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CLINICAL SAFETY OF THE USAGE OF MATERIALS FOR IMPLANTATION SURGERY BASED ON TITANIUM AND ITS ALLOYS ACCORDING TO THE BIOCOMPATIBILITY INDEXES

Dolgalev Al.Al.[1], Vorobyov M.S.[2], Prokopenko N.A.[2], Choniashvili D.Z.[3], Gezuev G.K.[4], Avanisyan V.M.[1], Piskov S.I.[5], Rzhepakovsky I.V.[5]

[1] Stavropol State Medical University, Stavropol, Russian Federation
[2] Institute of High Current Electronics, Siberian Branch of Russian Academy of Sciences, Tomsk, Russian Federation
[3] Kosta Levanovich Khetagurov North Ossetian State University, Vladikavkaz, Russian Federation
[4] DENTA-CITY LLC, Grozny, Chechen Republic
[5] North Caucasus Federal University, Stavropol, Russian Federation

SUMMARY
Biocompatibility is one of the most important characteristics of materials for implantation. It has been scientifically confirmed that the physical and chemical properties of the surface of metal implants or their elements can have an impact on clinical safety. The structure of the material (porosity, smoothness, geometry) can influence its incorporation into surrounding tissues. This requires conducting experiments with a longer contact interval of the tested materials under in vivo conditions.

KEYWORDS: *titanium, VT6, embryotoxicity, biocompatibility, chicken embryos.*

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КЛИНИЧЕСКАЯ БЕЗОПАСНОСТЬ ИСПОЛЬЗОВАНИЯ МАТЕРИАЛОВ ДЛЯ ИМПЛАНТАЦИОННОЙ ХИРУРГИИ НА ОСНОВЕ ТИТАНА И ЕГО СПЛАВОВ ПО ДАННЫМ ПОКАЗАТЕЛЕЙ БИОСОВМЕСТИМОСТИ

Долгалев Ал.Ал.[1], Воробьев М.С.[2], Прокопенко Н.А.[2], Чониашвили Д.З.[3], Гезуев Г.К.[4], Аванисян В.М.[1], Писков С.И.[5], Ржепаковский И.В.[5]

[1] Ставропольский государственный медицинский университет, Ставрополь, Российская Федерация
[2] Институт сильноточной электроники Сибирского отделения Российской Академии наук, Томск, Российская Федерация
[3] Северо-Осетинский государственный университет имени Коста Левановича Хетагурова, Владикавказ, Российская Федерация
[4] ООО «ДЕНТАЛ-СИТИ», Грозный, Чеченская Республика
[5] Северо-Кавказский федеральный университет, Ставрополь, Российская Федерация

РЕЗЮМЕ
Биосовместимость выступает одной из важнейших характеристик материалов для имплантации. Научно подтверждено, что физические и химические свойства поверхности металлических имплантатов или их элементов могут оказывать влияние на клиническую безопасность. Структура материала (пористость, гладкость, геометрия) может оказывать влияние на его включение в окружающие ткани. Это требует проведения экспериментов при более длительном интервале контакта тестируемых материалов в условиях in vivo.

КЛЮЧЕВЫЕ СЛОВА: титан, ВТ6, эмбриотоксичность, биосовместимость, куриные эмбрионы.

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Introduction

Medical materials science includes the development and research of materials that are used in medicine, created to compensate for the loss of organs or tissues. The subject of inorganic medical materials science are metals or metal alloys in the form of load-bearing structures or diagnostic preparations; materials of inert metals, oxide materials intended for the treatment of bone tissue defects or the cultivation of cell cultures when applied to appropriate metal surfaces [2, 8].

The biomaterial should be biocompatible and can be biodegradable [1, 9]. Biocompatible materials are materials that have a nonbiological origin and are used in medicine to achieve interaction with a biological system. They have the ability to function with the appropriate reaction of the host body in a particular case of use, without causing inflammation or necrosis of the surrounding tissues [3, 4, 7]. Biocompatible materials and devices act or function harmoniously and coherently when in contact or inside a living body, without causing serious diseases or complications.

