Ministry of Education and Science of the Russian Federation
Federal Independent Educational Institution
«NATIONAL RESEARCH TOMSK POLYTECHNIC UNIVERSITY»

Research School of Chemical and Biomedical Technologies
Program/specialty 12.04.04 «Biotechnical systems and technologies»

MASTER'S THESIS

Topic of the work
Модификация поверхности имплантов органическими функциональными группами **Implant surface modification with organic functional groups**

UDC 615.461:616-089.843

Student

Group	Full name	Signature	Date
9DM8I	Alasheva Umut Rasylbekovna		

Scientific Supervisor and Technical Advisor

Position	Full name	Academic degree, rank	Signature	Date
Assistant professor	Postnikov Pavel Sergeyevich	Candidate of Chemical Sciences		
Assistant scientist	Surmeneva Maria Alexandrovna	Candidate of Physical and Mathematical Sciences		

ADVISORS:

Section «Financial Management, Resource Efficiency and Resource Saving»

Position	Full name	Academic degree, rank	Signature	Date
Associate Professor of Department of Social and Human Sciences	Menshikova Ekaterina Valentinovna	Candidate of philosophy Sciences		

Section «Social Responsibility»

Position	Full name	Academic degree, rank	Signature	Date
Associate Professor of General Technical Disciplines	Mikhail Vladimirovich Gorbenko	Candidate of Engineering Sciences		

ADMIT OT DEFENSE:

Head of Department	Full name	Academic degree, rank	Signature	Date
Industrial and Medical Electronics	Gubarev Fedor Aleksandrovich	Candidate of Engineering Sciences		

Tomsk – 2020

Planned program learning outcomes

Код результата	Результат обучения (выпускник должен быть готов)	Требования ФГОС, критериев и/или заинтересованных сторон
Профессиональные компетенции		
Р1	Применять глубокие специальные естественнонаучные, математические, социально-экономические и профессиональные знания в инновационной инженерной деятельности при разработке, производстве, исследовании, эксплуатации, обслуживании и ремонте современной биомедицинской и экологической техники	Требования ФГОС (ОК-2, ОПК-2), Критерий 5 АИОР (п. 5.2.1), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
Р2	Ставить и решать инновационные задачи инженерного анализа и синтеза с использованием специальных знаний, современных аналитических методов и моделей	Требования ФГОС (ОПК-1, 3; ПК- 1 – 4), Критерий 5 АИОР (п. 5.2.2), согласованный с требованиями международных стандартов EUR-ACE и FEANI
Р3	Выбирать и использовать необходимое оборудование, инструменты и технологии для ведения инновационной практической инженерной деятельности с учетом экономических, экологических, социальных и иных ограничений	Требования ФГОС (ОК-9, ПК-10, 14, 18). Критерий 5 АИОР (пп. 5.2.3, 5.2.5), согласованный с требованиями международных стандартов EUR-ACE и FEANI
Р4	Выполнять комплексные инженерные проекты по разработке высокоэффективной биомедицинской и экологической техники конкурентоспособной на мировом рынке	Требования ФГОС (ОК-2, 3; ПК-5 – 11, 14), Критерий 5 АИОР (пп. 5.2.3, 5.2.5), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
Р5	Проводить комплексные инженерные исследования, включая поиск необходимой информации, эксперимент, анализ и интерпретацию данных с применением глубоких специальных знаний и современных методов для достижения требуемых результатов в сложных и неопределенных условиях	Требования ФГОС (ОК-2, 3; ОПК-5, ПК-1 – 4). Критерий 5 АИОР (пп. 5.2.2, 5.2.4), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
Р6	Внедрять, эксплуатировать и обслуживать современное высокотехнологичное оборудование в предметной сфере биотехнических систем и технологий, обеспечивать его высокую эффективность, соблюдать правила охраны здоровья и безопасности труда, выполнять требования по защите окружающей среды	Требования ФГОС (ОПК-1, 2), Критерий 5 АИОР (пп. 5.2.5, 5.2.6), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
Универсальные компетенции		
Р7	Использовать глубокие знания в области проектного менеджмента для ведения инновационной инженерной деятельности с учетом юридических аспектов защиты интеллектуальной собственности	Требования ФГОС (ОПК-2; ПК-14, 15). Критерий 5 АИОР (п. 5.3.1), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
Р8	Владеть иностранным языком на уровне, позволяющем активно осуществлять коммуникации в профессиональной среде и в обществе, разрабатывать документацию, презентовать и защищать результаты инновационной инженерной деятельности	Требования ФГОС (ОК-1), Критерий 5 АИОР (п. 5.3.2), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
Р9	Эффективно работать индивидуально и в качестве члена и руководителя команды, состоящей из специалистов различных направлений и квалификаций, с делением ответственности и полномочий при решении инновационных инженерных задач	Требования ФГОС (ОК-3, ОПК-3; ПК-3, 12, 13), Критерий 5 АИОР (п. 5.3.3), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
Р10	Демонстрировать личную ответственность, приверженность и готовность следовать профессиональной этике и нормам ведения инновационной инженерной деятельности	Критерий 5 АИОР (п. 5.3.4), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
Р11	Демонстрировать глубокие знание правовых социальных, экологических и культурных аспектов инновационной инженерной деятельности, компетентность в вопросах охраны здоровья и безопасности жизнедеятельности	Критерий 5 АИОР (п. 5.3.5), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
Р12	Самостоятельно учиться и непрерывно повышать квалификацию в течение всего периода профессиональной деятельности	Требования ФГОС (ОК-2, 4; ОПК-4), Критерий 5 АИОР (п.5.3.6), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*

Ministry of Education and Science of the Russian Federation
Federal Independent Educational Institution
«NATIONAL RESEARCH TOMSK POLYTECHNIC UNIVERSITY»

Research School of Chemical and Biomedical Technologies
Program/specialty 12.04.04 «Biotechnical systems and technologies»

APPROVED BY
Head of the Program
_____ _____ F.A.Gubarev
09.03.2020

ASSIGNMENT
For the Master's Thesis completion

In the form:

Master's Thesis

For a student:

Group	**Full Name**
9DM8I	Alasheva Umut Rasylbekovna

Topic of the work:

Модификация поверхности имплантов органическими функциональными группами **Implant surface modification with organic functional groups**	
Approved by the order of the Head (date, number)	

Deadline for completion of the Master's Thesis:	03.06.2020

TERMS OF REFERENCE:

Initial data for work: *(the name of the object of research or design; performance or load; mode of operation (continuous, periodic, cyclic, etc.); type of raw material or material of the product; requirements for the product, product or process; special requirements to the features of the operation of the object or product in terms of operational safety, environmental impact, energy costs; economic analysis, etc.).*	The object of the research: scaffold on based titanium alloy Ti6Al4V. Subject of the research: surface modification of titanium scaffolds to improve adhesion and osseointegration processes. The research results can be applied in the creation of biocompatible implants in the field of traumatology, orthopedics and dentistry to increase the osseointegration of the implant with the body tissue.
List of the issues to be investigated, designed and developed *(analytical review of literary sources in order to elucidate the achievements of world science and technology in the field under consideration, the formulation of the problem of research, design, construction, the content of the procedure of the research, design, construction, discussion of the performed work results, the name of additional sections to be developed; work conclusion).*	Conducting and writing a literature review on the topic. Investigation of existing problems. Setting goals and planning the stages of the experiment. Conducting experiments and research methods. Discussion of the results of the study. Assessment of the economic efficiency of the project and environmental safety. Conclusion.
List of graphic material *(with an exact indication of mandatory drawings)*	
Advisors on the sections of the Master's Thesis	

Chapter	Advisor
Section «Financial Management, Resource Efficiency and Resource Saving»	Associate Professor, Department of Social and Human Sciences Menshikova Ekaterina Valentinovna
Section «Social Responsibility»	Associate Professor of General Technical Disciplines Mikhail Vladimirovich Gorbenko

Date of issuance of the assignment for Master's Thesis completion according to a line schedule	09.03.2020

The task was issued by the Scientific Supervisor and Technical Advisor:

Position	Full Name	Academic degree, rank	Signature	Date
Assistant professor	Postnikov Pavel Sergeyevich	Candidate of Chemical Sciences		09.03.2020

The assignment was accepted for execution by the student:

Group	Full Name	Signature	Date
9DM8I	Alasheva Umut Rasylbekovna		09.03.2020

TASK FOR SECTION
«FINANCIAL MANAGEMENT, RESOURCE EFFICIENCY AND RESOURCE SAVING»

To the student:

Group	Full name
9DM8I	Alasheva Umut Rasylbekovna

School	RSCBMT	**School Department**	
The level of education	Master	**Direction / specialty**	12.04.04 / Biotechnical systems and technologies

Topic of the work

Модификация поверхности имплантов органическими функциональными группами **Implant surface modification with organic functional groups**	
Input data to the section «Financial management, resource efficiency and resource saving»:	
1. *Resource cost of scientific and technical research (STR): material and technical, energetic, financial and human*	Salary costs – 432770.7 rub STR budget – 113919.1 rub
2. *Expenditure rates and expenditure standards for resources*	Electricity costs – 5,8 rub per 1 kW
3. *Current tax system, tax rates, charges rates, discounting rates and interest rates*	Labor tax – 27,1 %; Overhead costs – 30%;
The list of subjects to study, design and develop:	
1. *Assessment of commercial and innovative potential of STR*	Comparative analysis with other researches in this field;
2. *Development of charter for scientific-research project*	SWOT-analysis;
3. *Scheduling of STR management process: structure and timeline, budget, risk management*	Calculation of working hours for project; Creation of the time schedule of the project; Calculation of scientific and technical research budget;
4. *Resource efficiency*	Integral indicator of resource efficiency for the developed project.
A list of graphic material *(with list of mandatory blueprints):*	
1. *Competitiveness analysis* 2. *SWOT- analysis* 3. *Gantt chart and budget of scientific research* 4. *Assessment of resource, financial and economic efficiency of STR* 5. *Potential risks*	

Date of issue of the task for the section according to the schedule	

Task issued by adviser:

Position	Full name	Scientific degree, rank	Signature	Date
Associate professor	E.V. Menshikova	PhD		

The task was accepted by the student:

Group	Full name	Signature	Date
9DM8I	Alasheva Umut Rasylbekovna		

ASSIGMENT FOR THE SECTION
«SOCIAL RESPONSIBILITY»

For the student:

Group	Full name
9DM8I	Alasheva Umut Rasylbekovna

School	RSCBMT	**Department (SEC)**	
Level of education	Master's degree	**Program / specialty**	12.04.04 «Biotechnical systems and technologies»

Topic of the work

Модификация поверхности имплантов органическими функциональными группами **Implant surface modification with organic functional groups**	
Initial data for the section "Social Responsibility":	
1. *Characteristic of the object of study (substance, material, device, algorithm, methodology, working area) and its fields of application*	The object of the study are biocompatible coatings of 4-carboxybenzenediazonium tosylates and a metal-organic framework for functionalizing the surface of scaffolds based on the titanium alloy.Ti6Al4V. Fields of application are biomedicine, in particular traumatology, orthopedics, and dentistry.
The list of issues subject to research, design and development	
1. Legal and organizational security issues: *1.1 Special legal norms of labor legislation;* *1.2 Organizational measures for the layout of the working area.*	1) The Labor Code of the Russian Federation dated December 30, 2001 N 197-FZ. 2) Ergonomic requirements for the correct location and layout of the researcher's working area.
2. Industrial safety: *2.1. Analysis of harmful and dangerous factors that may arise in the laboratory during research.* *2.2. Justification of measures to protect personnel from the action of dangerous and harmful factors*	Harmful factors: 1) Toxic substance: DMF, ethyl ether, acetone, acetic acid, etc. 2) Ultrasonic vibrations. Dangerous factors: 3) Explosive and fire hazard. 4) High temperature.
3. Environmental safety *3.1. Analysis of the impact of the research object on the environment.* *3.2. Analysis of the environmental impact of the research process.* *3.3. Justification of environmental protection measures*	Analysis of the impact of the substances used on the atmosphere, hydrosphere, lithosphere. Methods of waste recycling and chemical waste disposal.
4. Safety in emergency situations: *4.1. Analysis of probable emergencies that the object of research can initiate.* *4.2. Analysis of probable emergencies that may occur in the laboratory during research.* *4.3. Substantiation of measures to prevent emergencies and development of a procedure for action in case of occurrence*	When performing work, the most likely types of emergency are: 1) Poisoning with gases and solvent vapors. 2) Chemical burns. 3) Fire and thermal burns.

Date of assignment for the section on a linear schedule		

Assignment issued by a consultant:

Position	Full name	Degree	Signature	Date
Associate professor of General Technical Disciplines	Gorbenko Mikhail Vladimirovich	Candidate of Engeneering sciences		

The task was accepted for execution by the student:

Group	Full name	Signature	Date
9DM8I	Alasheva Umut Rasylbekovna		

Abstract

This Master's thesis contains 86 pages, 15 figures, 31 tables, 120 reference.

Key words: implant, scaffold, Ti6Al4V, surface modification, organic functional group, arenediazonium tosylates, metal-organic frameworks, adhesion, osseointegration.

The object of the research: scaffold on based titanium alloy Ti6Al4V.

Subject of the research: surface modification scaffolds to improve adhesion and osseointegration processes.

Aim of research to develop methods for covalent modification of scaffold surfaces using arenediazonium tosylates and metal-organic frameworks for better implant survival in the human body.

Analysis of the wettability of the surface of scaffolds after functionalization with arenediazonium tosylates (ADT-COOH) showed a significant decrease in the contact angle from unmodified 85° to modified 35 ° for electrochemistry and 38 ° using visible light. The contact angle after modification with metal-organic frameworks (MOFs) was from 25 ° to 0 °, when for the initial one it was 114 ° and functionalized with ADT - 64 °. The chemical composition and structure of MOF-Ca-BDC are confirmed by IR spectroscopy and XRD analysis. Assessment for cytotoxicity showed improved cell adhesion and growth on scaffolds; after ADT modification the cell density was 523 mm^2 and MOF 661 mm^2 and for pure scaffold 474 mm^2, respectively.

Three methods for the scaffold surface modification have been developed using 4-carboxybenzenediazonium tosylates and electrochemical, thermal and visible light stimuli as activator. A new method for modification of the scaffold the calcium-based metal-organic frameworks is proposed.

This work is significant in the creation of biocompatible implants in the field of traumatology, orthopedics and dentistry.

In the future, it is planned to conduct tests for cytotoxicity using human mesenchymal stem cells.

Definitions, designations, abbreviations, normative references

References to the following standards are used in this work:

1. The Labor Code of the Russian Federation dated December 30, 2001 N 197-FZ (as amended on December 27, 2018).
2. GOST 12.2.033-78 SSBT. The workplace when performing work while standing. General ergonomic requirements.
3. SanPiN 2.2.4.3359-16. Sanitary and epidemiological requirements for physical factors in the workplace.
4. GOST 12.1.005-88. General sanitary and hygienic requirements for working area air.
5. GOST 12.1.007-76 SSBT. Harmful substances. Classification and general safety requirements.
6. GOST 12.1.010-76 SSBT. Explosion proof. General requirements.
7. GOST 12.4.011-89 SSBT. Protective equipment for workers. General requirements and classification.
8. GOST 12.1.038-82 SSBT. Electrical safety. Maximum permissible levels of touch voltages and currents.
9. GOST 12.1.004-91 SSBT. Fire safety. General requirements.

The following abbreviations are used in the work:

ADT - Arenediazonium tosylate;

MOF – Metal-organic frameworks;

BDC – 1, 4-benzenedicarboxylate;

AM – Additive manufacturing;

EBM – Electron beam melting.

Contents

INTRODUCTION

Nowadays, the design of high-quality and accessible personalized implants is an extremely relevant and urgent task, which connected with the development of such a vital area for a person as personalized biomedicine. The application of endoprostheses and implants in traumatology, orthopedics and dentistry favorably affects the quality and longevity of a person.

The modern development of biomedical materials science, first, is aimed at design biocompatible materials that can exclude the implant rejection by the body, as well as maintain functional properties for a long period.

The efficiency of implantation largely depends on the nature and degree of interaction of living biological tissues and fluids with the implant biomaterial. To increase the efficiency of the interaction between implant and the body the surface implant functionalization is one of the convenient and effective methods for improving biological and mechanical compatibility.

Thus, the need to design effective functionalization methods that allow to control the properties of implant surfaces for the effective and safe application of materials in biomedicine becomes quite obvious. This direction is devoted to the alleged scientific work.

Novelty:

1. Three methods for the scaffold surface modification have been developed using 4-carboxybenzenediazonium tosylate and electrochemical, thermal and visible light stimuli as activator.
2. A new method for modification of the scaffold via surface-assisted growth of calcium-based metal-organic frameworks is proposed.

Aim of research: to develop methods for the modification of titanium scaffold surfaces using arenediazonium tosylates and surface-assisted growth of metal-organic frameworks for improvement of implant survival in the human body.

To achieve this goal, it is necessary to solve the following **tasks:**

1. To develop methods for covalent modification of scaffolds surface using 4-carboxybenzenediazonium tosylate (ADT-COOH).

2. To develop a method for covalent modification of the scaffolds surface using metal-organic scaffolds MOF-Ca-BDC.

3. To characterize and investigate the structure and properties of prepared scaffolds.

4. To carry out the biological evaluation to assess cell viability and adhesion on scaffold surfaces.

CHAPTER 1. LITERATURE REVIEW

Today, the design of biomaterials for implants is a strategically important task for materials science and biomedicine in general. Biomaterials are widely used to development implants and endoprostheses for the cardiovascular system (heart valves and blood vessels), traumatology (bones and cartilage), dentistry (teeth and jaw) and rheumatology (artificial skin). Polymers [1], ceramics [2], as well as metals and their alloys [3] could be implemented as materials for implants and prostheses. Polymers and ceramic materials are applied in less exercised parts of the body [4], or in a composite with other materials [5,6]. Metals and their alloys are widely used to replace bone joints, due to their high fatigue strength, stability and deformation ability [7], especially for replacing the hip bone and knee joint, which undergone the mechanical stress under the action of the upper body.

