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Composition and toxicity of damaging fragments in gunshot and mine-blast spine injuries

V.P. Orlov[1], Yu.A. Nashchekina[2], A.V. Nashchekin[3], S.D. Mirzametov[1], S.M. Idrichan[1], M.N. Kravtsov[1], D.V. Svistov[1]

[1] Kirov Military Medical Academy, Saint Petersburg, Russia;

[2] Institute of Cytology of the Russian Academy of Sciences, Saint Petersburg, Russia;

[3] Ioffe Physical-Technical Institute, Saint Petersburg, Russia

ABSTRACT

The feasibility of removing damaging fragments from the spine in gunshot and mine-blast injuries is assessed based on the data of their composition and cytotoxicity. Four damaging fragments removed from the spine and paravertebral tissues were analyzed. Elemental analysis was performed using a scanning electron microscope. The composition of the damaging fragments was studied using spectral analysis. The cytotoxicity of the medium with damaging fragments was evaluated using the methyl tetrazolium test, comparing to the control medium. Morphological changes in cells were assessed using optical light microscopy, comparing to the control. Elemental analysis showed that all studied fragments consisted of alloys of various metals and other chemical elements. During the first few weeks of incubation in a complete nutrient medium, metals underwent fairly active oxidation, producing an orange precipitate. During further incubation, the oxidation of metals continued quite intensively, leading to a change in the nutrient medium and reducing cell proliferation. Moreover, morphological examination showed that cells exposed to metal oxides were rounded, while control sample cells were elongated and spindle-shaped. The methyl tetrazolium test revealed high cytotoxicity of all the fragments studied. All fragments were found to release toxic metal oxides into the nutrient medium, significantly reducing cell viability, regardless of their elemental composition. to prevent complications associated with possible local and/or systemic toxicity of metal fragments, as well as early and late infections, it is recommended to remove projectiles to the maximum extent feasible.

Keywords: mine-blast injuries; gunshot spine injuries; wounding projectile; fragment toxicity; methyl tetrazolium test; elemental analysis of wounding projectiles; scanning electron microscopy; spectral analysis.

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Определение состава и токсичности поражающих элементов при огнестрельных и минно-взрывных ранениях позвоночника

В.П. Орлов[1], Ю.А. Нащекина[2], А.В. Нащекин[3], С.Д. Мирзаметов[1], С.М. Идричан[1], М.Н. Кравцов[1], Д.В. Свистов[1]

[1] Военно-медицинская академия имени С.М. Кирова, Санкт-Петербург, Россия;

[2] Институт цитологии Российской академии наук, Санкт-Петербург, Россия;

[3] Физико-технический институт им. А.Ф. Иоффе, Санкт-Петербург, Россия

АННОТАЦИЯ

Оценивается целесообразность удаления поражающих элементов, полученных при огнестрельных и минно-взрывных ранениях позвоночника, на основании результатов исследования их состава и цитотоксичности. Исследованы 4 поражающих элемента, удаленных из позвоночника и паравертебральных тканей. Элементный анализ проводили с помощью сканирующего электронного микроскопа. Состав поражающих элементов изучен с помощью спектрального анализа. С применением метилтетразолиевого теста определяли цитотоксичность среды с поражающими элементами по сравнению с контрольной средой. Морфологические изменения клеток оценивали с помощью оптической световой микроскопии, сравнивая с контрольной средой. По результатам элементного анализа все исследуемые осколки представлены сплавами различных металлов и других химических элементов. В процессе инкубирования осколков в полной питательной среде происходит достаточно активное окисление металлов в течение первых нескольких недель с появлением оранжевого осадка. При дальнейшем инкубировании осколков процесс окисления металлов продолжается достаточно интенсивно. Изменения в питательной среде, вызванные таким окислением, снижают пролиферацию клеток. Кроме того, наблюдается разница в морфологической структуре клеток, культивируемых в присутствии оксидов металлов и в контрольном образце, где клетки имеют характерную для них вытянутую веретеновидную форму, а в экспериментальных образцах — более округлую форму. По результатам метил-тетразолиевого теста выявлена высокая цитотоксичность всех исследованных осколков. Установлено, что все осколки в питательной среде выделяют токсичные окислы металлов, значительно снижающие жизнеспособность клеток независимо от элементного состава исследованных осколков. Для профилактики осложнений, связанных с возможной местной и/или системной токсичностью металлических осколков, а также ранних и поздних инфекционных осложнений, необходимо стремиться к максимальному удалению ранящих снарядов.

Ключевые слова: минно-взрывные ранения; огнестрельные ранения позвоночника; ранящий снаряд; токсичность осколков; метил-тетразолиевый тест; элементный анализ ранящих снарядов; сканирующая электронная микроскопия; спектральный анализ.