The biocompatibility of materials includes:

1. immunological compatibility, which is mainly related to the selection of antigen-compatible tissues, cells, and bioengineered structures;
2. morphofunctional compatibility (embedding, integration with surrounding tissues);
3. biomechanical compatibility (the ability to withstand mechanical, hydrodynamic and other types of loads) [2, 10].

Biocompatibility is a complex selective property of an organism, in which the possibility of coexistence of a biomaterial and a biosystem is mediated with the preservation of all tissue functions and its ability to regenerate [6]. The use of biomaterials becomes vital due to their special effect on the quality and duration of human life. Implants should not cause a local inflammatory reaction, systemic pathological processes, exacerbate complications, and must preserve the declared properties during their service life, thus confirming their clinical safety while using [5].

Materials and methods of research

The studied samples were titanium washers of the Ti VT 6 brand, similar to Ti-6Al-4V, with a diameter of 6 mm and a thickness of 1.5 mm with a functional coating. The film was pure titanium (Ti) and zirconium (Zr), as well as titanium oxides (TiO_2) and zirconium (ZrO_2). The thickness of the functional film was 3 microns. The coating was applied by the vacuum arc plasma-assisted method on the vacuum experimental installation "QUINTA" [11], which is included in the list of unique installations "UNIQUUM" (https://ckp-rf.ru/catalog/usu/434216/). The samples were mounted in special equipment, which allows covering > 98% of the surface area of the sample.

The biocompatibility of the product samples was studied using the chorioallantoid membrane (CAM) model of a chicken embryo in accordance with the method described in [7, 9]. For the experiment, fertilized eggs were treated with 70% ethanol and incubated in an automatic incubator Rcom Maru Deluxe Max 380 (AUTOELEX CO., LTD, Korea). On the 3rd day of incubation, 3 ml of protein was aspirated from the sharp pole of the egg through a drilled hole. The hole was sealed with sterile paraffin. On the 8th day of incubation, a square window measuring ≈2.5 cm^2 was cut out in the shell and sterile products of four variants (n = 8) were inserted into the CAM, one sample per egg. Sterile round cover glasses of a similar diameter (5 mm) based on silicate glass were used as a control. The hole in the shell was sealed with transparent tape to prevent dehydration, and the eggs were incubated for another 6 days.

All manipulations with the CAM of the chicken embryo were performed in a box of abacterial air medium BAVnp-01-"Laminar-S"-1.5 (LORICA, CJSC "Laminar Systems", Russia). The experiment included eight embryos per group. On day 14, the embryos were euthanized in a gas chamber (70% CO_2, 30 minutes). The sealing tape was removed, the eggs were opened, the implantable products were excised with the surrounding areas of CAM tissue. Macroscopic assessment of CAM areas in contact with implantable products was performed using an Axio ZOOM.V16 macroscope equipped with the AxioCam MRc5 image visualization system and the Zen 2 Pro software package (Carl Zeiss Microscopy, Oberkochen, Germany). CAM tissues in contact with product samples were carefully separated and fixed in a buffered solution of 10% formalin for subsequent histological analysis.

Assessment of the condition and visualization of CAM blood vessels in dynamics 5 minutes after applying the test solutions to CAM was performed using an ARSTEK SZ0850 stereo microscope (China) and image fixation using a digital camera (38 MP Samega V6) and the S-EYE2.0 program (YOUNG WIN Technology Co., Ltd.).

All quantitative data were analyzed using univariate analysis of variance. The Biostat software package (version 4.03) was used. The differences were considered statistically significant at $p < 0.05$.