Metal biomaterials are very promising from a technological point of view since their properties can be controlled by the process and manufacturing method for design implants with the most diverse and complex forms. Thus, metals are more preferred and promising biomaterials in the implants manufacture.

The metals for implants must be biocompatible or bioinert and integrate well with human tissue. Biological compatibility of metallic materials is evaluated by two main parameters: corrosion resistance and toxicity [8].

In the field of medicine, the following metal alloys are widely used for the implants manufacture:

- Stainless steel (iron-chromium-nickel).
- Cobalt based alloys.
- Titanium based alloys.

Stainless steels have the best strength characteristics [9] but are toxic to the body and have a higher corrosion rate compared with other alloys (Co and Ti). They are mainly used to temporarily maintain bone fractures (screws, nails, spokes and bayonets) and as blood vessel stents.

Cobalt-based alloys (CoCrMo, CoCrWNi) demonstrate high corrosion resistance and improved mechanical properties. However, the recent researches have shown that alloys of Co, Cr, Mo and Ni are carcinogenic confirmed by their increased content in the blood. Cobalt-based alloys are more commonly used to replace cups of the hip and knee [10].

Titanium and its alloys are most widely used due to their unique physic, mechanical and chemical properties. Titanium is a corrosion-resistant metal due to the formation of thin (approximately 4 nm) surface oxide layer. Moreover, Ti-based materials are distinguished by low weight, high heat resistance, perfect stability, high tensile strength and low temperature expansion And in most cases have significantly exceeded the performance and service life indicators [11].

Among the known alloys of titanium, the titanium-aluminum-vanadium Ti6Al4V has a leadership position in application due to its unique properties such as high strength fatigue, processability, low weight and bioinertness.

In implants designed the surface topography plays an important role what directly affected the adhesion, proliferation and differentiation of cells. Today, scaffolds [12,13,14]- the porous biomaterials - have a great interest and prospect through all metal materials. The advantage of scaffolds is the ability to control characteristics and structures of implants for maximizing their structure with the anatomical structure of the body. The high porous structure of scaffolds could stimulate the process of osseointegration for increasing the rate of new tissue formation and the regeneration [15].

1.1 Methods of implant production. Additive manufacturing

Today, the production of implants consumes the application of casting [16], forging, stamping [17] and additive manufacturing [18].

Among the aforementioned methods, the additive manufacturing are currently widely used in the field of materials design for implantation due to the ability to produce complex and multifunctional parts according to a pre-designed model based on the layer-by-layer deposition (fig. 1). The additive manufacturing has such

advantages as high manufacturing speed, the ability to develop the complex shapes, lightweight design, economical consumption of materials and no usage an additional processing [19].

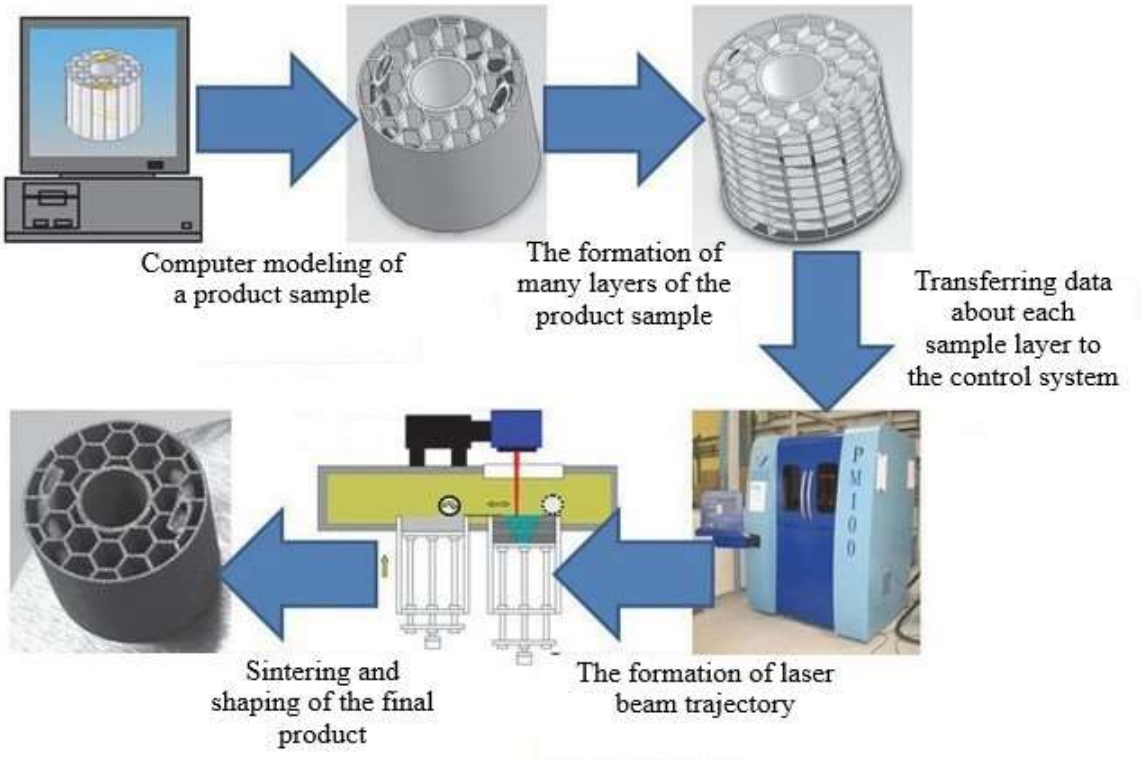


Fig.1 Additive Manufacturing

There are the following additive manufacturing technologies have developed: [20,21,22]: fused deposition modeling (FDM) [23]; stereolithography [24]; inkjet printing [25]; laminated object manufacturing (LOM) [26]; powder bed fusion (PBF): selective laser sintering (SLS) [27], selective laser melting (SLM) [28], electron beam melting (EBM) [29]; direct energy deposition (DED) or laser engineered net shaping (LENS) [30].

Among the described AM technologies, PBF, DED and LOM are suitable for metals. As a feedstock, PBF technologies require metal powders [31], DED - mainly wires [32] and LOM - metal sheets [33].

In bone engineering, PBF (SLS, EBM and SLM) and DED (LENS) (fig.2) [34] methods are the most used for the design of three-dimensional scaffolds from metals, in particular titanium and its alloys. These technologies allow to produce the highly porous scaffolds with good durability and exact geometric shape [35].

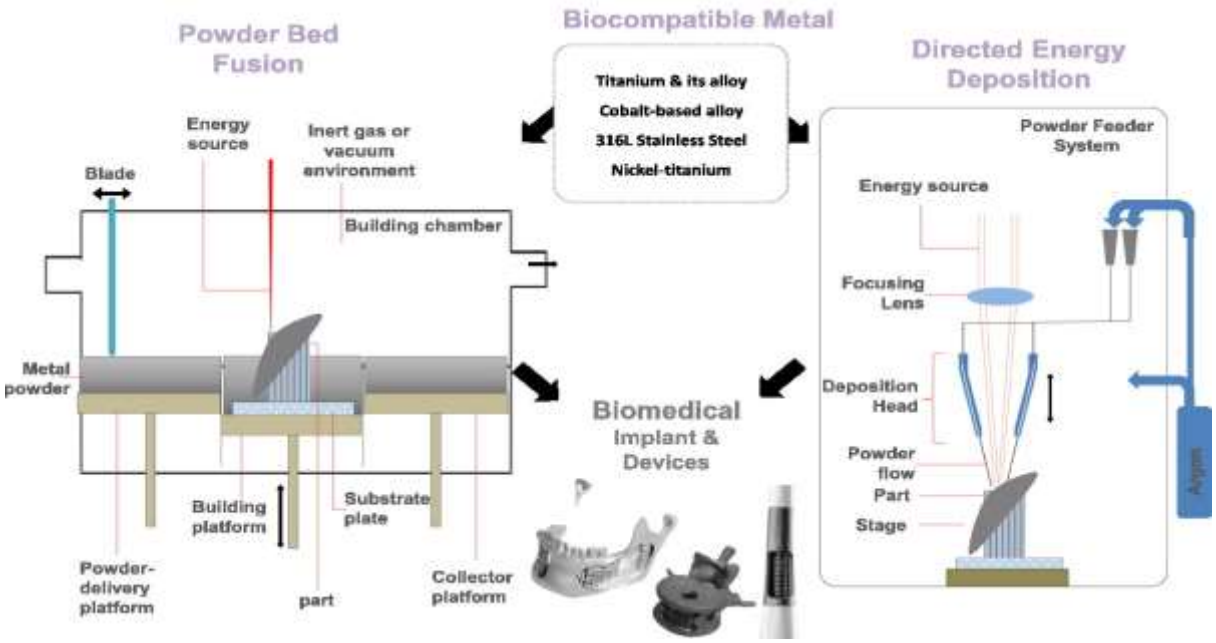


Fig.2 Schematic illustration of PBF and DED technologies.

1.1.1 Selective laser sintering

SLS can be considered as a most popular process in the modern additive manufacture [36]. This method is based on layer-by-layer sintering: partial melting of powder materials using the thermal energy of the laser beam. The advantages of this technology are the accuracy and durability of manufacturing objects with complex geometric shapes, the possibility of manufacturing large objects and high productivity.

The main advantage of this technology is the ability of waste-free application of wide range of powders. The disadvantage of this technology is that the materials are not completely dense. There is a possibility of the defects formation and deformation due to the difference between the laser temperature and the melting temperature of the material. That why is important that the process takes place at a constant temperature close to the melting point of the material.

Today, there is a certain number of studies dedicated to the scaffolds development using this method [37] for design artificial tissues and implants [38].

1.1.2 Selective laser melting

Selective laser melting (SLM) is also widely used in the manufacture of three-dimensional products, including the design of scaffolds [39].This method is based on the melting of metal powder particles by a high-power laser beam through the lens; the powder melts and hardens quickly. In order to prevent metal oxidation in the chamber an inert gas such as argon or nitrogen [40] is used.

Selective laser melting is similar to selective laser sintering in which parts are built line by line using the thermal energy of laser but SLM has the ability to completely melt the metal powder, when in SLS the powder is partially melted.

In addition, SLM is used to manufacture parts only from metals such as chromium, cobalt, titanium and steel while SLS also allows to use ceramic and polymer powders. Due to the complete melting of metal particles, the SLM method allows to design products with improved mechanical properties and more complex geometry [41]. However, rapid melting and cooling of the metal can lead to residual stresses and cracks can form on the parts during operation [42].

1.1.3 Electron beam melting

The electron beam melting (EBM) is a relatively new additive manufacturing technology that allows to produce the complex, multifunctional metal or alloy monoliths using CAD-selective melting of powder precursor [43]. This method is based on the application of high-power electron beams for melting a metal powder in a vacuum chamber with the formation of consecutive layers (fig. 3). The main difference from SLS and SLM lies in the application of electron beams as energy source instead of a laser. Obviously, the electron beam requries vacuum in the chamber instead of inert gases. The advantage of EBM is the ability to control the porosity and stiffness of parts. In addition, the ability to control the temperature of energy in the process allows to design internal parts and thin mesh structures that are difficult in SLS and SLM technologies [44].

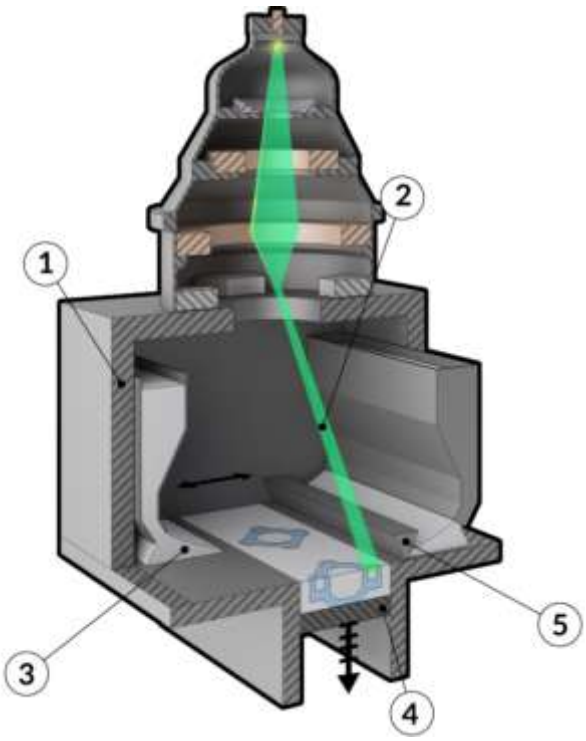


Fig.3 Scheme of the process EBM: 1-vacuum chamber; 2- electron beam; 3- metal powder; 4-platform; 5- rake.

Since the EBM combines all the necessary features such as high productivity, high scanning speed and moderate operating costs, in recent years this technology has attracted increasing interest from various industries. Currently, many research groups are studying EBM technology from different sides and for various applications [45].

1.1.4 Laser engineered net shaping

DED or LENS technology is based on the supply of metal powder or wires from a nozzle and their melting by a laser or electron beam and deposition on a platform in the inert gas atmosphere for prevention oxidation process. [46].

The LENS method allows to control the microstructure and composition of the material providing excellent mechanical properties to the product. Compared with PBF, this method has a higher melting and deposition rate. However, rapid cooling and solidification as is the case with SLM can lead to residual stresses [47]. The disadvantages of this technology are the requirement of a large amount of inert gas and the resulting products should be post-processed. In addition, the powder

from the nozzle is not completely melted, therefore, the disposal of the material is required, which leads to a decrease in the efficiency.

However, metal details produced by the additive technologies methods cannot be directly involved in the implantation process due to their bioinertness. To eliminate this problem preliminary surface modification is required, which is able to endow implants the spontaneous binding ability with live bone [48].

1.2 Surface modification

The material surface plays a particularly important role in the design of artificial medical devices implanted in the human body due to ability to control surface properties such as roughness, microtopography, nanotopography, porosity and surface energy [49].

At the stages of manufacturing titanium implants, an oxidized contaminated layer forms on the surface and often undergoes to stress and plastic deformation, which leads to heterogeneity. For this reason, the main objective of surface modification is to improve the corrosion resistance and osseointegration of the implant [50]. The surface modification maintains all physical characteristics of titanium and its alloys such as elastic modulus and strength, while improving the specific surface properties necessary for various clinical applications such as tissue integration and implant fixation. Surface modification provides a favorable environment for the adhesion, differentiation and proliferation of osteogenic cells [51]. In accordance with various clinical needs, various methods have been proposed for modifying and coating the surface of implant materials [52]:

- Mechanical - polishing, sandblasting, grinding, etc.
- Physical - thermal, plasma spraying, physical vapor deposition, ion coating and deposition, etc.
- Chemical - acid etching, alkaline treatment, sol-gel technology, oxidation method, electrochemical, chemical vapor deposition, etc.
- Biochemical - the addition and binding of biomolecules to the surface of the material.

Methods of *mechanical* modification are used to obtain certain roughness and topographyachieved by physical processing such as deformation, formation and removal of a layer from the surface.

Physical modification methods are high-speed atomization, deposition using thermal and electrical energy.

Chemical methods are mainly used to improve the biocompatibility of the material and corrosion resistance. Chemical methods as oxidation and electrochemistry are used to protect against corrosion, which flow under voltage to form oxide films. Acid and alkaline treatments are used to remove contaminants from the surface and obtain a smoother surface.

Biochemical methods are covalent binding of molecules to the surface of a material such as protein, peptides, functional groups and others.

However, recently a promising approach is surface modification using nanostructures, which can significantly affect the processes of osteogenesis due to the proportionality of osteoblasts.

Such nanostructures can be classified into the following categories [53]:

- Inorganic - calcium phosphate materials (hydroxyapatite), alumina, titanium dioxide, nanoparticles of Au, Ag, etc.
- Organic functional groups, collagen, biopolymers, enzymes, extracellular matrix components, etc.
- Mixed (inorganic and organic).

1.2.1 Calcium phosphates

One of widely used materials for surface implants coating is calcium phosphates (CaP). Calcium phosphates are the most common family of biologically active synthetic materials for biomaterials to improve bone strength and regeneration rate. Among biocompatible calcium phosphates – hydroxyapatite (HA) is the most commonly used due to good stability at a physiological pH of 7.2–7.6. Hydroxyapatites are attracting considerable attention of researchers because of

their structural similarity with natural bone. The physical and chemical properties of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ provide ideal biocompatibility as well as perfect stimulation of osteogenesis and bone restoration [54,55].

However, hydroxyapatite coatings on implants have very low mechanical durability and fracture toughness. Therefore, HA should be used for implants with low load (dental implants) or in a composite with other nanostructures. The main methods of coating by calcium phosphate materials include sol-gel [56,57] and magnetron sputtering [58].

1.2.2 Extracellular matrix

Extracellular matrix (ECM) is one of the common materials for the coating of metal implants [59]. Extracellular matrixes make up the extracellular structure of connective tissue, which form the intercellular junction and promote cell adhesion. The extracellular matrixes grafted onto the surface of the material are in contact with the ECM cells of the body via cell receptor adhesion- integrin, which affects not only adhesion but also cell proliferation and differentiation due to intracellular signal transmission.

The recent contributions in the field of Ti modification have dedicated to the coating by collagen [60], glycosaminoglycans [61], fibronectin, vitronectin [62], laminin [63] and hyaluronic acid [64] and others.