Как цитировать

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脊柱枪伤和地雷爆炸伤杀伤碎片的毒性和成分测定

V.P. Orlov[1], Yu.A. Nashchekina[2], A.V. Nashchekin[3], S.D. Mirzametov[1], S.M. Idrichan[1], M.N. Kravtsov[1], D.V. Svistov[1]

[1] Kirov Military Medical Academy, Saint Petersburg, Russia;

[2] Institute of Cytology of the Russian Academy of Sciences, Saint Petersburg, Russia;

[3] Ioffe Physical-Technical Institute, Saint Petersburg, Russia

摘要

根据脊柱枪伤和地雷爆炸伤中杀伤碎片的成分和细胞毒性的研究结果，对清除杀伤碎片的合理性进行评估。研究了从脊柱和椎旁组织中取出的4枚杀伤碎片。使用扫描电子显微术进行了元素分析。利用光谱分析法研究了杀伤碎片的成分。采用甲基四氮唑试验，确定了与对照培养基相比杀伤碎片培养基的细胞毒性。与对照培养基相比，用光学显微镜评估了细胞的形态变化。根据元素分析结果，所研究碎片都是各种金属和其他化学元素的合金。在完全营养培养基中培养碎片的过程中，最初几周会发生相当活跃的金属氧化，并出现橙色沉淀。 在碎片的进一步培养过程中，金属氧化过程会继续进行。 这种氧化引起的营养培养基变化会降低细胞的增殖。此外，在细胞形态结构上，有金属氧化物存在的培养的与对照样品中的存在差异，对照样品中的细胞呈特征性的细长纺锤形，而实验样品中的细胞则呈更圆润的形状。甲基四氮唑试验的结果表明，所有研究的碎片都具有很强的细胞毒性。已确定，所有碎片在营养培养基中都会释放出有毒的金属氧化物，极大降低了细胞的存活能力，与所研究碎片的元素组成无关。为防止金属碎片可能引起的局部和/或全身毒性并发症，以及早期和晚期感染并发症，必要最大限度地清除致伤弹丸。

关键词：地雷爆炸伤；脊柱枪伤；致伤弹丸；弹片毒性；甲基四氮唑试验；致伤弹丸的元素分析；扫描电子显微术；光谱分析。

引用本文

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INTRODUCTION

Gunshot wounds to the spine and spinal cord are a devastating combat injury, characterized by high mortality rates at all stages of spinal cord injury and significant long-term disability in most affected individuals [1]. However, gunshot wounds to the spine and spinal cord are relatively rare in combat settings. During the Great Patriotic War, their incidence depended on the type of military operations and ranged from 0.5% to 3% [2]. Data from localized conflicts, such as military operations in Afghanistan and the Chechen Republic, show an incidence of 4.7%–5.1% for gunshot wounds to the spine and spinal cord [3]. Recent high-tech localized armed conflicts demonstrated that most injuries are caused by mine explosions and shrapnel, often presenting as combined and multiple traumas, which exacerbates the severity of the wounded. in recent years, surgeons have adopted a highly effective active treatment approach. Additionally, treatment should be comprehensive, particularly for combined injuries [4, 5].

Modern gunshot wounds differ from those in past wars owing to their increased variety and the extensive tissue damage that extends beyond the wound canal [6–8]. in contemporary localized conflicts, combat operations frequently involve advanced weaponry, with each region exhibiting its own combat characteristics. Shrapnel are metallic fragments from aerial bombs, artillery shells, rockets, grenades, or landmines.

Currently, localized military conflicts heavily rely on artillery and strike drones, which function as explosive devices. The use of depleted uranium munitions has been reported. Concerns about the health and environmental effects of depleted uranium have prompted several nations to seek alternative materials for armor-piercing munitions, leading to the development of tungsten-based substitutes. However, experimental studies on laboratory rodents have demonstrated that highly aggressive malignant rhabdomyosarcomas developed after implantation of military-grade composite granules (e.g., tungsten, nickel, and cobalt) into limb muscles [9]. Furthermore, during the Gulf War, inhalation of desert dust particles led to outbreaks of respiratory diseases of unknown etiology, referred to as “severe acute pneumonitis.” Detailed analysis of Iraqi desert dust revealed that these particles contained a clay or quartz core surrounded by an inorganic calcium carbonate layer, incorporating various metals such as (in descending order of concentration) aluminum, iron, uranium, nickel, cobalt, copper, lead, and chromium [10].

Lead, which is commonly present in bullets, is a heavy metal classified as a chemical element that causes damage through ionic mimicry, intracellular calcium homeostasis disruption, nitric oxide synthesis inhibition, oxidative stress production, and gene transcription alterations, including the formation of subacute and delayed abscesses [11].