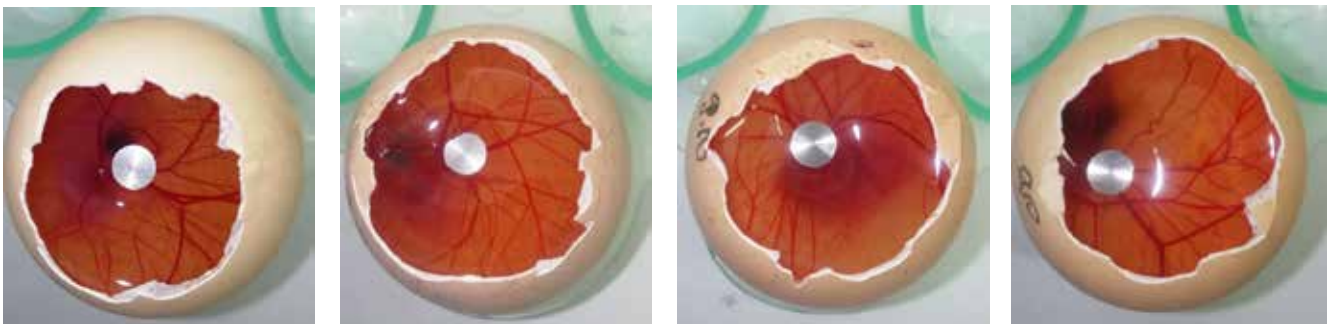
Results and discussion

The chemical composition, structure and texture of the surface of a material usually determine its main characteristics. The interaction of these properties in vivo is difficult to predict, so the final results can only be obtained empirically.

Among CAM's in vivo experiments, analysis is a fairly fast, simple, reproducible and practical method for studying the primary biocompatibility reaction of materials. The CAM system and the developing chicken embryo are recognized as a useful tool for assessing the toxic properties of engineering structures in vivo. The absence/presence of inflammatory and other reactions at the implantation site, as well as the mortality of the chicken embryo, are accepted as a method for assessing the biocompatibility of implants.

Biocompatibility is one of the most important characteristics of implantation materials. It has been scientifically confirmed that the physical and chemical properties of the surface of metal implants or their components can affect clinical safety. The structure of the material (porosity, smoothness, geometry) can affect its incorporation into surrounding tissues. This requires conducting experiments with a longer contact interval of the tested materials under in vivo conditions.

Considering the proven similarity of tissue reactions (vascular permeability, acute and chronic inflammation, granulation tissue formation and fibrosis) CAM and mammalian biocompatibility of the tested products *in vivo* was studied by implanting the products on the CAM of a chicken embryo (Figure 1).