One of the most commonly used extracellular matrices for coating the titanium surface is collagen.

Collagen is a protein-based connective tissues such as bone, tendon and cartilage. Collagen coating improves cell adhesion, promotes bone formation and significantly increases its growth rate. Collagen can be immobilized on the surface by adsorption or covalent grafting. Covalent grafting of collagen on titanium exhibits the higher capability to regulate the osteogenic activity of human mesenchymal stem cells than adsorption immobilization [65]. The main

disadvantage of the ECM is the biological synthesis way increasing the possibility of microbes getting into the implantation process.

1.2.3 Titanium dioxide nanotubes

Titanium dioxide nanotubes (TiO_2 NTs) can be used to modify the surface, including in a composite with metal nanoparticles, hydroxyapatites and biomolecules [66].

The authors of the work demonstrated functionalization of scaffolds with TiO_2 nanotubes enhances cell adhesion and cell growth on the surface due to the close structure of TiO_2 to the extracellular matrix [67]. The authors demonstrated the elastic modulus of a surface coated with nanotubes was much closer to the bone than for unmodified implant [68]. The surface modification of titanium with TiO_2 nanotubes is carried out by methods such as sol-gel and anodizing [69].

1.2.4 Metal nanoparticles

Metal nanoparticles are used for surface modification of the implant to improve the process of osteogenesis, which additionally often have an antibacterial effect.

A group of authors studied the surface of titanium coated with nanostructured particles of noble metals Ag, Au and Pd to study the process of osseointegration and their antibacterial activity [70]. The study demonstrated coatings improve osseointegration, due to their topography as well as their hydrophilic nature of the interaction. Due to the antibacterial properties of metals, the close interaction of the titanium surface with nanoparticles and tissues promotes to the antimicrobial effect.

Park et al. investigated surface modification of implants with strontium nanoparticles, which led to improved osseointegration due to Sr ions could significantly increase the activity of osteoblasts, i.e. enhance cell differentiation [71].

However, the application of metal nanoparticles is limited due to their toxicity [72].

1.2.5 Multilayer polyelectrolyte films and capsules

Polyelectrolyte (PE) films and capsules are widely used to cover the surface of implants due to their biocompatibility and biodegradability [73]. Polyelectrolytes are prepared from biopolymers by the method of alternating layer-by-layer deposition of positively and negatively charged nano- and micro-sized polyelectrolytes, which seem to be promising materials for biomimetic coatings due to their similarity with human tissues. Coating with PE promotes cell adhesion and growth [74], while the biomolecules encapsulated in them provide a slow and controlled release of therapeutic agents.

The authors showed that polyelectrolyte coatings based on poly (4-styrene sulfonic acid), poly (L-glutamic acid) and poly (L-lysine) have good biocompatibility with bone cells [75]. Research has shown that polyelectrolytes improve cell wettability and cell adhesion. Wang et al. demonstrated a modification of the titanium surface by multilayer microcapsules of poly (dopamine) (PDA) with BMP-2 proteins, which enhanced cell adhesion and growth due to the high microporous capsule structure [76].

The authors modified the surface of the Ti-Ni alloy by calcium phosphate polyelectrolyte, which led to increase the surface hydrophilicity while encapsulated the chitosan and heparin acted as antibacterial and anticoagulating agents [77]. Peterson et al. used a polyelectrolyte coating based on poly (meth acrylic acid), poly-L-histidine with BMP-2 protein and fibroblasts, which significantly improved adhesion to the titanium surface [78]. Guillot et al. developed multilayer polyelectrolyte films of encapsulated BMP-2 [79]. Porous titanium (Ti-6Al-4V) film-coated substrates demonstrated improved formation of new bone tissue.

1.2.6 Metal-organic frameworks

Metal-organic frameworks (MOFs) are porous coordination polymers consisting of a combination of negatively charged organic components or linkers with positively charged metal ions or clusters (fig.4) [80]. These materials are

attractive because they have an infinite frameworks structure and the ability to adjust their size and composition.

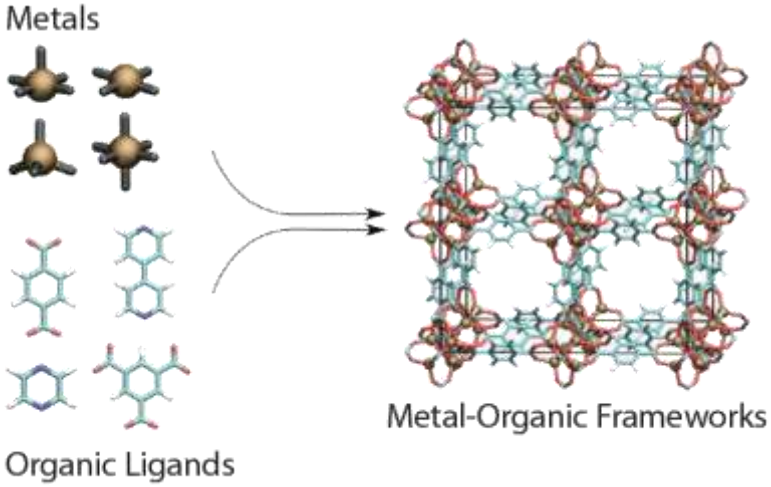


Fig.4 Schematic representation of a metal-organic framework

At present, metal-organic frameworks (MOFs) are widely used in various fields such as catalysis, gas sorption and biomedicine [81] due to their high porosity, structure control, large surface area [82], hypotoxicity and functionalization of pores [83]. Metal-organic frameworks can be a promising modifying agent for faster osteogenesis on metal implants in the early stages of the implantation period [84]. There are a number of works on the surface modification of metal implant based on titanium by metal-organic frameworks.

The team of authors modified the titanium surface with nanosized zeolite-imidazolate scaffolds-8 (ZIF-8) to enhance osseointegration [85]. According to the results, increased cellular activity and improved osteogenesis were observed on the surface with ZIF-8, as evidenced by increased extracellular matrix mineralization (ECM), collagen secretion and increased expression of osteogenic genes (Alp, Col1, Opg and Runx2) and proteins associated with osteogenesis (ALP and OPG).

Later, the authors reported the modification of the titanium surface with nanofilms of MOF-ZIF-8 (zeolite imidazolate frameworks) based on zinc synthesized by hydrothermal and solvothermal methods [86]. Porous nanosized ZIF-8 showed good cell adhesion and improved osseointegration due to nanostructure topography and gradual biodegradation of zinc led to an increase in the adhesion and

proliferation of bone cells of osteoblasts. As is known, zinc has antibacterial properties and the ZIF-8 films deposited demonstrated antibacterial properties.

Shen et al. synthesized Mg / Zn metal-organic frameworks Mg / Zn-MOF-74 on titanium implants to improve antibacterial and anti-inflammatory properties as well as rapid bone regeneration. The results demonstrated high antibacterial properties of the obtained MOF due to its decomposition lead to the formation of an alkaline medium [87].

The authors suggested surface modification of orthopedic implants with metal-organic scaffolds with the release of a drug - naringin, which promoted osseointegration and had an antimicrobial effect [88]. According to the results, the adhesion, proliferation and osteogenic differentiation of mesenchymal stem cells (MSCs) on the titanium surface were significantly improved and the controlled release of naringin acted against bacteria as *Staphylococcus aureus*.

Ran and colleagues proposed a drug delivery method for dexamethasone-zeolite imidazolate nanoparticles (DEX-ZIF-8) on titanium substrates to improve osseointegration and osteogenesis [89]. DEX-ZIF-8 nanoparticles were immobilized on titanium substrates by a silk fibroin membrane through covalent interaction. In addition to the binding role, silk fibroin was used as a protection of nanoparticles from detaching in the case of mechanical stress. The results of the work showed high viability and differentiation of osteogenic cells.

The authors studied MOFs based on Ca and Sr for the regeneration of bones, which are osteoinductive metals [90]. Metal-organic scaffolds were able to decompose and release calcium and zirconium ions. In addition, Sr and Ca ions were able to provide signaling pathways that lead to the differentiation of bone cells and osteoblasts. The results showed that Ca-Sr-MOF promoted large cell growth and resulted in high proliferation. It has been shown that MOFs are able to activate osteogenic cells in human mesenchymal stem cells. The ability of MOFs to encapsulate low molecular weight molecules has also been demonstrated. The authors noted that MOFs could be used to cover implants that can decompose and promote bone growth.

A group of scientists synthesized a Fe-based metal-organic framework - MIL-101 (Fe) and NH_2-MIL-101 (Fe) with titanium [91]. To prepare Ti / MIL-101 (Fe), titanium (IV) butoxide (TBT) was added to the MIL-101 (Fe) powder. Antioxidant tests have shown that Ti / MIL-101 has higher radical scavenging activity than other does. Their activity against bacteria was also tested, in which Ti / MIL-101 (Fe) showed the highest antimicrobial ability against *L. Pneumophila*. There are studies on the role of iron on bone metabolism, which in a certain concentration of Fe positively affects the activity of osteoblast therefore, Ti / MIL-101 (Fe) could be used to develop implants [92].

CHAPTER 2. MATERIALS AND RESEARCH METHODS

2.1 Materials

2.1.1 Scaffolds manufacturing

Scaffolds (fig.5) based on the Ti6Al4V titanium alloy were fabricated by electron beam melting technology at the ARCAM A2 facility (Mölndal, Sweden) [93]. Porous titanium alloy scaffolds are shaped as solid thin-walled cups having an outer diameter of 7 mm and an overall height of 5 mm with the lattice inside. Dense scaffolds are cylindrical with the same outer dimensions. These structures were built layer-by-layer using the precursor Ti6Al4V (ELI) powder, with an average particle diameter of 70μm. Corresponding EBM equipment was described in detail earlier [94,95]. The first stage consisted of loading the powder, then chamber have been evacuated until 10^{-4} Pa. Melting was carried out at a temperature of 730º C for 8 hours, the beam energy was 60 kW, the power was 2.5 kW and the diameter of the electron beam was 0.1- 0.2 mm.

Fig.5 Scaffolds based on the Ti-6Al-4V titanium alloy manufactured by electron beam melting technology at the ARCAM A2 EBM installation (30 mm on the left and 7 mm in diameter on the right).

2.1.2 Arenediazonium tosylate

The method of preparation of 4-carboxybenzenediazonium tosylate (ADT-COOH)

The procedure is based on a previously report [96]. To a solution of 4-toluenesulfonic acid (1.249 g, 6 mmol) in 20 mL of AcOH was added 0.713 mL (6 mmol) of tert-butyl nitrite under stirring, followed by the immediate addition of 0.745 g (5 mmol) of p-aminobenzoic acid. The reaction mixture was stirred during 1 h and the resulted MeOH-solution was poured into 200 mL of cold diethyl ether. The precipitate was washed by decantation 3 times by cold diethyl ether and dried under vacuo.

Methods for surface modification of scaffolds with ADT-COOH. The scaffolds were modified in 1 mM ADT solution using activation methods (stimuli) such as visible light, temperature and electrochemistry.

- *Visible light*. The 4-carboxyphenyl organic functional groups (OFGs) were grafted under visible light (blue light) by soaking of the scaffolds in 1 mM freshly prepared solution of 4-carboxybenzenediazonium tosylate in deionized water for 5,10,15,20 and 30 min. After modification, scaffolds were rinsed under sonication sequentially with deionized water, ethanol, and acetone for 10 min to remove unreacted salts and dried in desiccator for 3 h.

- *Temperature*. The modification was carried out in a beaker with ADT-COOH solution-on a plate at a constant temperature of 60 degrees for an hour. After the sample was washed with water, ethanol and acetone then dried.

- *Electrochemistry* (cyclic voltammetry) [97]. Electrografting on scaffold surface was performed in 1 mM-water solutions of by the cyclic voltammetry (CVA) method in a three-electrode cell (PalmSens4 potentiostat with PCTrace 5 software) with the following parameters: scan speed 0.1 V / s, scan range from -0.3 to 0.7 V and scan step 0.002 V. We used a silver Ag / AgCl as a reference electrode, a platinum wire as a counter electrode and a scaffold as the working electrode (fig. 6). After, the samples were washed in the same way as the previous ones.

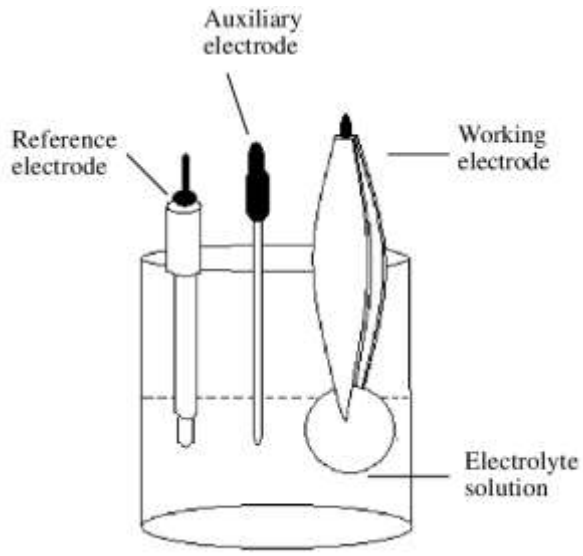


Fig.6 Cyclic voltammetry

2.1.3 Synthesis of metal-organic frameworks

The metal-organic framework Ca-BDC was synthesized according to the method of Mazaj et al. [98].Two solutions: 0.368 g (1.56 mmol) of $Ca(NO_3)_2 \cdot 4H_2O$ (99%) in 2 ml of demineralized water and 0.166 g (1 mmol) of terephthalic acid ($C_8H_6O_4$) (95%) in a 8 ml (100 mmol) of N'N-dimethylformamide (99%, DMF) was mixed and and solvothermally treated under autogenous pressure in an autoclave at 150 °C for 24 hours. After 24 hours, the suspension was left for slow cooling for another 24 hours. The resulting powder (rod-shaped crystals) was washed with DMF and ethanol 3 times. After the powder was left in the oven, it is dried at a temperature of 80-85 ° C until complete evaporation of DMF.

Method for surface modification of scaffolds with MOF-Ca-BDC

The surface modification of scaffolds with MOF was carried out by soaking in the mother liquid of Ca-BDC. Metal-organic frameworks were grown on the surface of scaffolds with pre-functionalized 4-carboxyphenyl groups using visible light as a stimulus (15 minutes) in a 1 mM ADT-COOH solution. Samples were placed vertically in the mother liqiud for 1, 3, 5 and 10 days. After soaking, the samples were washed 2 times with N’N-dimethylformamide, ethanol and demonized

water. Then the samples were dried in an oven under vacuum for 8 hours at a temperature of 80 degrees.

2.2 Characterization of materials

2.2.1 Contact wetting angle and free surface energy

The contact angles (CA) were measured by an Easy drop goniometer using DSA4 software. The static CA was recorded using a sessile drop method in air at room temperature. A minimum of 10 droplets (2 μL) of water, glycerol and ethylene glycol were examined for each sample, and the mean values of CA and surface free energy were calculated. The surface free energy and its dispersive and polar components were estimated using the Owens-Wendt-Rabel-Kaelb method [99]. The deviation between measurements was used for the calculation of measurements errors.

2.2.2 IR spectroscopy

The chemical composition and structure of Ca-MOF were determined by an Agilent Cary 630 IR Fourier spectrometer in the range 500-4000 cm^{-1}. Instrument control, measurement and data analysis were performed using Agilent MicroLab Expert software.

2.2.3 X-ray diffraction analysis

The crystal structure of MOF-Ca (BDC) was determined by X-ray diffraction analysis. The MOF X-ray powder diffraction data were collected on an X-ray diffractometer (Shumadzu XRD 7000S) using CuKα1 radiation λ = 1.5418 Å, voltage 40 kV and current strength 30 mA. Scanning was carried out in the range from 5 to 90 ° 2θ in increments of 0.014 ° per minute.

2.2.4 Resazurin assay

Assessment of cell viability on the surface of scaffolds was performed using the Resazurin assay. For testing, mouse fibroblasts 3T3-L1 were used as a cell model. The seeding density of the cells was 25 thousand per well on a 24-well culture plate.

CHAPTER 3. EXPERIMENTAL PART

3.1. Surface modification of scaffolds with arenediazonium tosylates

In the present work, the Ti-6Al-4V scaffolds surface were functionalized by arenediazonium tosylates, which are widely used in covalent modification of metal surfaces due to the safety, storage stability, the effectiveness of method and a wide range of functional groups for functionalization onto the surface [100].

A team of scientists conducted studies on the modification of the surface of the titanium alloy Ti-6Al-4V by compounds of diazonium salts and investigated the effect of ascorbic acid and thermal reaction on the formation of an organic layer, as a result of which high temperature led to the formation organic layer on the surface titanium [101].

Mesnage et al.modified titanium oxide nanoparticles (NPs TiO2) with polymers based on the reduction of diazonium salts and demonstrated stable grafted polymers on the surface of NPs TiO2 through diazonium salt radicals [102].

Zeb et al. functionalized the surface of titanium nitride with diazonium salts by immersion in an acidic solution and as a result of monolayer of aminophenylene spontaneously grafted onto the TiN surface [103]. The team of authors reported functionalization of titanium surface by electrochemical grafting method using aryldiazonium salts in which aryl groups were successfully grafted onto the Ti surface [104].

In the present work, the Ti6Al4V scaffolds surface was functionalized using 4-carboxybenzenediazonium tosylates. The salt synthesis was carried out according to the procedure [96]. Three methods for activation of the modification reaction using 4-carboxybenzenediazonium tosylates (ADT-COOH) were proposed: electrochemical, thermal and visible light (blue) (fig. 7).