This STUDY AIMED to evaluate the feasibility of extracting metallic fragments resulting from gunshot and mine-blast spinal injuries by analyzing their composition and cytotoxicity in relation to mesenchymal stromal cells (MSCs).

MATERIALS AND METHODS

Damaging fragments removed from the spine and paravertebral tissues were studied. Indications for the removal of metallic fragments in spinal and spinal cord injuries included blind penetrating wounds of the spine and spinal cord, cauda equina root injuries, and blind nonpenetrating and paravertebral spinal wounds. Accessible metallic fragments were extracted using minimally invasive techniques, such as tubular retractors and endoscopic procedures (Fig. 1).

Four samples were selected for composition and cytotoxicity analysis. The first stage of the cytological studies involved preparing the fragments. The metallic fragments extracted from the spinal cord and vertebrae were washed in running water and mechanically cleaned of organic material adhering to their surfaces. Subsequently, oxides were removed from the fragment surfaces using a metal brush and were washed in running water, dried, and prepared for analysis. Elemental analysis was performed using a JSM-7001F scanning electron microscope (Jeol, Japan). The composition of the fragments was determined through spectral analysis at the A.F. Ioffe Physical-Technical Institute of the Russian Academy of Sciences (St. Petersburg, Russia).

For cytotoxicity testing, the fragment samples were sterilized in 70% ethanol and incubated in a complete nutrient medium (Dulbecco’s Modified Eagle Medium [DMEM/F12], Gibco, USA), supplemented with 1% essential amino acids, 10% heat-inactivated fetal bovine serum (HyClone, USA), 1% L-glutamine, 50 IU/ml penicillin, and 50 μg/ml streptomycin.

For cytotoxicity assessment, the FetMSCs human mesenchymal stromal cell line (Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia) was used. The cells were cultured in a CO_2 incubator at 37 °C in a humidified atmosphere containing 5% CO_2 in DMEM/F12 medium. The methyl tetrazolium (MTT) assay was conducted using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.1 mg/ml) to quantitatively assess the cytotoxicity of metal oxides.

For the experiment, 5×10^3 cells per 100 μl per well were seeded in 96-well plates and cultured for 24 hours to allow cell attachment. After 24 hours, the medium was