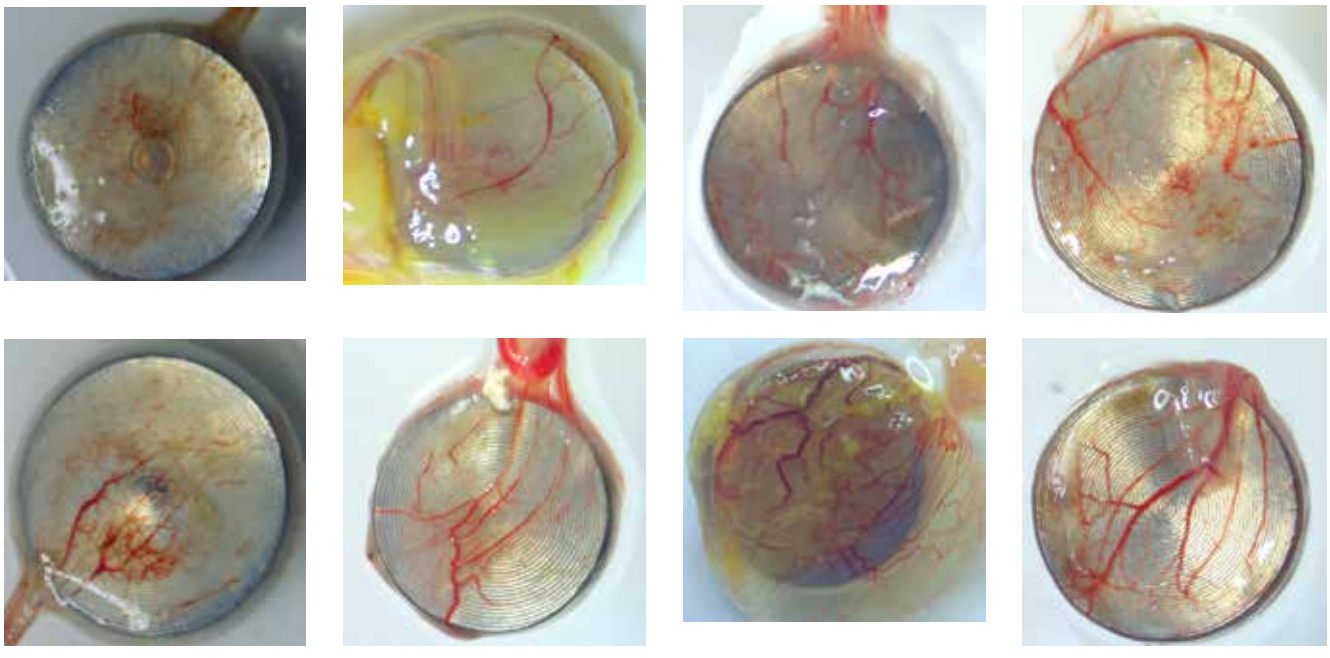
VT6 VT6+Ti VT6+TiO_2 VT6+ZrO_2

Fig. 1. Implantation of test products on the CAM of a chicken embryo on the 8th day of incubation

As in experiment of other researchers [4], because of their weight, the products placed on the CAM gradually dropped within an hour after implantation. Flooding of the implants was accompanied by their complete confinement in the CAM tissue. As a result, all the surfaces of the implanted product were in contact with the vascular network of the CAM (Figure 2).

Fig. 2. Immersion of the implanted product in CAM tissues

Macroscopic assessment of CAM tissues in contact with samples of metal products was performed after excision on the 14th day of incubation. Images of implanted articles and adjacent CAM sites for macroscopic assessment of the vasoproliferative response were made for each sample (Figure 3).



VT6 VT6+Ti VT6+TiO_2 VT6+ZrO_2

Fig. 3. Appearance of metal structures extracted from CAM 6 days after implantation