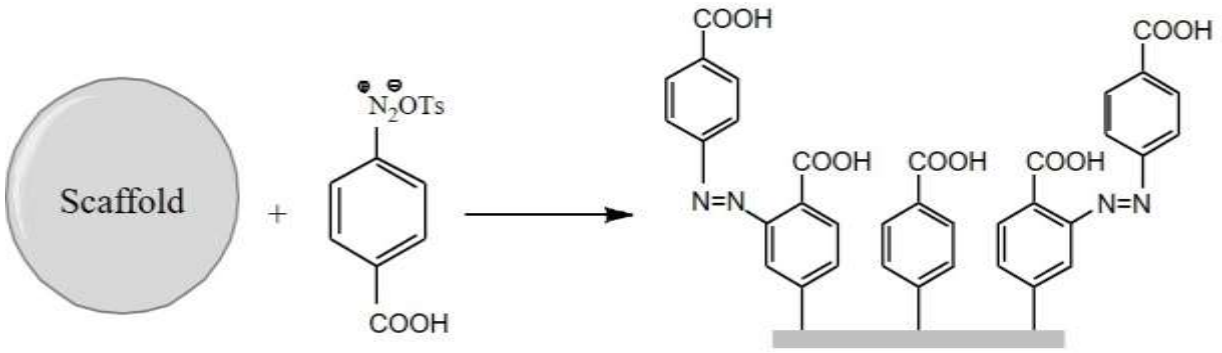


Fig.7 Scheme of surface modification of scaffolds with ADT-COOH.

The methods differ in the nature of the interaction of diazonium salts with the surface, i.e. grafting of organic functional groups to the surface and the number of phenylene layers [105,106,107]. Organic functional groups were grafted onto the surface of scaffolds using electrochemical grafting, temperature and visible light in an aqueous solution with the formation of covalently bonded polyphenylene layers [108]. Modification methods differ in the nature of the interaction of the surface with diazonium salts and the mechanism. In the electrochemisty, diazonium salts are reduced upon imposing a potential [108]; Irradiation with visible light causes excitation of atoms, which leads to photochemical transformations [109].Modification using stimuli usually leads to the formation of polyphenylene layers, when during the spontaneous reaction [110] only a monolayer is formed.

To confirm the presence of OFGs the contact angle was determined which an indirectly evidence a change in surface properties of scaffold. Wettability is an important surface property characterized the nature of the interaction between surface and external environment and affected the biocompatibility of the material, the viability and adhesion of the cells [111].

According to the water CA measurements, modification under visible light stimulus led to significant changes in the contact angle from 85 ° for the starting material to 38 ° for the modified one (table 1). The smallest wetting angle was observed in sample No. 4 with duration of light exposure of 20 minutes, at which the wetting angle was 38 ± 3°.

Table 1. Water contact angle of the scaffolds surface modified with ADT-COOH by visible light activation

№	Time, min	Contact angle, degree						
		1	2	3	4	5	Average	Error, ±
1	5	68	73	68	76	73	72	3
2	10	64	69	72	65	69	67	3
3	15	58	62	56	54	61	58	3
4	20	38	35	39	41	37	38	3
5	30	38	43	39	44	38	41	2
6	0	85	84	86	86	86	86	1

Table 2. Water contact wetting angle of the surface of scaffolds after modification with ADT-COOH

Parameters	Contact angle θ, degree	Image
Control (pristine)	85 ± 2	
Electrochemical activation	35 ± 3	
Visible light	38 ± 3	
Thermal activation	85 ± 3	

According to the wettability analysis, modification with ADT-COOH led to a significant decrease of contact angle that related with covalently grafted OFGs onto the scaffold, which influence the hydrophilicity of the surface. Among activation methods the smallest contact angle, i.e. high hydrophilicity of the surface was observed in the samples after electrochemistry- 35 ± 3 ° and visible light (time - 20 min) - 38 ± 3 ° (table 2).

Calculation of the free surface energy. Surface free energy at the interface between a solid and a liquid, is the sum of intermolecular interactions that determine the adhesion of a surface between two media. Due to the initial hydrophobicity of the titanium surface, the interaction of the implant with the bioliquid requires more time, which negatively affects the osseointegration process [112]. One of the goals of the modification is to increase surface energy. Surface energy data are needed to determine the correlation between wettability and biological responses.

Surface free energy of scaffold was calculated by the Owens-Wendt method based on contact angles with various liquids: water, ethylene glycol and glycerin. The Owens-Wendt method considers a solid surface of two components: dispersive and polar. The dispersion component includes van der Waals forces and the polar component includes strong interactions such as hydrogen and covalent bonds. These components determine the activity of the surface during adhesion.

According to calculations (table 3), the surface energy after modification with ADT-COOH by electrochemistry was 80.1 ± 2.5 mN /m and visible light activation was 67.7 ± 1.2 mN /m [113,114].

According to the results of the analysis, an increase in the surface energy from the initial 21.1 ± 1.2mN / m to the modified 80.2 ± 2.5mN / m confirms a successful scaffold surface modification with arenediazonium salts.

Table 3. Surface free energy data calculated by Owens-Wendt method

Parameters		Ti6Al4V	Ti6Al4V + ADT-COOH + electrochemistry	Ti6Al4V + ADT-COOH + visible light
Contact angle θ,°	Water	86±1 	35±3 	38±3 
	Ethylene glycol	61±2 	9±2 	33±3 
	Glycerol	97±2 	78±3 	91±1 
Surface free energy, mN/m		21.1±1.2	80.1±2.5	67.7±1.2
Polar, mN/m		16.2±3.4	80.1±2,6	66.1±1.3
Dispersive, mN/m		4.8±2.2	0.1±2.6	1.6±0.6

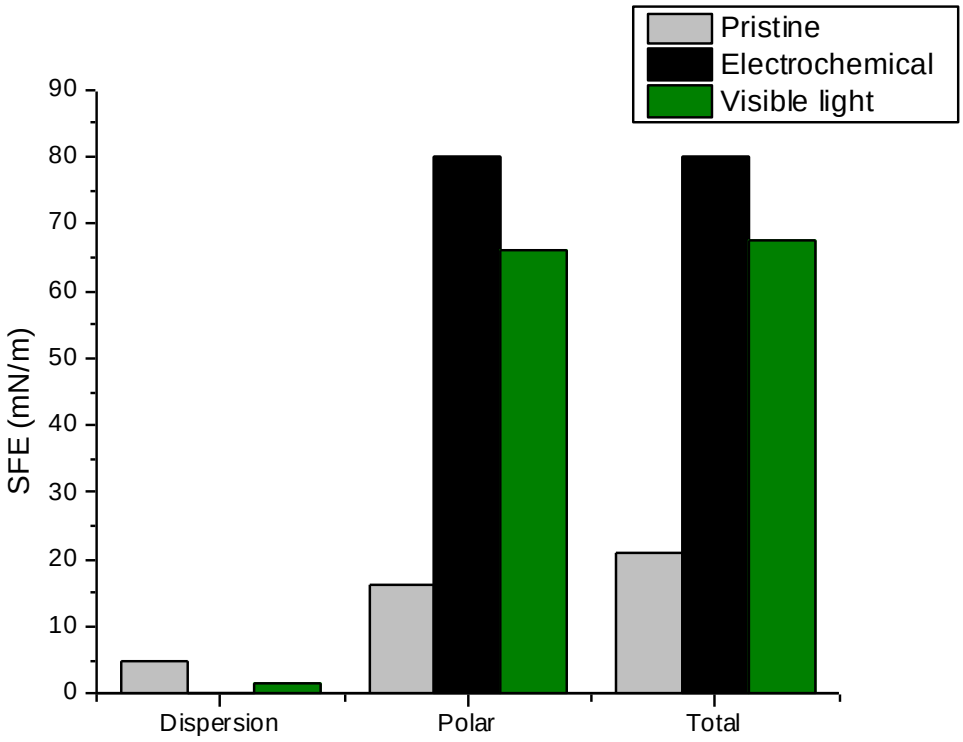


Fig. 8 Diagram of the obtained surface energy

3.2 Surface modification of scaffolds with MOFs

In our research, he surface of scaffolds modified by metal-organic frameworks based on calcium (MOF-Ca-BDC) since calcium is one of the main components of bone tissue and promotes cell growth on the surface of the material. The MOF synthesis was carried out by the solvothermal method Mazaj et al. [98] (fig. 9).

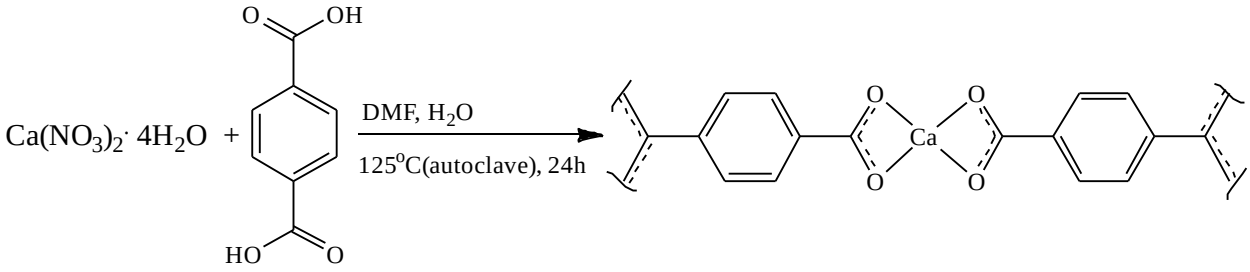


Fig. 9 Synthesis of metal-organic framework Ca-BDC.

The surface modification of scaffolds with MOF was carried out by the soaking method [115] in the Ca-BDC mother liquid (fig.10). The metal-organic frameworks with calcium were grown on the scaffolds surface previously functionalized with 4-carboxyphenyl groups using a diazonium modification (visible light stimuli, 1 mmol and 15 minutes exposure).

For modification, the samples were placed vertically in the mother liquid for 1, 3 and 10 days. At the end, the samples were washed with N'N-dimethylformamide (DMF) and ethanol two times then dried in an oven under vacuum at a temperature of 80 degrees for eight hours.

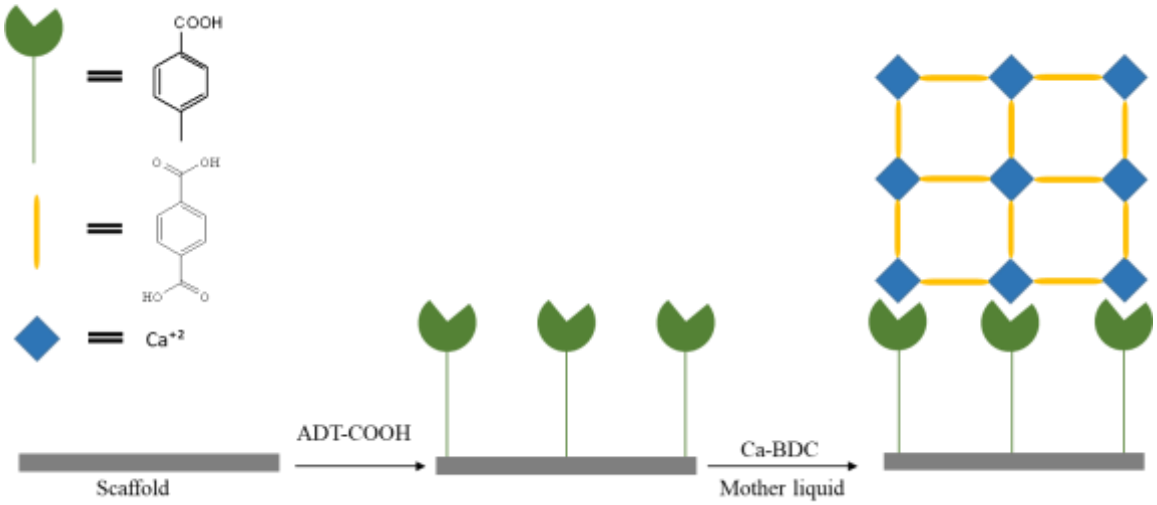


Fig. 10 Scheme of a modification of the surface of scaffolds with ADT-COOH and MOF-Ca –BDC.

As a result of the wettability of the surface of scaffold after the Ca-MOF modification the contact angle with water was 25 ± 2 ° with a soaking time of 1 day whereas for non-functionalized scaffolds the angle was 114 ± 3 ° and modified ADT-COOH - 98 ± 2 °, respectively (table 4). After 3 and 10 days of soaking the samples, complete spreading of water over the surface was observed and the contact angle of wetting was 0 degrees.

The complete wetting on the surface of the scaffold means that MOFs favorably affect the wettability due to their porous structure promoting the absorption of liquid.

Table 4. Water contact angle of the scaffolds surface after modification with Ca-MOF

Parameters	Control	Ti6Al4V+ADT	Ti6Al4V+ADT+MOF		
			1 day	3 days	10 days
Contact angle θ, grad	114,2 ± 2,5 	64,8 ± 3,1 	25,5±2,2 	0 	0

To study the chemical composition and structural of MOF was used the FTIR spectroscopy method (fig. 11). There are the peaks characterized for Ca-MOF: OH groups (3500–3200 cm^{-1}), the symmetric and asymmetric vibrations of carboxylate groups (1397 cm^{-1}), C-H ring (751.3 cm^{-1}), C=C bond (1559 cm^{-1}), C = O (1656 cm^{-1}), C – O group (1104 cm^{-1}) and C – OH (671 cm^{-1}).

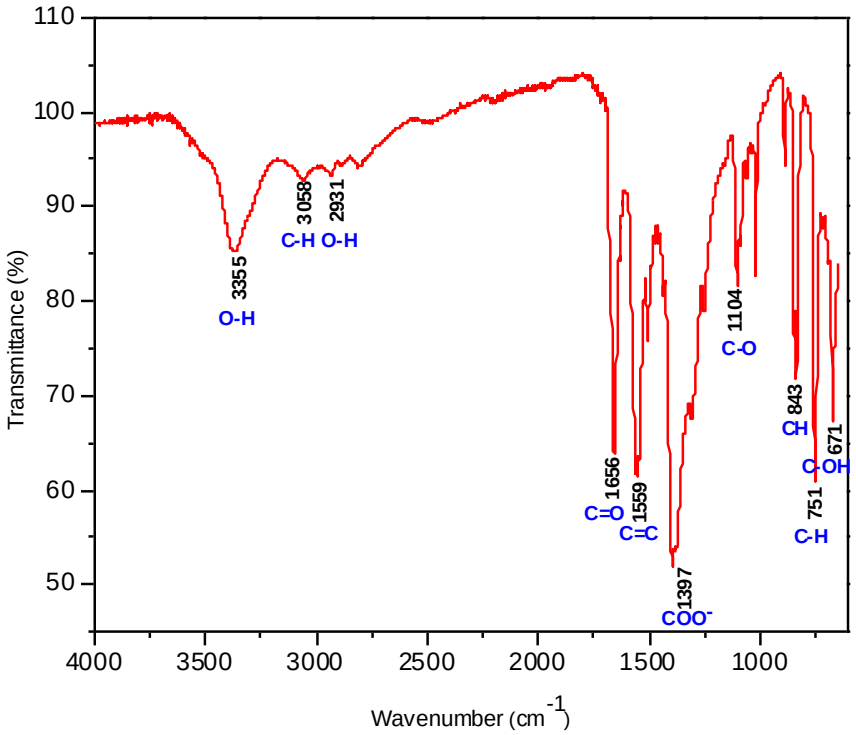


Fig.11 IR spectrum MOF-Ca-BDC

The Ca-BDC X-ray powder diffraction data exhibit strong peaks at angles of 9.5, 16, 19, 26.5, 34, 36 and 38 (fig. 12) that confirmed with previous obtained data [98].

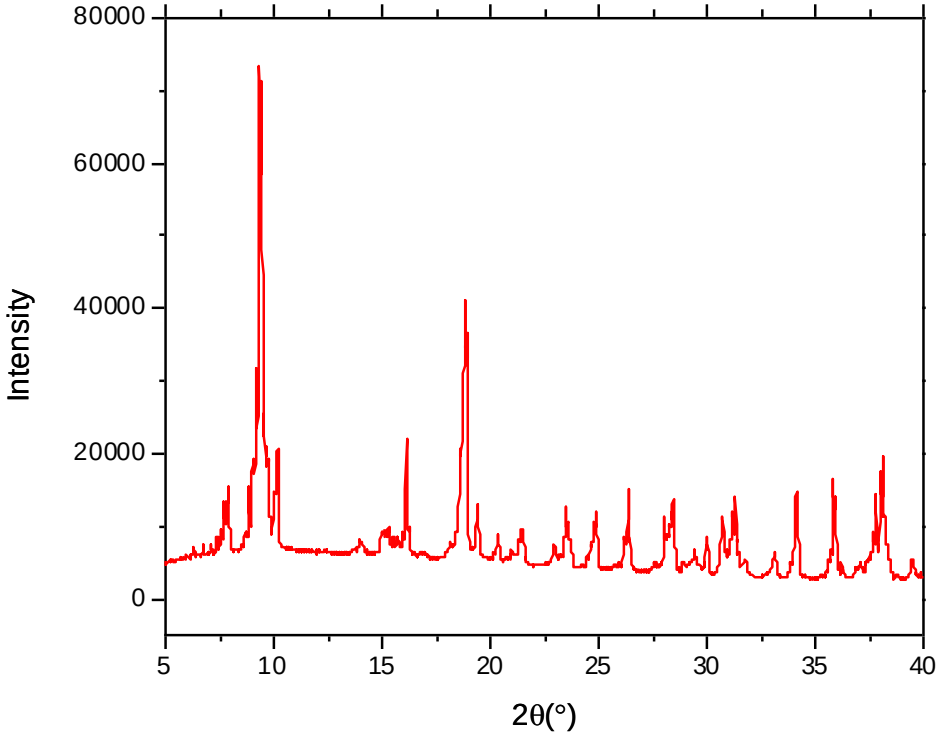


Fig. 12 X-ray powder pattern MOF-Ca-BDC

3.3 Cytotoxicity test

Cytotoxicity tests were performed on five different scaffold samples: pristine scaffold modified ADT-COOH, modified Ca-MOF, acid-etched scaffold, acid-etched scaffold modified ADT-COOH. An adhesive plastic (a special surface for cell growth) was used as a control.