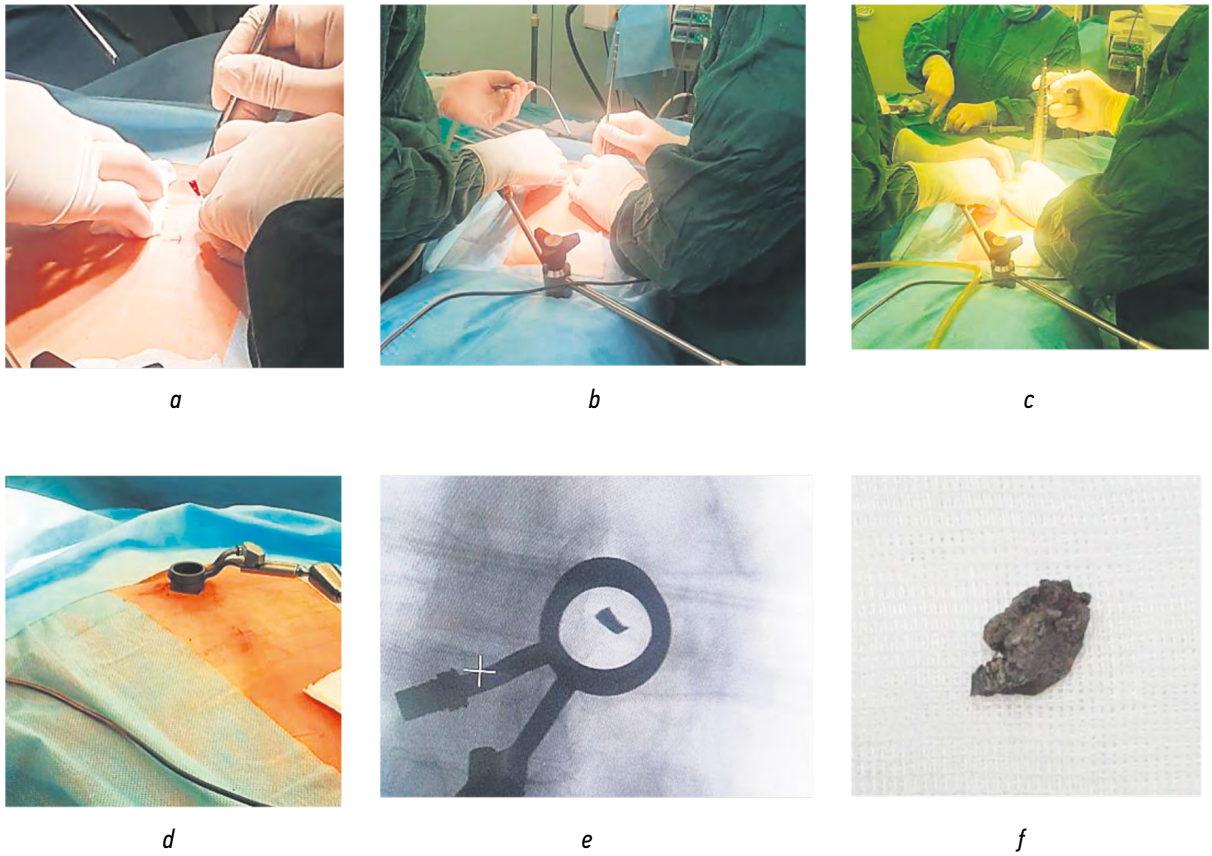


Fig. 1. Minimally invasive removal of a fragment from paravertebral soft tissues; *a, b, c, d* — stages of preparing access using tubular retractors and installation of a working sheath (21 mm in diameter); *e* — *X*-ray guided adjustment of the sheath position; *f* — removed wounding projectile

Рис. 1. Минимально инвазивное удаление осколка из паравертебральных мягких тканей: *a*, *b*, *c*, *d* — этапы выполнения доступа с применением тубулярных ретракторов и установки рабочего тубуса (диаметр 21 мм); *e* — коррекция положения тубуса под контролем рентгенографии; *f* — внешний вид удаленного ранящего снаряда

removed, and the wells were replenished with complete nutrient medium containing the metal fragments, which were incubated for 3 weeks. After 3 days, the medium was removed, and 50 µl of DMEM/F12 containing MTT was added to each well [12]. The cells were incubated in a CO_2 incubator for 2 hours at 37 °C. After removing the supernatant, formazan crystals, which were produced by metabolically active cells, were dissolved in 50 µl of dimethyl sulfoxide per well and transferred to clean wells. Then, MSC viability was assessed by measuring the optical density at 570 nm using a plate spectrophotometer. Similar experiments were conducted with the medium after an incubation period of 3 months. Polynomial regression analysis was performed using Microsoft Excel (Microsoft Corporation, USA) to calculate the MSC viability.

Statistical processing was conducted using recommended methods for medical, pharmaceutical, and biomedical research, and Microsoft Excel 2010 (Microsoft Corporation, USA) was utilized. The sample size was not precalculated.

RESULTS AND DISCUSSION

The examined fragments were alloys composed of various metals and other chemical elements (Fig. 2, Table 1).

Each fragment contained oxygen (O_2) in the form of oxides, along with iron (Fe) (samples 1–4) or copper (Cu) (samples 1, 3, and 4). Moreover, a significant amount of carbon (C) was detected in all the samples. The magnetic properties of the fragments varied depending on the metal composition. Fragments with a higher iron content demonstrated stronger magnetic properties.

During the incubation of the fragments in the complete nutrient medium, active oxidation of the metals occurred within the first few weeks, as evidenced by a change in medium color and formation of an orange precipitate (Fig. 3*a*). Metal oxidation remained intense as the incubation period continued, leading to a more pronounced color change and accumulation of a dense orange precipitate (Fig. 3*b*).

