli S., Cangini I., Maso A., Speziale P. et al. Orthopedic implant infections: incompetence of Staphylococcus epidermidis, Staphylococcus lugdunensis, and Enterococcus faecalis to invade osteoblasts. *J Biomed Mater Res A*. 2016;104(3):788-801. doi: 10.1002/jbm.a.35564.
30. Stracquadanio S., Musso N., Costantino A., Lazzaro L.M., Stefani S., Bongiorno D. Staphylococcus aureus internalization in osteoblast cells: mechanisms, interactions and biochemical processes. What did we learn from experimental models? *Pathogens*. 2021;10(2):239. doi: 10.3390/pathogens10020239.
31. Hinton P.V., Rackard S.M., Kennedy O.D. In vivo osteocyte mechanotransduction: recent developments and future directions. *Curr Osteoporos Rep*. 2018;16(6):746-753. doi: 10.1007/s11914-018-0485-1.
32. Maycas M., Esbrit P., Gortázar A.R. Molecular mechanisms in bone mechanotransduction. *Histol Histopathol*. 2017;32(8):751-760. doi: 10.14670/HH-11-858.

33. Somemura S., Kumai T., Yatabe K., Sasaki C. Fujiya H., Niki H. et al. Physiologic mechanical stress directly induces bone formation by activating glucose transporter 1 (GLUT 1) in osteoblasts, inducing signaling via NAD+-dependent deacetylase (Sirtuin 1) and Runt-Related Transcription Factor 2 (Runx2). *Int J Mol Sci.* 2021;22(16):9070. doi: 10.3390/ijms22169070.

34. Soldatov Yu.P., Stogov M.V., Ovchinnikov E.N., Gubin A.V., Gorodnova N.V. [Evaluation of clinical efficacy and safety of the Ilizarov apparatus for external fixation (literature review)]. *Genij Ortopedii.* 2019;25(4):588-599. (In Russian). doi: 10.18019/1028-4427-2019-25-4-588-599.

Authors' information

✉ *Maksim V. Stogov* — Dr. Sci. (Biol.)
Address: 6, M. Ulyanova str., Kurgan, 640014, Russia
https://orcid.org/0000-0001-8516-8571
e-mail: stogo_off@list.ru,

Andrey A. Emanov — Cand. Sci. (Vet.)
https://orcid.org/0000-0003-2890-3597
e-mail: a_eman@list.ru

Natalia V. Goodovykh
https://orcid.org/0000-0001-8512-4165
e-mail: natalia_nvn@mail.ru

Evgenyi N. Ovchinnikov — Cand. Sci. (Biol.)
https://orcid.org/0000-0002-5595-1706
e-mail: omu00@list.ru

Natalia V. Tushina — Cand. Sci. (Biol.)
https://orcid.org/0000-0002-1322-608X
e-mail: ntushina76@mail.ru

Viktor P. Kuznetsov — Dr. Sci. (Tech.)
https://orcid.org/0000-0001-8949-6345
e-mail: wpkuzn@mail.ru