To compare the effect of roughness on cell viability and adhesion, acid-etched samples were prepared. Scaffolds were treated in a mixture of reagents: hydrofluoric acid (HF), nitric acid (HNO_3) and water (H_2O) in a ratio of 1: 2: 1. In acid-treated samples, the contact angle with water was 46 ± 1° before modification and after functionalization with ADT 17 ± 1 °.

Testing was carried out on a suspension of mouse fibroblasts 3T3L1. The seeding density was 25 thousand per well on a 24-well plate. Cell growth dynamics were observed after 24, 48 and 72 hours incubation time (Table 5).

Table 5. Number of cell on the scaffolds surface after 24, 48 and 72 hours, calculation per one mm^2

Time	Ti6Al4V	Ti6Al4V +ADT	Etched Ti6Al4V	Etched Ti6Al4V +ADT	Ti6Al4V +MOF	Control (adhesion plastic)
24 h	78 ±11	103 ± 48	81 ± 20	206 ±33	115 ±38	560 ±274
48 h	224 ± 41	292 ± 21	78 ± 24	321 ±79		718 ±12
72 h	473 ± 266	523 ± 105	726 ± 35	747 ±213	661 ± 146	1579 ±168

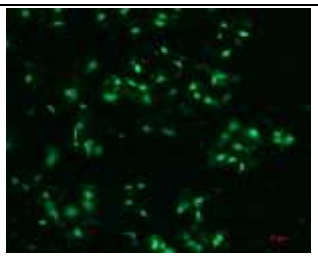
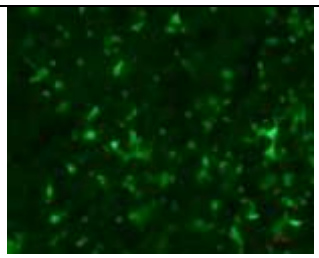
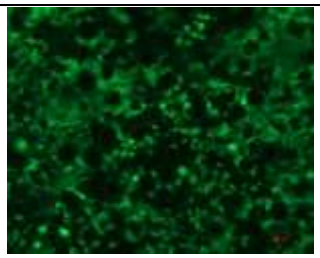
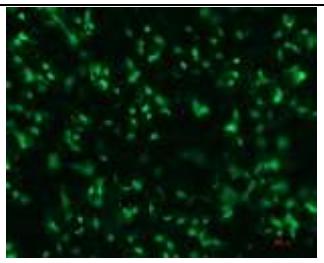
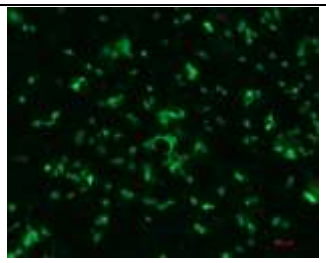
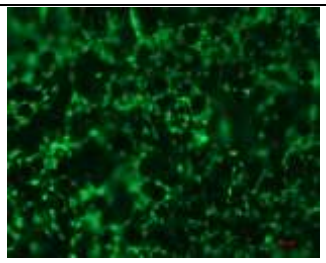
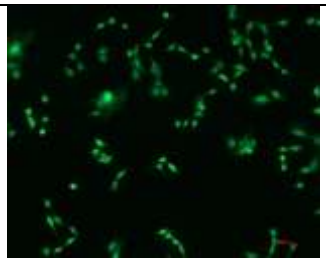
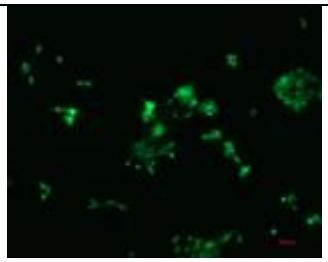
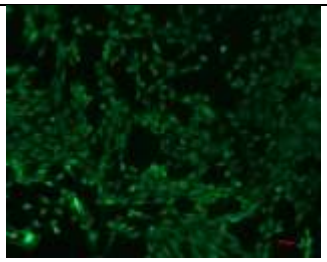
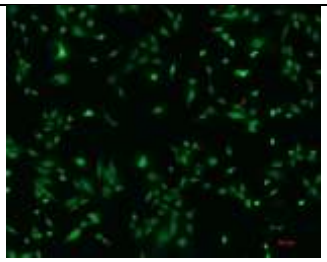
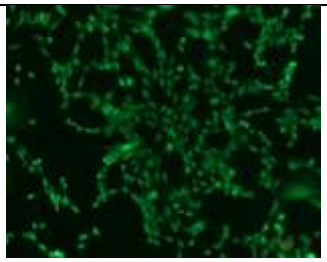
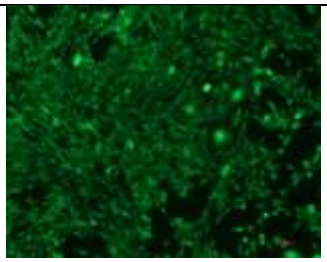
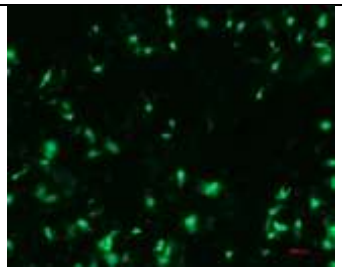
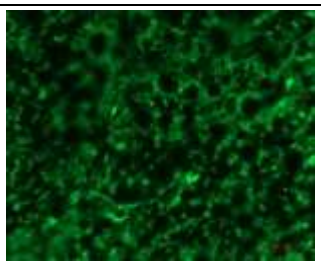
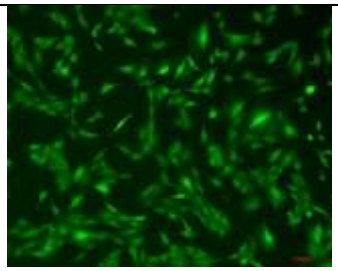
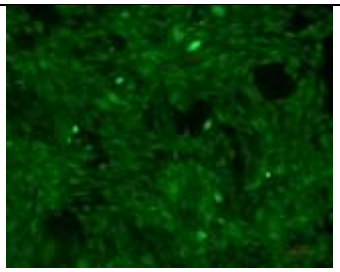
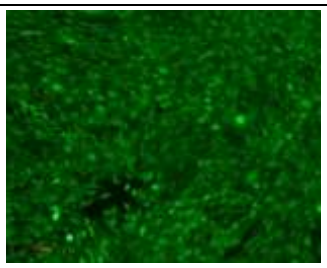
Parameters	24 h	48 h	72 h
Ti6Al4V			
Ti6Al4V +ADT			
Etched Ti6Al4V			
Etched Ti6Al4V +ADT			
Ti6Al4V +MOF			
Control (adhesion plastic)			

Fig. 13 Images of cells on scaffolds after 24, 48 and 72 hours of incubation obtained by fluorescence microscopy using dye Resazurin.

According to the obtained data, low cell adhesion on all acid-etched scaffolds is visually noticeable (Fig. 13). Cells departed from the surface with little exposure, impact or disc dropping (on samples at 24 and 48 hours) butthe cells are physically viable. In general, the performance of acid-etched scaffolds is relatively higher due to the even surface morphology; the growth density was 78 ± 24 mm^2 (48 h) and 726 ± 35 mm^2 (72 h). On untreated titanium, cell adhesion is better but growth density is lower due to developed rough morphology.

The best results for cell growth and adhesion were shown by scaffolds modified with modified MOF with a cell density of 115 ± 38 mm^2 (24h) and 661 ± 146 mm^2 (72h).

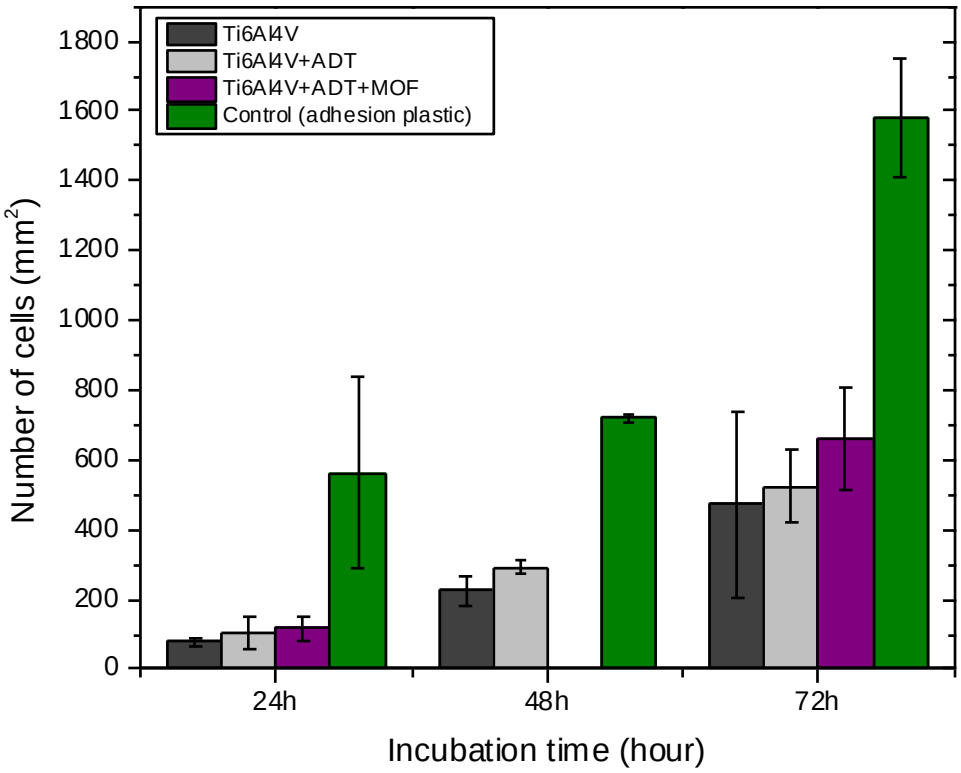


Fig.14 Cell growth chart

The study demonstrated that the scaffolds surface also plays an important role in cell adhesion. Untreated scaffolds had the best adhesion, although the acid-etched scaffolds with less roughness provided a hydrophilic surface and high cell growth but were not able to adhere cells.

CHAPTER 4. FINANCIAL MANAGEMENT, RESOURCE EFFICIENCY AND RESOURCE SAVING

4.1 Analysis of the project

4.1.1 Potential consumers of research results

This master's thesis is devoted to obtaining a biocompatible coating on the surface of scaffolds by covalent bonding with diazonium salts and metal-organic frameworks.

The target market for the project is implant manufacturers.

4.1.2 Competitiveness analysis of technical solutions

In order to find sources of financing for the project, it is necessary, first, to determine the commercial value of the work. Analysis of competitive technical solutions in terms of resource efficiency and resource saving allows to evaluate the comparative effectiveness of scientific development. This analysis is advisable to carry out using an evaluation card.

First of all, it is necessary to analyze possible technical solutions and choose the best one based on the considered technical and economic criteria.

The main competitors of metal implants are ceramic materials – I_1 and polymers – I_2. The criteria for comparing and evaluating resource efficiency and resource conservation presented in table 6. The position of the study and competitors is evaluated for each indicator on a five-point scale. Where one is the weakest position and five is the strongest. The weights of the indicators defined in the total should be one. Analysis of competitive technical solutions is determined by the formula:

$$C = \sum W_i \cdot P_i \quad (1)$$

C - the competitiveness of research or a competitor;

W_i– criterion weight;

P_i – point of i-th criteria.

Table 6. Evaluation card for comparison of competitive technical solutions

Evaluation criteria	**Criterion weight**	**Points**			**Competitiveness**		
		P_f	P_{i1}	P_{i2}	C_f	C_{i1}	C_{i2}
1	2	3	4	5	6	7	8
Technical criteria for evaluating resource efficiency							
1. Biocompatibility	0,15	4	5	4	0,6	0,75	0,6
2. Biosafety	0,12	5	4	4	0,6	0,48	0,48
3. Wear resistance	0,13	5	4	3	0,65	0,52	0,39
4. Regeneration time	0,14	4	4	3	0,56	0,56	0,42
5. Ease of manufacture	0,11	4	4	3	0,44	0,44	0,33
6. Lifetime	0,1	5	3	4	0,5	0,3	0,4
Economic criteria for performance evaluation							
7. Cost	0,09	4	3	4	0,36	0,27	0,36
8. Competitiveness of the implant	0,05	4	5	3	0,2	0,25	0,15
9. Availability of development certification	0,11	5	5	5	0,55	0,55	0,55
Total	**1**	**40**	**37**	**32**	**4,46**	**4,12**	**3,68**

Table 6 shows that the competitiveness of metal implants is higher than that of ceramic materials I1 and polymers I2. Compared to metals, ceramic production is quite expensive, which leads to higher prices. In addition, ceramic materials are fragile and of low strength, which can lead to destruction of the implant and repeated replacement of the implant and additional operations. Polymers are soft materials with low wear resistance, which limits their use. The main advantages of metal implants are that they are biocompatible, high wear resistance and low costs.

4.1.3 SWOT analysis

Complex analysis solution with the greatest competitiveness is carried out with the method of the SWOT analysis: Strengths, Weaknesses, Opportunities and Threats.

Table 7. SWOT analysis

	Strengths: S1. Claimed economy and energy efficiency of technology S2. Lower production cost compared to other technologies. S3. Environmentally friendly technology. S4. Qualified staff S5. Short study time	**Weaknesses:** W1. The lack of a prototype of scientific development W2. Long delivery of materials and components used in the conduct of scientific research W3. Lack of funds W4.Biological test limitations
Opportunities: O1. Use of TPU innovation infrastructure O2. The emergence of a more advanced competitive method O3. Increase the cost of competitive development O4. The availability of raw materials used in scientific research	This study is cost-effective, efficient and relevant, and the project is considered attractive for promotion on the market The availability of the necessary equipment and materials in TPU allows research in a short time.	Develop a material delivery schedule Attraction of interested persons in this area for financial support.
Threats: T1. Lack of demand for new production technologies T2. Lack of sufficient funding. T3. Developed competition of production technologies	The lack of sufficient funding is offset by the availability of necessary resources in TPU. The low costs and effectiveness of the technology, as well as the short lead time for research, can be a key factor in competitiveness.	Untimely delivery of raw materials and lack of financial resources can lead to a delay in research and the emergence of a more advanced method, which will lead to the loss of research.

The analysis has several stages. The first stage consists of describing the strengths and weaknesses of the project, identifying opportunities and threats to the project that have emerged or may appear in its external environment. The second stage consists of identifying the compatibility of the strengths and weaknesses of the project with the external environmental conditions. This compatibility or incompatibility should help to identify what strategic changes are needed.

4.2 Project Initiation

The initiation process group consists of processes that are performed to define a new project or a new phase of an existing one. In the initiation processes, the initial purpose and content are determined and the initial financial resources are fixed. The internal and external stakeholders of the project who will interact and influence the overall result of the research project are determined.

Table 8. Stakeholders of the project

Project stakeholders	**Stakeholder expectations**
Implant manufacturers	Improving the adhesion and osseointegration characteristics of the implant surface

Table 9. Purpose and results of the project

Purpose of project:	Study of biocompatible coatings on titanium scaffolds using diazonium salts and metal-organic frameworks by covalent linking
Expected results of the project:	Obtaining positive results on the study of cell growth and adhesion on the surface of scaffolds
Criteria for acceptance of the project result:	1. Reduced surface wetting angle 2. Increase in surface energy 3. Improving cell growth and adhesion
Requirements for the project result:	Getting positive results: • Show changes in the contact angle of surface wetting after modification with ADT and MOF. • Show surface energy increase after modification. • Show high cell growth on a modified scaffold surface.

4.2.1 The organizational structure of the project

When initiating a project, a working group of the project is needed, to determine the role of each participant in this project, and to assign the functions of participants and their number of working hours in the project (table 10).

Table 10. Structure of the project

№	Participant	Role in the project	Functions	Labor time, h
1	Postnikov Pavel Sergeevich NR TPU, cand. chem.sci., PhD.	Supervisor	Leadership and coordination of the work of the working group. Monitoring the status of the project. Checking Results.	56
2	Surmeneva Marina Alexendrovna NR TPU, cand. phys.math. sci.	Supervisor	Coordination of the work of the working group. Monitoring the status of the project. Checking Results.	56
3	Sviridova Elizaveta Vitaleevna NR TPU, graduate student	Assistant	The approval of the main sections, the issuance of tasks for execution	240
4	Chudinova Ekaterina Alexandrovna, NR TPU, graduate student	Assistant	The approval of the main sections, the issuance of tasks for execution	24
5	Alasheva Umut Rasylbekovna, NR TPU, student	Executor	Performance of research	1528

4.2.2 Project limitations

Project limitations are all factors that can be as a restriction on the degree of freedom of the project team members.

Table 11. Project limitations

Factors	Limitations / Assumptions
3.1. Project's budget	800000
3.1.1. Source of financing	NR TPU
3.2. Project timeline:	1,5 years
3.2.1. Date of approval of plan of project	25.10.2020
3.2.2. Completion date	25.05.2020

4.2.3 Project Schedule

As part of the planning of a scientific project, it is necessary to build a calendar schedule (table 12) and a Gantt chart.