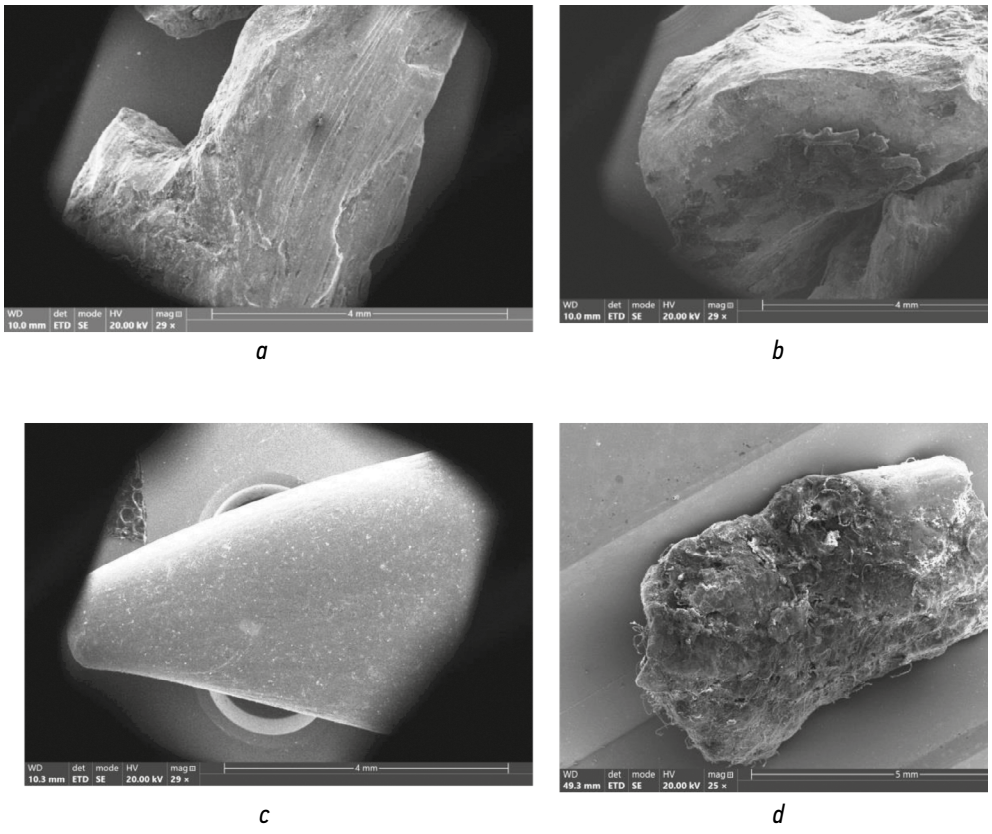


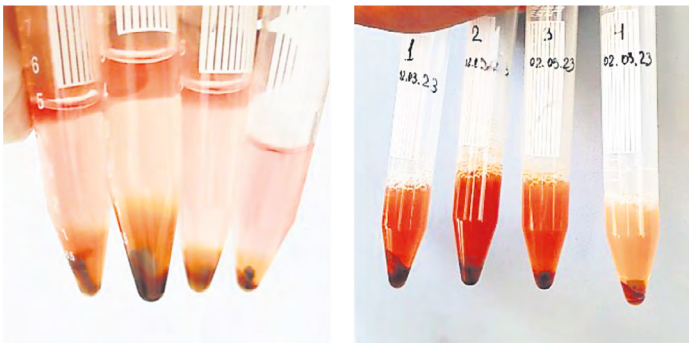
Fig. 2. Scanning electron microscopy of the surface of four fragment samples; *a* — Sample 1; *b* — Sample 2; *c* — Sample 3; *d* — Sample 4
Рис. 2. Сканирующая электронная микроскопия поверхности четырех образцов осколков: *a* — образец 1; *b* — образец 2; *c* — образец 3; *d* — образец 4

Table 1. Elemental composition of four fragment samples
Таблица 1. Элементный состав четырех образцов осколков

Sample 1		Sample 2		Sample 3		Sample 4	
Element	Mass %	Element	Mass %	Element	Mass %	Element	Mass %
C	35.2	C	43.69	C	47.77	C	38.49
O	22.25	O	26	O	17.79	O	14.87
F	4.53	Na	2.41	F	1.75	F	4.43
Mg	0.19	Al	0.18	P	0.74	Na	2.01
Al	0.29	Si	0.59	Ca	1.1	P	0.27
Si	1.88	P	0.65	Mn	0.9	Cl	0.21
P	0.19	K	0.3	Fe	23.82	Ca	0.27
S	0.16	Ca	0.76	Cu	3.64	Cr	0.43
K	0.15	Cr	0.36	Zn	2.15	Fe	36.79
Ca	0.69	Fe	24.3	Mo	0.34	Co	0.08
Mn	0.45	Cu	0.37	–	–	Tb	2.15
Fe	27.26	Mo	0.38	–	–	–	–
Cu	4.16	–	–	–	–	–	–
Zn	2.6	–	–	–	–	–	–