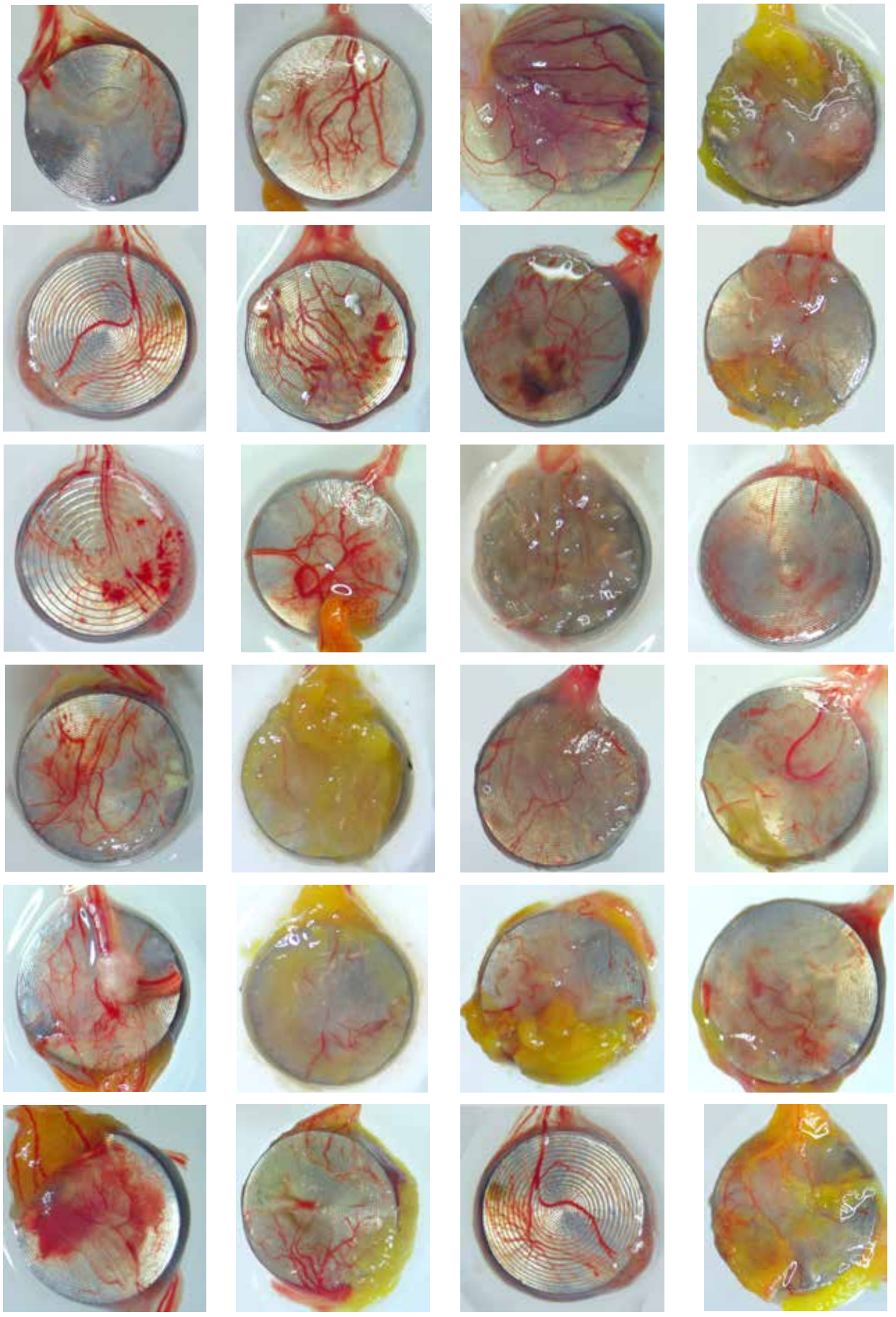


Fig. 3 (cont.). Appearance of metal structures extracted from CAM 6 days after implantation

Toxic substances can cause pronounced morphological and vascular changes in the CAM. Implantation of a material with toxic properties can be accompanied by coagulation, hemorrhage, discoloration of the contacting CAM tissues, the appearance of ghost vessels, and embryo mortality. Implantation of VT6, VT6+Ti, VT6+TiO_2, VT6+ZrO_2 samples on CAM did not critically affect the survival of embryos up to 14 days of development. In these groups and in the control group, the percentage of embryo mortality did not exceed 15%, which is not out of the reference range for the conditions of conducting such experiments and, presumably, is associated with manipulations with the incubation egg, rather than with the toxicity of implanted materials.

Macroscopic visualization of the extracted implants clearly shows vascularization and proliferation of contacting tissues around the products, which confirms their biocompatibility under CAM conditions. It was visually noted that the samples VT6+TiO_2, VT6+ZrO_2 were characterized by the highest rate of capsule formation from CAM tissues around the implanted products VT6+TiO_2, VT6+ZrO_2.

According to the presence of signs of vascular lysis, which is one of the signs of a toxic reaction, the implant samples were distributed in the order of its decrease as follows:

1. VT6;
2. VT6 + ZrO_2;
3. VT6 + Ti = VT6 + TiO_2.

Signs of CAM hemorrhage were also recorded for all product groups. To reduce the appearance of signs of hemorrhage, the samples were arranged as follows:

1. VT6;
2. VT6 + Ti = VT6 + ZrO_2;
3. VT6 + TiO_2.

It should be borne in mind that registered hemorrhages of CAM in contact with samples can be regarded not so much as a consequence of the toxic effect of the products, but rather as a result of mechanical injury and stretching of the CAM vessels. This fact is described in some works [9] and logically confirmed by the recorded hemorrhages in the control group (Figure 4).

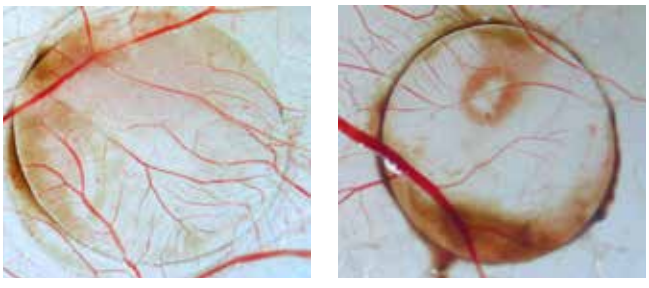


Fig. 4. Appearance of CAM and silicate glass samples (control) implanted on CAM after treatment on the 14th day of incubation

Conclusion

Summarizing the results of the evaluation of biocompatibility of coatings based on titanium, zirconium and their alloys deposited on implants using vacuum-arc sputtering with plasma assistance, we can conclude the following.