Table 12. Project Schedule

№	Job title	Duration, working days	Start date	Date of completion	Participants
1	Drawing up technical specifications	4	25.09.19	30.09.19	Postnikov.P.S. Surmeneva M.A.
2	Literature study	12	30.09.19	13.10.19	Alasheva.U.R
3	Surface modification of scaffolds with 4-carboxybenzenediazonium tosylates	27	13.10.19	13.11.19	Alasheva U.R. Sviridova E.V.
4	Surface study after modification with ADT	30	13.11.19	17.12.19	Alasheva U.R.
5	Analysis and processing of research results data	13	17.12.19	09.01.2020	Alasheva U.R.
6	Surface modification of the scaffold with MOFs	20	09.01.20	01.02.19	Alasheva U.R.
7	Study of the modified surface by various methods	20	01.02.20	24.02.20	Alasheva U.R.
8	Biological research	14	24.02.20	10.03.20	Plotnikov E.V.
9	Processing received data	18	10.03.20	29.03.20	Alasheva U.R.
10	Discussion of the results of the study.	3	29.03.20	03.04.20	Postnikov P.S. Surmeneva M.A. Chudinova E.A. Sviridova E.V. Alasheva U.R.
11	Compilation of a report	8	03.04.20	10.04.20	Alasheva U.R.
12	Writing thesis	40	10.04.20	25.05.20	Alasheva U.R.

A Gantt chart, or harmonogram, is a type of bar chart that illustrates a project schedule. This chart lists the tasks to be performed on the vertical axis, and time intervals on the horizontal axis. The width of the horizontal bars in the graph shows the duration of each activity.

Table 12. A Gantt chart

№	Activities	Participants	T_c	Duration of the project																	
				Sep		Oct		Nov		Des		Jen		Feb		Mar		Apr		May	
				1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	Drawing up technical specifications	Postnikov.P.S. Surmeneva M.A.	4																		
2	Literature study	Alasheva.U.R	12																		
3	Surface modification of scaffolds with 4-carboxybenzenediazonium tosylates	Alasheva U.R. Sviridova E.V.	27																		
4	Surface study after modification with ADT	Alasheva U.R.	30																		
5	Analysis and processing of research results data	Alasheva U.R.	13																		
6	Surface modification of the scaffold with MOFs	Alasheva U.R.	20																		
7	Study of the modified surface by various methods	Alasheva U.R.	14																		
8	Biological research	Plotnikov E.V.	20																		
9	Processing received data	Alasheva U.R.	18																		
10	Discussion of the results of the study.	Postnikov P.S. Surmeneva M.A. Chudinova E.A. Sviridova E.V. Alasheva U.R	3																		
11	Compilation of a report	Alasheva U.R.	8																		
12	Writing thesis	Alasheva U.R.	40																		

4.3 Scientific and technical research budget

The amount of costs associated with the implementation of research work is the basis for the formation of the project budget. To form the final cost, all expenses are added up. In the process of budgeting, the following grouping of costs by items is used:

1) Material costs of scientific and technical research.
2) Costs of special equipment for scientific work (Depreciation of equipment used for design).
3) Basic salary.
4) Additional salary.
5) Labor tax.
6) Overhead.

4.3.1 Calculation of material costs

The calculation of material costs is carried out according to the formula:

$$C_{m1} = (1 + k_1) \cdot \sum_{i=1}^{m} P \cdot N \qquad (2)$$

where

m – the number of types of material resources consumed in the performance of scientific research;

N_{consi} – the amount of material resources of the i-th species planned to be used when performing scientific research (units, kg, m, m^2, etc.);

P_i – the acquisition price of a unit of the i-th type of material resources consumed (rub./units, rub./kg, rub./m, rub./m^2, etc.);

k_T – coefficient taking into account transportation costs.

Table 13. Material cost

№	Name	Unit	Amount	Price per unit, rub	Material cost, rub
1	Acetic acid (glacial)	ml	1000	0,34	340
2	Aceton	ml	1000	0,125	125
3	Calcium nitrate tetrahydrate	g	100	0,53	53,2
4	DMF	ml	900	0,26	235
5	Distilled water	ml	5000	0,02	112
6	Ethanol	ml	1000	0,049	49
7	Ethyl ether	ml	700	0,94	662
8	Para-toluene sulfonic acid	g	200	0,15	30
9	Tret-butyl nitrite	ml	25		85
10	Nitrile gloves	couple	15	10,4	156
Total for materials					1847,2
Transportation and procurement costs (3-5%)					92,4
Total					1939,6

4.3.2 Costs of special equipment

This point includes the costs associated with the acquirement of special equipment (instruments, stands, devices and mechanisms) necessary to carry out work on a specific topic. Table 10 presents the cost calculations for special equipment required for research.

Calculation of the depreciation:

$$A = \frac{C_{\text{перв}} * \text{H}_\text{a}}{100} \quad (3)$$

where

A – annual amount of depreciation;

$C_{перв}$ – initial cost of the equipment;

$\text{H}_\text{a} = \frac{100}{\text{T}_{\text{сл}}}$ – rate of depreciation;

$T_{сл}$ – life expectancy.

Table 14. Calculation of equipment costs

№	Equipment identification	Quantity of equipment	Total cost of equipment, rub.	Life expectancy, year	Depreciation for the duration of the project, rub.
1.	Magnet Stirrer with Heating	1	18150	5	18150
2.	OHAUS EX124 Explorer Analytical Balance	1	223 632	4	4659
3	Memmert Vacuum Drying Oven	1	456785	6	6303
4	Ultrasonic bath ”Sapphire”	1	28800	5	28800
	Total				59912

4.3.3 Basic salary

This item includes the basic salary of participants directly involved in the performance of work on this study. The magnitude of the cost of wages is determined based on the complexity of the work performed and the current system of remuneration.

The basic salary (BS) is calculated by the formula:

$$S_b = S_a \cdot S_w \quad (4)$$

where S_b – basic salary per participant; T_w – the duration of the work performed by the scientific and technical worker, working days; S_d - the average daily salary of an participant, rub.

The average daily salary is calculated by the formula:

$$S_d = \frac{S_m \cdot M}{F_v} \quad (5)$$

where S_m – monthly salary of an participant, rub; M – the number of months of work without leave during the year: at holiday in 48 days, M = 11.2 months, 6 day per week; F_m – valid annual fund of working time of scientific and technical personnel (244 days).

Table 15. The valid annual fund of working time

Working time indicators	
Calendar number of days	365
The number of non-working days - weekend - holidays	 52 14
Loss of working time - vacation - isolation period - sick absence	 48 7
The valid annual fund of working time	244

Monthly salary is calculated by formula:

$$S_{month} = S_{base} \cdot (1 + k_{premium} + k_{bonus}) \cdot k_{reg} \quad (6)$$

where S_{base} – base salary, rubles; $k_{premium}$ – premium rate (30 % of the basic salary); k_{bonus} – bonus rate (in research institutes and industrial enterprises for professional skills and harmful conditions: 15% of the basic salary); k_{reg} – regional rate equal to 1.3 (in the Tomsk region).

Table 16. Calculation of the base salaries

Perfomers	S_{base}, rub	K_{prem}	k_{bonus}	k_{reg}	S_{month}, rub	W_d, rub	T_p, work days	W_{base}, rub
Postnikov P.S.	35120	0.3	0.15	1.3	66201.6	2758.4	7	19308.7
Surmeneva M.A.	30847	0.3	0.15	1.3	58146.6	2422.8	7	16959.4
Plotnikov E.V.	35120	0.3	0.15	1.3	66201.2	2758.4	14	29947.4
Sviridova E.V.	22701	0.3	0.15	1.3	42701.4	1782.9	30	53487.0
Chudinova E.A.	22701	0.3	0.15	1.3	42791.4	1782.9	3	5348.9
Alasheva U.R.	17890	0.3	0.15	1.3	33722.7	1405.1	191	268376.5
Total								393427.9

4.3.4 Additional salary

This point includes the amount of payments stipulated by the legislation on labor, for example, payment of regular and additional holidays; payment of time associated with state and public duties; payment for work experience, etc.

Additional salaries are calculated based on 10-15% of the base salary of workers:

$$W_{add} = k_{extra} \cdot W_{base} \quad (7)$$

where W_{add} – additional salary, rubles; k_{extra} – additional salary coefficient (10%); W_{base} – base salary, rubles.

Table 17. Additional salary

Perfomers	W_{base}, rub	k_{extra}, %	W_{add}, rub
Postnikov P.S.	19308.7	10	1930.9
Surmeneva M.A.	16959.4	10	1695.9
Plotnikov E.V.	29947.4	10	2994.7
Sviridova E.V.	53487.0	10	5348.7
Chudinova E.A.	5348.9	10	534.9
Alasheva U.R.	268376.5	10	26837.7
Total			39342.8

4.3.5 Labor tax

Tax to extra-budgetary funds are compulsory according to the norms established by the legislation of the Russian Federation to the state social insurance (SIF), pension fund (PF) and medical insurance (FCMIF) from the costs of workers.

Payment to extra-budgetary funds is determined of the formula:

$$P_{social} = k_b(W_{base} + W_{add}) \quad (8)$$

where k_b – coefficient of deductions for labor tax.

In accordance with the Federal law of July 24, 2009 No. 212-FL, the amount of insurance contributions is set at 30%. Institutions conducting educational and scientific activities have rate - 27.1%.

Table 18. Labor tax

Perfomers	Coefficient of deductions	Salary (basic and additional), rub	Labor tax, rub
Postnikov P.S.	27,1	21239.6	5755.9
Surmeneva M.A.	27,1	18655.3	5055.6
Plotnikov E.V.	27,1	32942.1	8927.3
Sviridova E.V.	27,1	58835.7	15944.5
Chudinova E.A.	27,1	5883.8	1594.5
Alasheva U.R.	27,1	295214.2	80003.0
Total			117280.8

4.3.6 Overhead costs

Overhead costs include other management and maintenance costs that can be allocated directly to the project. In addition, this includes expenses for the maintenance, operation and repair of equipment, production tools and equipment, buildings, structures, etc.

Overhead costs account from 30% to 90% of the amount of base and additional salary of employees.

Overhead is calculated according to the formula:

$$C_{ov} = k_{ov} \cdot (W_{base} + W_{add}) \quad (9)$$

where k_{ov} – overhead rate.

Table 19. Overhead

	Overhead rate	Salary, rub	Overhead, rub
Postnikov P.S.	30	21239.6	6371.9
Surmeneva M.A.	30	18655.3	5596.6
Plotnikov E.V.	30	32942.1	9882.6
Sviridova E.V.	30	58835.7	17650.7
Chudinova E.A.	30	5883.8	1765.2
Alasheva U.R.	30	295214.2	88564.3
Total			129831.3

4.3.7 Other direct costs

Energy costs for equipments are calculated by the formula:

$$C = P_{ei} \cdot P \cdot F_{eq} \quad (10)$$

where P_{ei}– power rates (5.8 rubles per 1 kWh); P – power of equipment, kW; F_{eq} – equipment usage time, hours.

Table 20. Energy cost

№	Equipment identification	Power of equipment, kW	Power rates, per 1 kWh	Equipment usage time, h	Depreciation for the duration of the project, rub.
1.	Magnet Stirrer with Heating	0.63	5.8	58	211.9
2.	OHAUS EX124 Explorer Analytical Balance	0,11	5.8	132	84.2
3	Memmert Vacuum Drying Oven	1.2	5.8	96	668.2
4	Ultrasonic bath ”Sapphire”	0.13	5.8	24	18.1
	Total				982.4

4.3.8 Formation of budget costs

The calculated cost of research is the basis for budgeting project costs.

Table 21. Full project cost estimate

Name	Cost, rubles
1. Material costs	1939
2. Equipment costs	59912
3. Basic salary	393427
4. Additional salary	39342
5. Labor tax	117280
6. Overhead	129831
7. Other direct costs	982
Total planned costs	**742713**

4.4. Evaluation of the comparative effectiveness of the project

Determination of efficiency is based on the calculation of the integral indicator of the effectiveness of scientific research. Its finding is associated with the definition of two weighted average values: financial efficiency and resource efficiency. The costs of development analogues of scientific research amounted to *№1* 628421 and *№2* 687934 rubles.

The integral indicator of the financial efficiency of a scientific study is obtained in the course of estimating the budget for the costs of three (or more) variants of the execution of a scientific study. For this, the largest integral indicator of the implementation of the technical problem is taken as the calculation base (as the denominator), with which the financial values for all the options are correlated.

The integral financial measure of development is defined as:

$$I_f^d = \frac{C_i}{C_{max}} \qquad (11)$$

where I_f^d– integral financial measure of development; C_i – the cost of the i-th version; $C_{\max}$ – the maximum cost of execution of a research project (including analogues).

1) $I_f^d = \frac{520117}{687934} = 0{,}76$; 2) $I_f^{a1} = \frac{628421}{687934} = 0{,}91$; 3) $I_f^{a2} = \frac{525869{,}8}{687934} = 1$

The obtained value of the integral financial measure of development reflects the corresponding numerical increase in the budget of development costs in times (the value is greater than one), or the corresponding numerical reduction in the cost of development in times (the value is less than one, but greater than zero).

Since the development has one performance, then $I_f^d = 1$.

The integral indicator of the resource efficiency of the variants of the research object can be determined as follows:

$$I_m^a = \sum_{i=1}^{n} a_i b_i^a, \qquad I_m^p = \sum_{i=1}^{n} a_i b_i^p \tag{12}$$

where I_m– integral indicator of resource efficiency for the i-th version of the development;

a_i– the weighting factor of the i-th version of the development;

b_i^a, b_i^p– score rating of the i-th version of the development, is established by an expert on the selected rating scale; n – number of comparison parameters.

The calculation of the integral indicator of resource efficiency is presented in the form of table 22.

Table 22. Evaluation of the performance of the project

Criteria	Weight criterion	Points		
		Project	Anologue1	Anologue 2
1. Energy efficiency	0,14	5	3	3
2. Reliability	0,18	5	4	3
3. Safety	0,11	5	4	4
4. Functional capacity	0,15	5	4	3
Economic criteria for performance evaluation				
1. The cost of development	0,11	5	3	3
2. Market penetration rate	0,12	5	3	3
3. Expected life	0,09	5	4	3
4. After-sales service	0,1	5	4	4
Total	1	40	29	26

$$I_m^p = 0{,}14 \cdot 5 + 0{,}18 \cdot 5 + 0{,}11 \cdot 5 + 0{,}15 \cdot 5 + 0{,}11 \cdot 5 + 0{,}12 \cdot 5 + 0{,}09 \cdot 5 + 0{,}1 \cdot 5 = 5$$

$$I_m^{a1} = 0{,}14 \cdot 3 + 0{,}18 \cdot 4 + 0{,}11 \cdot 4 + 0{,}15 \cdot 4 + 0{,}11 \cdot 4 + 0{,}12 \cdot 3 + 0{,}09 \cdot 3 + 0{,}1 \cdot 4 = 3{,}65$$

$$I_m^{a2} = 0{,}14 \cdot 3 + 0{,}18 \cdot 3 + 0{,}11 \cdot 4 + 0{,}15 \cdot 3 + 0{,}11 \cdot 3 + 0{,}12 \cdot 3 + 0{,}09 \cdot 3 + 0{,}1 \cdot 4 = 3{,}21$$

The integral indicator of the development efficiency (I_e^p) is determined based on the integral indicator of resource efficiency and the integral financial indicator using the formula:

$$I_e^p = \frac{I_m^p}{I_f^d} = \frac{5}{0{,}76} = 6{,}58; \quad I_e^{a1} = \frac{I_m^{a1}}{I_f^{a1}} = \frac{3{,}65}{0{,}91} = 4{,}01; \; I_e^{a2} = \frac{I_m^{a2}}{I_f^{a2}} = \frac{3{,}21}{1} = 3{,}21$$

Comparison of the integral indicator of the current project efficiency and analogues will determine the comparative efficiency. Comparative effectiveness of the project:

$$E_{c1} = \frac{I_e^p}{I_e^{a1}} = \frac{6{,}58}{4{,}01} = 1.64,$$

$$E_{c2} = \frac{I_e^p}{I_e^{a2}} = \frac{6{,}58}{3{,}21} = 1.98$$

Thus, the effectiveness of the development is presented in table 23.

Table 23. Efficiency of development

№	Indicators	Project	Anologue 1	Anologue 2
1	Integral financial measure of development	0,76	0,91	1
2	Integral indicator of resource efficiency of development	5	3,65	3,21
3	Integral indicator of the development efficiency	6,58	4,01	3,21

Comparison of the values of integral performance indicators allows us to understand and choose a more effective solution to the technical problem from the standpoint of financial and resource efficiency.

Conclusion

Thus, in this section was developed stages for design and create competitive development that meet the requirements in the field of resource efficiency and resource saving.

These stages includes:

- development of a common economic project idea, formation of a project concept;
- organization of work on a research project;
- identification of possible research alternatives;
- research planning;
- assessing the commercial potential and prospects of scientific research from the standpoint of resource efficiency and resource saving;
- determination of resource (resource saving), financial, budget, social and economic efficiency of the project.

Comparison of the integral performance indicators allows us to judge the acceptability of the existing solution to the technical problem posed in the master's thesis from the position of financial and resource efficiency.

CHAPTER 5. SOCIAL RESPONSIBILITY

Introduction

In this work, we study the functionalization of the surface of scaffolds based on a Ti6Al4V titanium alloy with diazonium salts and metal-organic frameworks to create biocompatible implants.

The scope of titanium scaffolds is traumatology, orthopedics and dentistry.