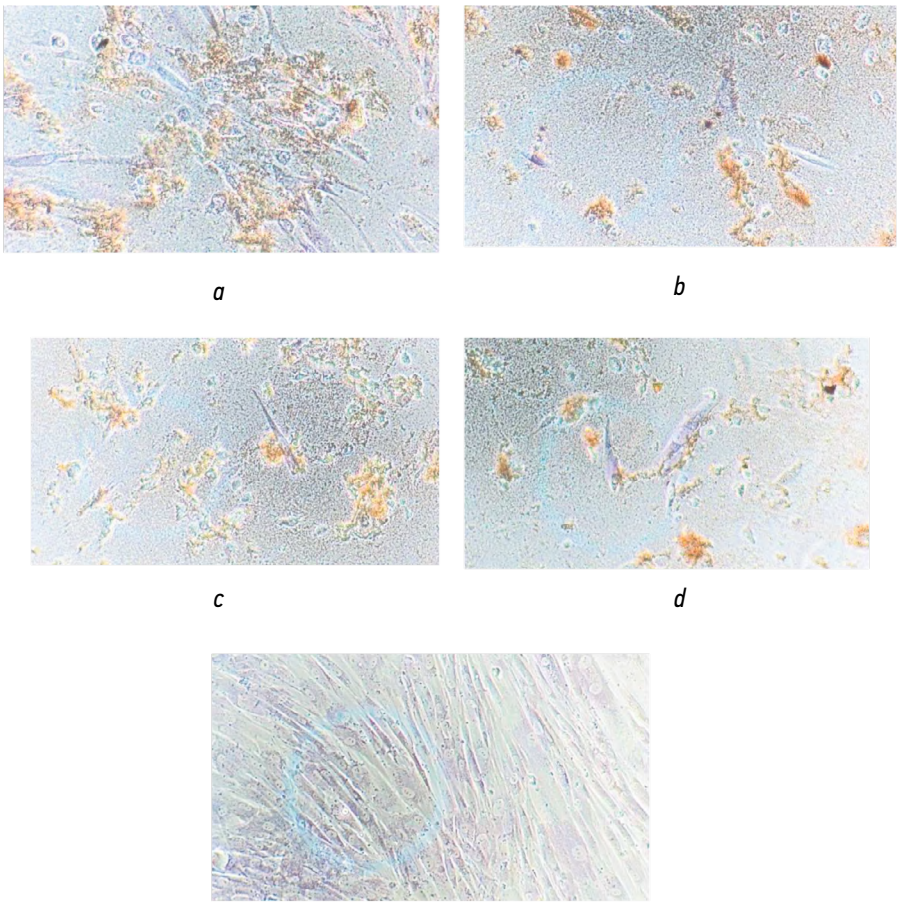
To assess the potential cytotoxicity of the fragments and metal oxides formed during incubation, the conditioned nutrient medium was added to the MSCs and cultured for 3 days. Then, cell morphology was evaluated through light microscopy (Fig. 4).

Figure 4 shows that all four samples contained metal oxide precipitates in the form of orange deposits. This precipitate reduced cell proliferation, as indicated by the lower cell density in the experimental samples compared with the control, where a monolayer of cells had formed by day 3. Additionally, cell morphology differed between the experimental and control samples. in contrast to the control sample, wherein cells exhibited their characteristic elongated, spindle-like shape,



a *b*

Fig. 3. External appearance of the nutrient medium; *a* — after 3-week incubation with tragments; *b* — after 3-month incubation with fragments
Рис. 3. Внешний вид питательной среды: *a* — после 3 нед. инкубирования осколков; *b* — после 3 мес. инкубирования осколков



a *b* *c* *d* *f*

Fig. 4. Light microscopy of mesenchymal stromal cells cultured in the medium after incubation with fragments for 3 days; *a* — Sample 1; *b* — Sample 2; *c* — Sample 3; *d* — Sample 4: *e* — control medium
Рис. 4. Световая микроскопия МСК при культивировании в среде после инкубирования с осколками в течение 3 сут: *a* — образец 1; *b* — образец 2; *c* — образец 3; *d* — образец 4: *e* — контрольная среда

the experimental samples showed more rounded cells. Sample 1 exhibited a higher number of adherent cells compared with samples 2–4.

Figure 5 presents a viability assessment diagram for MSCs cultured in the conditioned medium after 3 weeks of incubation with metal fragments. The MTT assay results was consistent with the light microscopy findings. The number of viable cells in samples 2–4 was significantly lower than that in the control sample. Although the number of viable cells was higher in sample 1 than in samples 2–4, it was still lower than that in the control group.

With longer incubation (3 months), metal oxide formation remained active. Metal oxides were observed in the culture medium after 3 days of cell culturing (Fig. 6).

In all four samples, no complete monolayer formation was observed. Moreover, the morphology of the cells

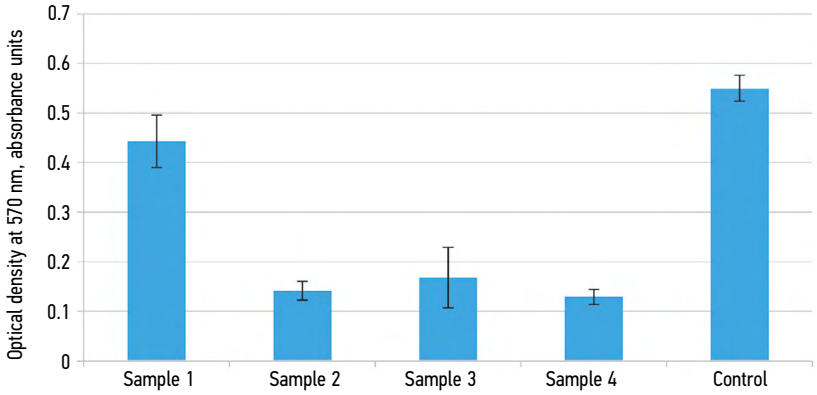


Fig. 5. Methyl tetrazolium test of mesenchymal stromal cells cultured for 3 days in the medium after incubation with fragments for 3 weeks

Рис. 5. МТТ МСК после 3 сут культивирования в присутствии питательной среды после инкубирования с осколками в течение 3 нед.