A series of experiments conducted to assess the biocompatibility of the tested products under the conditions of the CAM model of a chicken embryo confirmed the adequacy of its application. The size and weight of the studied samples were accompanied by subsidence deep into the CAM and their complete confinement in the tissue. As a result, all the surfaces of the implanted product were in contact with the vascular network of the CAM, which ensured the same conditions and the validity of conducting a comparative analysis of the tested samples. Mechanical action during implantation of the studied products on the surface of CAM did not affect the viability of chicken embryos up to 14 days of embryogenesis. Observations made at this stage of the study using specific in vivo techniques showed that the studied samples of products were compatible with the biological environment of CAM and did not show pronounced irritating, angiotoxic properties.

However, it should be borne in mind that this study has some limitations. The model of a developing chicken embryo and the CAM system may not always fully reflect the picture of the real clinical situation and act primarily as a preliminary screening test. The CAM model is mainly used for short-term studies due to the relatively short incubation period. In addition, substances introduced into the air chamber of the incubation egg may not correspond to the amount absorbed by the embryo, as they can spread to other egg structures. Therefore, to study the long-term effect and interaction, in some cases, it is obvious that it is advisable to expand the range of studies of the tested materials in mammalian models.

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INFORMATION ABOUT THE AUTHORS

Dolgalev Alexander Alexandrovich, *MD, Professor of the Department of General Dentistry and Pediatric Dentistry, Stavropol State Medical University, Ministry of Health of the Russian Federation, Stavropol, Russian Federation. ORCID: 0000-0002-6352-6750. E-mail: dolgalev@dolgalev.pro*

Vorobyov Maxim Sergeevich, *Doctor of Technical Sciences, Leading Researcher at the Institute of High-Current Electronics of the Siberian Branch of the Russian Academy of Sciences, Tomsk, Russian Federation. ORCID: http://orcid.org/0000-0001-5136-5905. E-mail: vorobyovms@yandex.ru*

Prokopenko Nikita Andreevich, *Junior Researcher at the Institute of High-Current Electronics of the Siberian Branch of the Russian Academy of Sciences, Tomsk, Russian Federation. ORCID: http://orcid.org/0000-0002-9381-872X. E-mail: prokopenko@opee.hcei.tsc.ru*

Choniashvili David Zurabovich, *Candidate of Medical Sciences, Associate Professor of the Department of Therapeutic, Surgical and Pediatric Dentistry with a course in Implantology, reconstructive Surgery of the oral cavity, pediatric CHLH, Dean of the Faculty of Medicine of the North Ossetian State University named after Kostya Levanovich Khetagurov (SOGU), Chief physician of the Clinical and Diagnostic Center Dentistry SOGU, Vladikavkaz, Russian Federation. E-mail: davidchoniashvili@mail.ru*

Avanisyan Vazgen Mikhailovich, *prosthodontist, Assistant at the Department of Organization of Dental Care, Management and Prevention of Dental Diseases, Stavropol State Medical University, Ministry of Health of the Russian Federation, Stavropol, Russian Federation. ORCID: https://orcid.org/0000-0002-0316-5957. SPIN code: 1207-9234. E-mail: avanvaz@yandex.ru*

Gezuyev Gimalai Kazbekovich, *freelance orthopedic dentist, dental surgeon of DENTA CITY LLC, Grozny, Chechen Republic. ORCID: 0009-0009-8612-4234. E-mail: denta_city@mail.ru*

Piskov Sergey Ivanovich, *PhD, Leading Researcher at the Interdepartmental Scientific and Educational Laboratory of Experimental Immunomorphology, Immunopathology and Immunobiotechnology of the Faculty of Medicine and Biology of the North Caucasus Federal University. E-mail: spiskov@ncfu.ru. ORCID: http://orcid.org/0000-0002-5558-5486*

Rzhepakovsky Igor Vladimirovich, *PhD, Associate Professor, leading researcher at the Interdepartmental Scientific and Educational Laboratory of Experimental Immunomorphology, Immunopathology and Immunobiotechnology of the Faculty of Medicine and Biology of the North Caucasus Federal University. ORCID: https://orcid.org/0000-0002-2632-8923. E-mail: 78igorr@mail.ru*