The study was carried out in the chemical laboratory №138 in the building №2 of the TPU. The diazonium salts and metal-organic frameworks used to functionalize the surface of scaffolds were obtained using organic substances and solvents such as acetic acid, dimethyl formamide, diethyl ether, tert-butyl nitrite, p-toluenesulfonic acid and others, which are toxic and flammable. If the rules and safety measures are not followed, they can cause poisoning and chemical burns, as well as the occurrence of an explosion and fire. For this reason, it is necessary to minimize the dangerous factors that can affect a chemical engineer, for which the necessary conditions must be provided that comply with the rules and regulations.

In this section, harmful and dangerous factors that can affect a person and the environment were analyzed and identified, as well as the organization of safety measures in emergency situations.

5.1 Legal and organizational security issues

4.1.1 Special legal norms of labor legislation

This section discusses labor law in relation to chemical laboratory conditions. The basic ergonomic requirements for the correct location and layout of the researcher’s working area, the designed working area in a production environment to create a comfortable working environment are given.

In relation to persons working with harmful substances, the legal system in accordance with the 1st part of Article No. 92 of the Labor Code of the Russian Federation provides:

- for workers employed in work with harmful and and in hazardous conditions should not exceed not more than thirty-six hours within 7 working weeks in working week - 8 hours, with a 30-hour working week or less - 6 hours;
- in the Article No. 147 for workers working in harmful and dangerous or other special conditions, increased wages are provided;
- an increase in the wages of workers employed in work with harmful and (or) dangerous working conditions is 4 % of the wage established for various types of work with normal working conditions;
- employees are granted additional leave - seven calendar days, in accordance with Article No.117 of the LC of the RF;
- working in harmful conditions is required to undergo extraordinary medical examinations, preliminary and intermediate (paid by the employer);
- special clothes, shoes and personal protective equipment are issued;
- Federal Law of 17.12.2001 No. 173-FL On Labor Pensions in the Russian Federation establishes that men are entitled to a retirement pension upon reaching 60 years of age and women upon reaching 55 years of age.

The efficiency of a person's labor activity and his performance largely depend on how fully the ergonomic requirements are taken into account when designing equipment and organizing workplaces. Failure to comply with these requirements leads to excessive labor efforts and movements, which contributes to the rapid development of fatigue and stress of the body functions of workers.

5.1.2 Organizational measures for the layout of the working area

Analysis of the workplace of a laboratory engineer

In the laboratory room, the ceiling height should be at least 3.2 m; the width of the corridor is at least 2.2 m so that equipment and objects can be moved; door width 1 m, if double 1.6 m (2 * 0.8). The average area for each working laboratory assistant should be at least 14 m^2 and the length of the workplace at least 1.5 m and the height of the working cabinet should be 0.93-0.98 m.

The parameters of the working room and place of work were calculated, which correspond to GOST 12.2.033-78. In the workplace, the ceiling height h = 3 m; the width of the corridor b = 3.3 m; door width 1 m; the average area of the workplace in the room is 12 m^2 and the length of the worktable is 1.2 m. The worktable has leg space 0.45 m deep, 0.65 m high and 0.6 m wide.

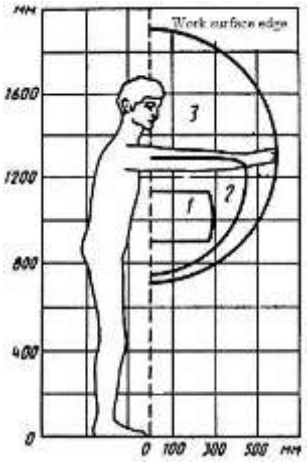
Laboratory floors should be made of refractory, wear-resistant and non-conductive current material as ceramics and stone.

In the laboratory, work is mainly carried out in a fume hood, and work on it represents the following rules:

- In laboratories of organic chemistry, the length of the fume hood should be 1-1.5 m and width 0.8-1 m.
- Laboratory premises must have a supply and exhaust ventilation with mechanical motivation.
- A fume hood should have a place for washing with water and sewage.
- Work desks and fume hoods designed to work with fire and explosive substances must be covered with fireproof material.
- Fume hoods in which work is carried out with substances emitting harmful and combustible vapors and gases must be equipped with upper and lower suction (the inclusion of suction must be regulated depending on the vapor density), with bumpers that prevent liquid from draining onto the floor.
- All moving parts of the laboratory facility mechanisms must be enclosed. there should not be any cluttering objects near the workplace for the possibility of free movement, necessary things and materials should be at arm's length.
- The fume hood should be adequately lit.

Work in the laboratory is mainly performed while standing and is a moderate physical work. For the worker, the relative position of the workplace must be provided and comply with anthropometric, physiological and psychological requirements.

Work in a fume hood includes manual operations, and the working space in the hood should be within easy reach and the optimal area of the motor field (fig. 1a and b).

a) 

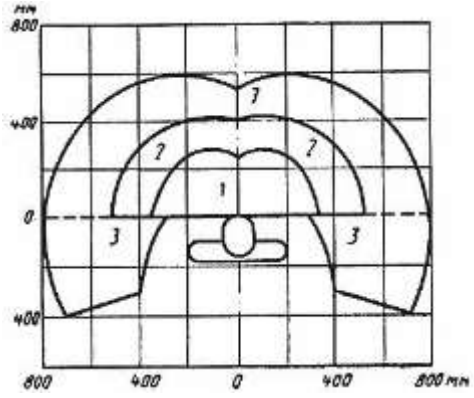
b) 

Fig. 15 a) the reach of the motor field in a vertical plane; b) the reach zone of the motor field in the horizontal plane.

The planning of the workplace and the designing of equipment should provide a direct and free position of the body of the body working or tilting it forward by no more than 15 °. To provide the most convenient approach to the table, leg space should be provided with dimensions of at least 0.15 m in depth, 0.15 m in height and 0.53 mm in width.

Requirements for the placement of controls (fig.15):

- When working with two hands, the controls are placed so that there is no crossing of the hands.
- Very often used and most important controls should be located in zone 1.
- Frequently used and less important controls are not allowed to be located outside the zone.
- Rarely used controls are not allowed to be located outside zone 3.

5.2 Industrial safety

5.2.1 Analysis of harmful and dangerous factors that can create an object of study

The object of study titanium scaffolds (Ti6Al4V) do not represent harmful and dangerous factors.

5.2.2 Analysis of harmful and dangerous factors that may arise in the laboratory during research

In laboratory conditions, harmful and hazardous production factors can affect a person in laboratory conditions. The table 1 below lists the potential factors that may arise during the production of diazonium salt and metal-organic frameworks.To identify possible risk factors, GOST 12.0.003-2015 «Dangerous and harmful production factors. Classification».

Table 24. Possible dangerous and harmful factors

Factors (GOST 12.0.003-2015)	Work stages			Regulations
	Development	Manufacture	Explotation	
1. The increased level of ultrasonic vibrations		+		The requirements for protection against ultrasound are established by SanPiN 2.2.4 / 2.1.8.582-96 «Hygienic requirements when working with air and contact ultrasound sources for industrial, medical and domestic purposes».
2. Exposure to chemical toxic substances		+	+	Requirements for the treatment of hazardous substances are established by GOST 12.1.007-76 Occupational Safety Standards System (SSBT). Harmful substances. Classification and general safety requirements (with Amendments N 1, 2).
3. Fire and explosion hazard		+	+	Requirements for the handling of explosive substances are established in conformity with GOST 12.1.010-76 Occupational safety standards system. Explosion proof. General requirements (as Amended by N 1).
4. Elevated temperature of equipment		+		The requirements for protection against elevated temperatures are established in accordance with GOST 12.4.011-89 (ST SEV 1086-88) Occupational safety standards system. Protective equipment for workers. General requirements and classification.

1) Increased level of ultrasonic vibrations

Researcher working with solid chemicals often have to use an ultrasonic bath to dissolve them, which can adversely affect the human body. According to the method of action on humans, ultrasonic vibrations are divided into:

A) Air - ultrasound acts on a person through the air. B) Contact - ultrasound acts on a person in contact with hands an ultrasound source.

Under industrial conditions, short-term and periodic contact exposure to ultrasound occurs when holding the instrument, loading products into bathtubs, unloading them, and other operations. Under the influence of ultrasound (air and contact), low intensities stimulate and activate, while medium and large ones suppress human functions. The most powerful action of ultrasound is upon contact, ultrasonic vibrations, penetrating deep into the body can cause serious local disturbances in the tissues: an inflammatory reaction and at high intensity tissue necrosis. Changes caused by the action of contact ultrasound are usually more pronounced in the contact area, most often these are fingers and hands.

The maximum permissible level (MPL) of ultrasound is the level that that during work, but not more than forty hours a week, should not cause harm to health and diseases. Compliance with ultrasound remote control does not exclude a violation of health in hypersensitive people. MPL ultrasound are shown in table 25.

Table 25. Maximum permissible levels of contact ultrasound in the workplace

Frequency subbands, kHz	Time-averaged peak spatial intensity, W / cm^2	Time-averaged peak spatial intensity for synergy air and contact ultrasound, W / cm^2
11.2-80	0.03	0.017
80-630	0.06	
0.63·10-5.0·10	0.1	

Direct human contact with the working surface of the ultrasound source and with the contact medium during the excitation of ultrasonic vibrations in it is prohibited. In order to protect hands from the effects of ultrasound when exposed to solid or liquid media, gloves and mittens (made of rubber and the inside of cotton) must be worn.

2) Exposure to chemical harmful substances. In the work on surface modification with diazonium salts and metal-organic frameworks, substances are used that are harmful to one degree or another (table 26).

Table 26. Harmful chemicals substances

№	Name	Hazard class	The effect on the body	MPC, mg/m^3
1	Dimethylformamide (DMF)	2	Irritating to eyes. The substance may cause effects on the liver and respiratory system [GOST 20289-74 S. 3 Dimethylformamide].	10
2	Glacial acetic acid	3	Irritating to mucous membrane of upper respiratory tract; causes skin burns [GOST 61-75 Reagents. Acetic acid].	5
2	Tert-butyl nitrite	4	Harmful by breathing and if swallowed [116].	
3	Diethyl ether	4	Toxic by inhalation [117].	300
4	Acetone	4	Acetone has a narcotic effect, affects the nervous system [GOST 2768-84. Technical Acetone].	200
5	Ethyl alcohol	4	Alcohol has a narcotic effect, causes dry skin; alcohol fumes irritate the mucous membranes of the eyes and upper respiratory tract [GOST 18300-87 Technical rectified ethyl alcohol. Specifications].	1000

According to the degree of exposure to harmful substances, they are divided into the following classes: extremely dangerous, especially dangerous, moderately dangerous and low-hazard substances. The indicators and standards of the hazard class of harmful substances are shown in table 27.

Table 27. The hazard class of harmful substances is established depending on the norms and indicators

Name of indicator	Norms for hazard class			
	1	2	3	4
Maximum permissible concentration (MPC) of harmful substances in the air of the working area, mg / m^3	< 0.1	0.1-1.0	1.1-10.0	> 10.0
The average lethal dose when introduced into the stomach, mg / kg	< 15	15-150	151-5000	> 5000
The average lethal dose when applied to the skin, mg / kg	< 100	100-500	501-2500	> 2500
The average lethal concentration in air, mg / m^3	< 500	500-5000	5001-50000	> 50000
Coefficient of possibility of inhalation poisoning	> 300	300-30	29-3	< 3
Acute area	< 6.0	6.0-18.0	18.1-54.0	> 54.0
Chronic area	> 10.0	10.0-5.0	4.9-2.5	< 2

When working with harmful substances, protective clothing should be selected specifically for each workplace, depending on the concentration and

quantity of hazardous substances used. Workers must use robes, rubber boots, rubber gloves (with a thickness of 0.7 mm for hazard level 2 substances), respirators and safety glasses with a sealed frame.

3) Fire and explosion hazard

In the process, the laboratory uses flammable and explosive substances, which can cause an explosion and fire. Most organic solvents are highly flammable, and their vapors are able to form explosive mixtures with air at room temperature (Table 28).

Table 28. Flammable and explosive substances depending on temperature

Name	Hazard category	t flash, °C	Fluid characteristic
Diethyl ether	1	-40	Flammable. Very volatile at room temperature.
DMF	2	59	Flammable. Vapors form explosive mixtures with air.
Acetone	2	-18	Flammable. Acetone with sodium peroxide or chromic anhydride ignites with an explosion.
Tert-butyl nitrite	2	-10	Flammable.
Glacial acetic	3	38	Flammable.
p-toluenesulfonic acid	3	>150 °C	Combustible. Form explosive mixtures with air.
Ethyl alhocol	3	-13	Flammable.

4) Elevated temperature

The work on the synthesis of metal-organic frameworks is carried out using a vacuum drying oven, which involves working with high temperatures and the possibility of obtaining a thermal burn. There is also a danger of an explosion due to gas mixtures on the working volume of the furnace, as well as around it. To prevent explosions, the stove must be placed indoors with ventilation systems.

To protect thermal burns, heat-insulating gloves and goggles should be used.

5.2.3 Justification of measures to protect personnel from the action of dangerous and harmful factors

In this subsection, solutions are developed that ensure the reduction of the impact of identified hazardous and harmful factors on workers. Measures are also proposed to ensure process safety and equipment operation

In each production, the implementation of labor protection instructions and the safe performance of work are important regulatory acts containing labor protection requirements. Those who work with toxic substances are instructed before going to work, and then periodically every six months. They must know the requirements for the safe conduct of the process; know the toxic properties of the compounds with which they work.

Depending on the working environment, it is necessary to consider the safety requirements for all types of work, installations and devices that form hazardous factors: microclimate, lighting, maximum permissible concentration of harmful substances in the air, noise, vibration, radiation. To envisage and develop measures and means to protect workers. Microclimatic conditions must be established in the working rooms according to the functional state of the person and his work. In which they will not cause damage or impairment of health, discomfort and poor performance.

In the production laboratory, the temperature should be maintained from 19 to 24 ° C, the air velocity - 0.1 m / s, relative humidity - from 40 to 75%. By measures to ensure the necessary microclimate in the working room, ventilation, air conditioning and heating systems must be properly organized.

Incorrect and inadequate lighting leads to fatigue, decreased concentration and damage to the visual organs. For inorganic and organic laboratories, illumination of 300 lux is recommended.

The maximum permissible level of sound in a work requiring concentration, measuring and analytical work in the laboratory is 70 dBA. In areas with noise levels above the limit, collective and individual protective equipment is used, which include special headphones, anti-noise helmets.

Analysis of air exchange in the workplace

Air exchange in chemical laboratories is necessary to clean the air of harmful substances such as gas, steam and dust. For each room, it is necessary to calculate the required air exchange for air cleaning using general ventilation.

The required air exchange is determined by the formula [118]:

$$L = \frac{G \times 1000}{x_{r-} x_p} \quad (1)$$

where L, m^3/h - the required air exchange; G, g /h - the amount of harmful substances released into the room air; x_r, mg / m^3 - the maximum permissible concentration of harmfulness in the air of the working area of the room, according to GOST 12.1.005-88; x_p, mg /m^3 - the maximum possible concentration of the same harmfulness in the air of populated areas (GN 2.1.6.133803).

The amount of volatile solvents released in indoor air is determined by the following formula:

$$G = \frac{a \times A \times m}{100} \quad (2)$$

where, a, m^2/ h - the average productivity of one worker (a=1.2 m^2 / h); A, g / m^2 - consumption of solvents (materials); m,% - percentage of volatile solvents contained in materials; number of workers - 2.

Table 29. Air exchange calculation

№	Name	Solvent consumption, g/m^2	The percentage of volatile solvents	G, g/h	L, m^3/h
2	Ethanol	250	95	570	572
3	Acetone	180	99.9	359	1798
4	Diethyl ether	230	99.5	549	1986

$G = \frac{a \times A \times m \times n}{100} = \frac{1.2 \cdot 250 \cdot 95 \cdot 2}{100} = 570$ g/h; $G = \frac{a \times A \times m \times n}{100} = \frac{1.2 \cdot 180 \cdot 99.9}{100} = 359.6$ g/h;

$G = \frac{a \times A \times m \times n}{100} = \frac{1.2 \cdot 230 \cdot 99.5}{100} = 549$g/h;

According to the formula (1) the required air exchange:

$$L = \frac{G \times 1000}{x_r - x_l} = \frac{570 \cdot 1000}{1000 - 5} = 572\ m^3/h; \qquad L = \frac{G \times 1000}{x_r - x_l} = \frac{359 \cdot 1000}{200 - 0.35} = 1798\ m^3/h;$$

$$L = \frac{G \times 1000}{x_r - x_l} = \frac{594 \cdot 1000}{300 - 1} = 1986\ m^3/h.$$

The concept of air exchange rate (n) is used which shows how many times in one hour the air is completely replaced in the room.

The rate of air exchange is determined by the formula:

$$n = \frac{L}{V_r} \qquad (3)$$

where, Vr is the internal volume of the room, m^3. The volume of the chemical laboratory premises is V = 280 m^3.

$$n = \frac{572}{280} = 2; \qquad n = \frac{1798}{280} = 6; \qquad n = \frac{1986}{280} = 7.$$

According to SN 245-71, the air exchange rate n> 10 is unacceptable. The calculation shows that the air exchange rate is less than 10, which corresponds to the standards.

5.2.3.1 Requirements and safety measures against exposure to harmful substances

In chemical laboratories, taking into account the fact that harmful substances are used, their danger to the human body must be taken into account. To ensure a safe working condition in the laboratory premises, the following measures should be provided [119]:

- hermetic equipment and installations must be constructed to prevent the release of gases and vapors of harmful substances into the room air;
- sanitary facilities and devices (heating, ventilation, water supply and sewage) were introduced;
- automated and remotely controlled technologies and process control are applied that would exclude contact with harmful substances;

- ventilation system was installed that would go to a safe place and systems were used to capture harmful impurities of gases and vapors for their cleaning or disposal;
- the room must be provided with a regular supply of fresh air to reduce the concentration of harmful substances;
- use separate rooms when working with incompatible substances;
- toxicological characteristics of harmful substances should be included in technological regulations.