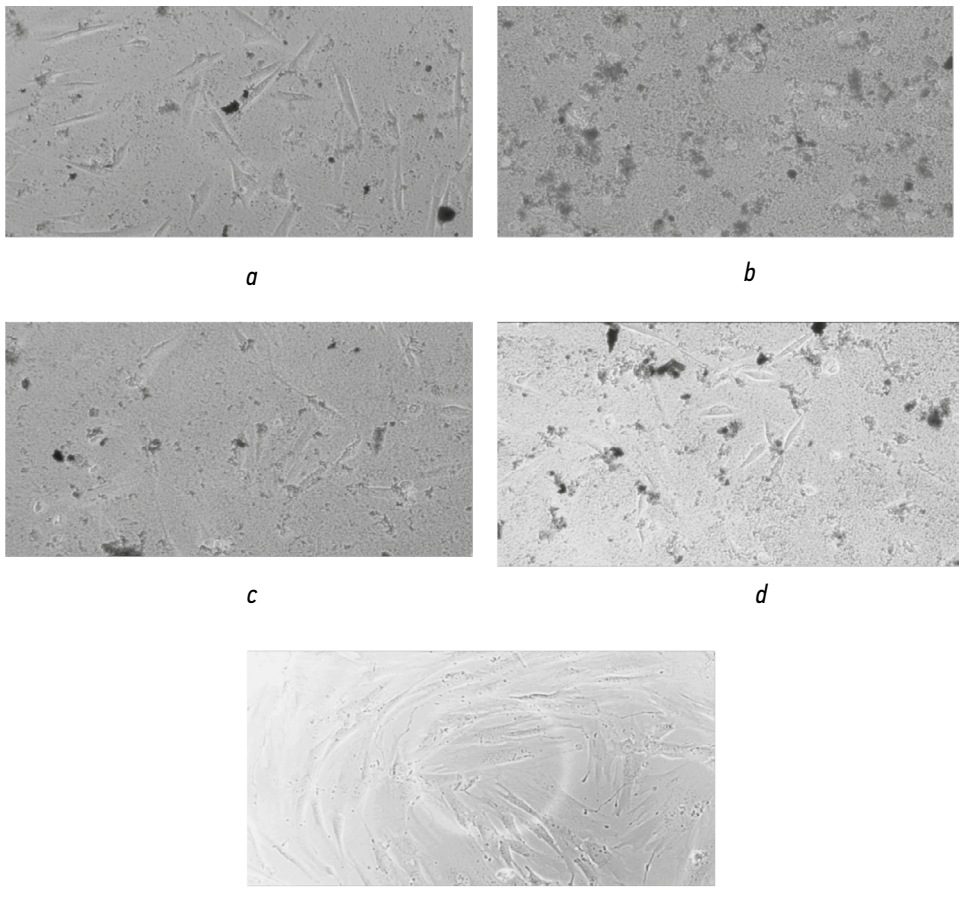


Fig. 6. Light microscopy of mesenchymal stromal cells cultured in the medium after incubation with fragments for 3 days; *a* — Sample 1; *b* — Sample 2; *c* — Sample 3; *d* — Sample 4; *e* — control medium

Рис. 6. Световая микроскопия МСК при культивировании МСК в среде после инкубирования с осколками в течение 3 сут: *a* — образец 1; *b* — образец 2; *c* — образец 3; *d* — образец 4; *e* — контрольная среда

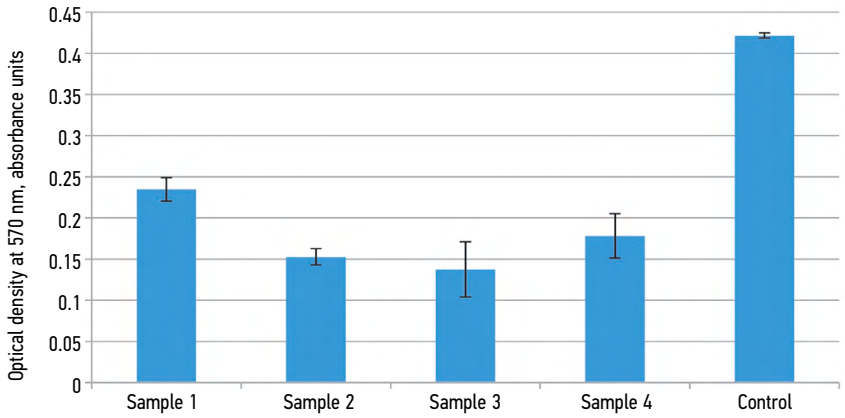


Fig. 7. Methyl tetrazolium test after 3-day cultivation in the medium after incubation with fragments for 3 months
Рис. 7. МТТ после 3 сут культивирования в присутствии питательной среды после инкубирования с осколками в течение 3 мес.

in samples 1–3 differed significantly from that of the control group. The number of viable cells in these media was <50% compared with the control, as confirmed by the MTT assay (Fig. 7).

All examined fragments consisted of multiple chemical elements and various metal alloys. A comparison of the elemental analysis and MTT assay results demonstrated that all fragments released toxic metal oxides into the nutrient medium, significantly reducing the viability of surrounding tissues, regardless of their elemental composition.

No adverse events were observed during the study. However, a limitation of the present study was the lack of a comparative cytotoxicity analysis between wounding projectiles and bioinert materials (e.g., implants).

CONCLUSION

The study results reveal that oxides of various metal alloys present in damaging fragments removed from the spine and paravertebral tissues exhibit toxicity to the human body, regardless of their elemental composition. It is crucial to maximize the removal of wounding projectiles whenever feasible to prevent complications associated with the potential local and/or systemic toxicity of metallic fragments and early and late infectious complications.

ADDITIONAL INFORMATION

Authors' contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

The contribution of each author. V.P. Orlov — development of a general concept; writing an article; Yu.A. Nashchekina — determination of the toxicity of fragments, data analysis; A.V. Nashchekin — assessment of the composition of wounding shells, data analysis; S.D. Mirzametov — removal of fragments, data analysis; S.M. Idrichan — removal of fragments; M.N. Kravtsov — removal of fragments; D.V. Svistov — study design, data analysis.

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

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AUTHORS INFO

***Saidmirze D. Mirzametov,** MD, Cand. Sci. (Medicine);
ORCID: 0000-0002-1890-7546; eLibrary SPIN: 5959-1988;
e-mail: vmeda-nio@mil.ru

Vladimir P. Orlov, MD, Dr. Sci. (Medicine), professor;
ORCID: 0000-0002-5009-7117; eLibrary SPIN: 9790-6804

Yuliya A. Nashchekina, MD, Cand. Sci. (Medicine);
ORCID: 0000-0002-4371-7445; eLibrary SPIN: 1138-8088;
e-mail: nashchekina.yu@mail.ru

Alexey V. Nashchekin, Cand. Sci. (Physics and Mathematics);
ORCID: 0000-0002-2542-7364; eLibrary SPIN: 6638-5243

Sergey M. Idrichan, MD, Cand. Sci. (Medicine);
ORCID: 0009-0007-9442-7281; eLibrary SPIN: 1474-1269

Maxim N. Kravtsov, MD, Dr. Sci. (Medicine);
ORCID: 0000-0003-2486-6995; eLibrary SPIN: 2742-6397;
e-mail: neuromax@mail.ru

Dmitry V. Svistov, MD, Cand. Sci. (Medicine), assistant professor;
ORCID: 0000-0002-3922-9887; eLibrary SPIN: 3184-5590

ОБ АВТОРАХ

***Саидмирзе Джамирзоевич Мирзаметов,** канд. мед. наук;
ORCID: 0000-0002-1890-7546; eLibrary SPIN: 5959-1988;
e-mail: vmeda-nio@mil.ru

Владимир Петрович Орлов, д-р мед. наук, профессор;
ORCID: 0000-0002-5009-7117; eLibrary SPIN: 9790-6804

Юлия Александровна Нащекина, канд. мед. наук;
ORCID: 0000-0002-4371-7445; eLibrary SPIN: 1138-8088;
e-mail: nashchekina.yu@mail.ru

Алексей Викторович Нащекин, канд. физ.-мат. наук;
ORCID: 0000-0002-2542-7364; eLibrary SPIN: 6638-5243

Сергей Михайлович Идричан, канд. мед. наук;
ORCID: 0009-0007-9442-7281; eLibrary SPIN: 1474-1269

Максим Николаевич Кравцов, д-р мед. наук;
ORCID: 0000-0003-2486-6995; eLibrary SPIN: 2742-6397;
e-mail: neuromax@mail.ru

Дмитрий Владимирович Свистов, канд. мед. наук, доцент;
ORCID: 0000-0002-3922-9887; eLibrary SPIN: 3184-5590

* Corresponding author / Автор, ответственный за переписку