When working with harmful substances, the following rules must be observed [120]:

- at least two people should work, in the case of first aid to one another;
- it is forbidden to smoke, drink and store food;
- it is forbidden to keep foreign objects on the laboratory table (books, papers);
- workers must know the location of personal protective equipment, first-aid kit and firefighting equipment;
- to work in cotton robes, goggles and gloves;
- before work to check the serviceability of equipment, circuit breakers, the presence of grounding etc.
- to avoid contact with chemicals on the skin;
- the work with organic solvents is carried out only in fume hoods;
- when working in a fume hood, the cabinet doors should be raised to a height of no more than 20-30 cm so that only hands are in the cabinet;
- it is forbidden to introduce reagents into the pipettes by mouth, rubber flasks or other devices should be used for this;
- when determining the smell of chemicals, you should carefully sniff the odors, directing fumes or gases to you with a hand movement;
- mixing or dilution of chemicals, accompanied by heat, should be carried out in heat-resistant or porcelain dishes;

- in no case should you heat liquids in flasks or devices that do not in contact with the atmosphere.

5.2.3.2 Safety measures against explosive and fire hazardous substances

Explosion-proof production processes must be provided with explosion prevention and explosion protection, organizational and technical measures. Explosion protection requirements for production processes should be established by regulatory and technical documentation.

To prevent the formation of an explosive atmosphere and the content of explosive substances not exceeding the lower concentration limit of ignition, taking into account the safety factor, the following must be achieved:

- the use of pressurized production equipment;
- the use of working and emergency ventilation;
- to removal of explosive atmospheres and substances that can lead to its formation;
- to control of the composition of the air and deposits of explosive dust.

To prevent the occurrence of an explosion source, it must be provided:

- heating equipment to the autoignition temperature of an explosive atmosphere;
- using of materials that do not create sparks upon impact, capable of initiating an explosion of an explosive atmosphere;
- using of protective equipment against electricity, stray currents, earth fault currents;
- elimination of dangerous thermal manifestations of chemical reactions and mechanical influences;
- used explosive substances must have a characteristic, solutions and means to ensure explosion prevention and explosion protection, maximum permissible explosion-proof concentrations of substances.

Rules and requirements for working with flammable liquids and flammable substances:

- check and prepare primary fire extinguishing means;
- carry out work only in a fume hood with ventilation in order to prevent flammable vapors from entering the atmosphere; all work with flammable liquids;
- do not heat substances in the water bath that may react with water as a result of explosion or gas evolution;
- make sure that containers with flammable liquids (FL), do not appear near heated objects and are not illuminated by direct sunlight;
- do not store organic solvents prone to peroxide formation in large bottles. Store in dark glass bottles, if possible, in the refrigerator; safety requirements correspond to bottles with a capacity of not more than 1 liter;
- do not store even small amounts of flammable liquids with a boiling point below 50 ° C in laboratory rooms;
- work with FL with the burners turned off, electrical devices and potential ignition sources to prevent ignition of the vapor-air mixture;
- turn off all electric heaters in case of leakage or ignition of the FL; if necessary, disconnect the laboratory by turning off the general switch;
- cover with dry sand the spill of the substance, then collect it with a wooden or plastic scoop; the use of metal scoops is prohibited;
- use water to extinguish organic peroxides; dry sand, powder formulations and carbon dioxide fire extinguishers should be used for inorganic peroxides.

5.2.3.3 Electrical safety

Technical instruments and equipment in a chemical laboratory must meet the requirements of electrical safety when working with electrical installations in accordance with GOST 12.1.019-79. According to the degree of danger, the room is especially dangerous due to the presence of an active organic environment. All

instruments and equipment: a heating cabinet, electric stoves, a drying cabinet, scales, and ultrasonic bath mixers operate at a voltage of 220 V from a common network. There is a danger of electric shock during operation. Parts that may be energized must be properly grounded and earthed. All electrical equipment must have: basic insulation; protective shells; protective barriers; protective shutdown; limitation of steady contact current and electric charge; electrical separation; warning light, sound alarm, safety locks and safety signs. To disconnect the power supply there must be circuit breakers or other accessible devices at the inputs.

In order to prevent electric shock, it is prohibited:

- to work on faulty electrical appliances and installations;
- to overload the power grid;
- to carry and leave unattended electrical appliances on;
- work near open parts of electrical installations, touch them;
- It is forbidden to touch the case of a damaged device or live parts with damaged insulation and to touch a damaged device while standing on a damp floor.

In case of electric shock, it is necessary to release the victim as soon as possible from the action of electric current by disconnecting the electrical appliance to which it is touching. Shutdown is carried out using a switch or circuit breaker. If it is impossible to quickly turn off the appliance, it is necessary to release the victim from live parts with a wooden or other non-conductive object, the source of the damage. In all cases of electric shock, you must call a doctor.

To provide protection against direct contact, it is necessary to use electrical protective devices and personal protective equipment. To ensure electrical safety the following are used: dielectric gloves, galoshes, tools with insulated handles, safety glasses, warning signs and current indicators.

5.3 Environmental safety

5.3.1. Analysis of the impact of the research object on the environment

The titanium scaffolds under study are not harmful to the environment.

5.3.2. Analysis of the environmental impact of the research process

In the work of the materials used the following solvents pose the greatest risk of pollution and environmental hazard: diethyl ether, dimethylformamide, acetone and acetic acid.

Atmosphere. Organic solvents (Tables 4) are toxic, if the necessary safety measures are not taken can lead to increased concentrations in the air.

Hydrosphere. The above listed substances should not be allowed to enter drains. They are toxic to fish and aquatic invertebrates. Their lethal dose of LD 50 for fish is shown in table 30.

Table 30. Water Toxicity

Name	Dose L50, mg/l	Time of exposure, h
Acetone	8.3	96
Diethyl ether	2.8	48
DMF	7.1	96
Acetic acid	300	96

4.3.3. Justification of environmental protection measures

To protect personnel, as well as to prevent organic solvent from entering the atmosphere, the following measures should be applied:

- sealing of technogenic equipment and machinery;
- environmental protection equipment (ventilation, local suction, gas traps, emission dispersion systems);
- local ventilation for trapping harmful substances directly at the source of occurrence;
- local suction, gas catchers, filters to ensure the purification of emissions of substances into the atmosphere;
- adsorbents for the absorption of gases and vapors;

- air control in the production area using self-recording automatic devices, not only recording concentrations of toxic substances but also in case of exceeding the MPC, including sound and light alarms for taking the necessary measures. The standard content of pollutants in the air is shown in table 31.

Table 31. Maximum Permissible concentrations in the atmospheric air of settlements areas (*mot* - maximum one-time, *ad*-average daily)

Substance	MPC*mot*, mg/m^3	MPC$_{ad}$, mg/m^3
Acetone	0.35	0.35
DMF	0.03	0.03
Diethyl ether	1.0	0.6
Acetic acid	0.2	0.06
Ethanol	5.0	5.0

To prevent substances (table 6) from entering sewage (including soil):

- Close the drain holes.
- If it is spilled to collect and pump out spilled liquids. Collect using a liquid adsorbent (sand, kieselguhr, an acid binder and universal binder).

Solvent of waste after washing, cleaning, diluting lose their physical properties. Therefore, for enterprises using these liquids in their process, they must be disposed of. Some organic solvents are still subject to purification, for example acetone and ethanol. Purification of solvents is carried out by distillation using distillers. For solvent cleaning, the following requirements apply:

- Do not allow large fractions of contamination to enter the solvent.
- Received material must be sorted. It is unacceptable to mix different types of solvents, water in one container. Substances must be left in their original packaging; must not be mixed with other wastes.
- The shipping container must not be damaged or leaked. This should be a sealed container indicating the type of substance.

5.4 Safety in emergency situations

5.4.1 Analysis of probable emergencies that the object of research can initiate

According to GOST R 22.0.02-94 emergency situation in a certain territory resulting from an accident, natural hazard, catastrophe, natural or other disaster that could result in or caused human casualties, damage to human health or the environment, significant material losses and violation of human conditions.

The object of study scaffolds (titanium disks) do not have any dangers for the environment and human life.

5.4.2 Analysis of probable emergencies that may occur in the laboratory during research

In this subsection, a brief analysis of possible emergency situations (ES) that can occur during the preparation of arenediazonium salts and metal-organic scaffolds is carried out.

Poisoning and chemical burns. When working with harmful substances at elevated concentrations, they can cause poisoning and some chemical burns.

Fire and thermal burns. Sources of fire and explosion may be flammable liquids and flammable substances (Table 4), gas cylinders and faulty electrical equipment.

The cause of *thermal burns* can be touching with unprotected hands to hot or very hot objects of laboratory equipment (oven, oven, and heater), ignition of flammable or combustible liquids.

5.4.3. Substantiation of measures to prevent emergencies and development of a procedure for action in case of occurrence

1. In case of poisoning (ethyl alcohol, diethyl ether, acetone) the victim must be given 0.03 g of phenamine or 0.1 g of corazole, or thirty drops of cordiamine, or 0.5 g of camphorbromide. After that, you need to give strong tea or coffee. If necessary, do artificial respiration. In case of a chemical burn with acids, rinse the burn with plenty of water, then with a 5% sodium bicarbonate solution or 2% soda solution.

2. If an emergency occurs:

- call the emergency service;
- before the arrival of the fire service, it is necessary to turn off the electrical equipment and heaters;
- Eliminate the source of ignition. Carbon dioxide and foam extinguishers, sand, asbestos blankets and water should be available in every workroom.

3. First aid for thermal burns:

- I degree - place sprinkled with sodium bicarbonate, starch or talc. Put cotton wool moistened with ethyl alcohol. Repeat wetting.
- II degrees (bubbles) - treat with 3-5% solution of potassium permanganate, soda or 5% solution of tannin. Ethyl alcohol wetting.
- III degree (tissue destruction). Cover the wound with a sterile dressing and urgently call a doctor.

Conclusion

In this paper, we consider the legal norms of an engineer-employee of a chemical laboratory; identify harmful and dangerous production factors and ways to protect from their effects. Possible sources of ecosystem pollution and methods for minimizing them are also considered. The analysis of the occurrence of possible emergency cases.

During the experiments, all production factors and safety precautions were observed. The laboratory room meets all standards and requirements. The laboratory has firefighting equipment, collective and individual protective equipment.

CONCLUSION

1. The functionalization of the scaffolds surface by 4-carboxybenzenediazonium tosylate was carried out under different stimuli and it was shown that ADT-COOH significantly improves the wettability of the surface of scaffolds. According to the results, the smallest contact angle was observed in the samples after electrochemical 35 ± 3 ° and visible light activation 38 ± 3 °, while for the initial scaffold it was 85 ± 2 °.
2. The surface was modified by metal-organic frameworks Ca-BDC and during of the study it was found that Ca-MOF improve the wettability of the surface from 25 ± 2 ° to 0 °, when the contact angle for the starting material was 114 ± 2 ° and treated ADT - 64 ± 3°.
3. The chemical composition and structure of Ca-MOF were confirmed by IR spectroscopy and XRD analysis.
4. Cytotoxicity test was performed, and it was shown that the best adhesion and cell growth was observed after surface modification with ADT-COOH - 523 ± 105 mm^2 and MOF - 661 ± 146 mm^2 and for pure scaffold - 473 ± 266 mm^2, respectively.
5. It was shown that surface modification of titanium scaffolds with ADT-COOH and MOF-Ca-BDC lead to an improvement in the process of osseointegration, which may allow us to consider the modification with ADT-COOH and MOF-Ca-BDC as a promising method of functionalization of materials for further biomedical applications.

REFERENCE

1 Middleton, J.C. Synthetic biodegradable polymers as orthopedic devices/ A.J. Tipton // Biomaterials. – 2000. – V.21. – P.2335-2346.

2. Potential of ceramic materials as permanently implantable skeletal prostheses/ S.F. Hulbert, F.A.Young, R.S. Mathews, J.J. Klawitter et.al. // J. Biomed. Mater. Res. – 1970. – V.4 – №3. – P.433-456.

3. Biocompatibility and osteogenesis of refractory metal implants, titanium, hafnium, niobium, tantalum and rhenium/H. Matsuno et.al. //Biomaterials. – 2001. – V.22 – № 11. – P.1253-1262.

4. Salinas, A.J. Bioactive ceramics: from bone grafts to tissue engineering /M.V. Regi // RSC Advan. – 2013. – V.3 – P.11116.

5. Wang, M. Developing bioactive composite materials for tissue replacement // Biamaterials. – 2003. – V.24. – №13. – P.2133-2151.

6. Biomedical applications of polymer-composite materials: a review/ S. Ramakrishna, J. Mayer, E. Wintermantel, K. Leong// Composites Science and Technology. – 2001. – V.61 – № 9. – P.1189-1224.

7. Ghosh, S. Metallic biomaterial for bone support and replacement / S. Sanghavi, P. Sancheti // Fund. Biomater: Met. – 2018. – P.139–165.

8. Модификация поверхности титановых имплантатов и ее влияние на их физико-химические и биомеханические параметры в биологических средах / В. В. Савич, Д. И. Сарока, М. Г. Киселев, М. В. Макаренко; под науч. ред. В. В. Савича. – Минск: Беларус. наука, 2012. – 244 с. – ISBN 978-985-08-1379-4.

9. Walczak, F.J. In vivo corrosion of 316L stainless-steel hip implants: morphology and elemental compositions of corrosion products/ F.H. Shahgaldi, F. Heatley // Biomaterials. – 1998. – V.19. – P.229-237

10. Verano, R. The effect of microstructure on the wear of cobalt-based alloys used in metal-on-metal hip implants/ J.D. Bobyn// Proc. Inst. Mech. Eng. H. – 2006. – V.2. – P.145-159.

11. Niinomi M. Mechanical properties of biomedical titanium alloys// Materials Science and Engineering: A. – 1998. – № 243 (2). – P.231-236.

12. Hutmacher, D.W. Scaffolds in tissue engineering bone and cartilage// Biomaterials. – 2000. – V.21 – № 24. – P.2529-2543.

13. Hollister, S.H. Porous scaffold design for tissue engineering"//Nature Mater. – 2005. – V.4. – P.518–524.

14. O'Brien, F.J. Review: Biomaterials and scaffolds for tissue engineering"// Materials Today. – 2011. – V.14 – №3. – P.88-95.

15. Osteogenesis of 3D printed porous Ti6Al4V implants with different pore sizes/ Q. Ran, W. Yang, Y. Hu et.al. // J. Mech. Behav. Biomed. Mater. – 2018. – V.84. – P.1-11.

16. Dynamic freeze casting for the production of porous titanium (Ti) scaffolds/ H.D. Jung, S.W. Yook et.al. // Mater. Sci. Eng. – 2013. – V.33. – № 1. – P.59-63.

17. Adamus J. Forming of the titanium implants and medical tools by metal working"// Arch. Mat. Sci. Eng. – 2007. – V.28 – № 5. – P.313-316.

18. Wong, K.V. A review of additive Manufacturing/ A. Hernandez// Mech. Engineering. – 2012. – V.5402. – P.10.

19. Additive manufacturing for medical and biomedical applications: advances and challenges/ A. Koptioug, L. Rännar, M. Bäckström, M. Cronskär // Materials science forum. Trans 2. Tech Publications. – 2014. – V.783. – P.1286-1291.

20. Gibson, I. Additive Manufacturing Technologies: 3D Printing, Rapid Prototyping, and Direct Digital Manufacturing / D. Rosen, B. Stucker. – New York: Springer. – 2015. – P.498. – URL: https://link.springer.com/content/pdf/10.1007/978-1-4939-2113-3.pdf. – Text: electronic.

21. Progress in additive manufacturing and rapid prototyping / J. Kruth et.al. // CIRP An. Manuf. Tech. – 1998. – V.47. – № 2. – P.525-540.

22. Additive manufacturing of metals / D. Herzog, V. Seyda, E. Wycisk, C. Emmelmann // Acta Mater. – 2016. – V.117. – P.371-392.
23. Fused deposition modeling of novel scaffold architectures for tissue engineering applications / I. Zein, D.W. Hutmacher, K.C. Tan, S.H. Teoh // Biomaterials. – 2002. – V.23. – № 4. – P. 1169-1185.
24. Stereolithographic Bone Scaffold Design Parameters: Osteogenic Differentiation and Signal Expression /K. Kim, A. Yeatts, D. Dean, J.P. Fisher// Tissue Engineering Part B: Reviews. – 2010. – V.16. – №5. – P.523-539.
25. Murr, L.E. 3D metal droplet printing development and advanced materials additive manufacturing / W.L. Johnson // J. Mater. Res. Tech. – 2017. V.6. – № 1. – P.77-89.
26. Quantification of surface roughness of parts processed by laminated object manufacturing / D. Ahn, J.H. Kweon, S. Lee // J. Mater. Pros. Tech. – 2012. – V.212. – № 2. – P.339-346.
27. A review on powder-based additive manufacturing for tissue engineering: selective laser sintering and inkjet 3D print / S. Shirazi, S. Gharehkhani, M. Mehrali et.al. // Sci. Tech. Advan. Mater. – 2015. – V.16 – № 3. – P.33502.
28. Analysis of defect generation in Ti–6Al–4V parts made using powder bed fusion additive manufacturing processes / H. Gong, K. Rafi, H. Gu et.al. //Add. Manufac. – 2014. – V.1 – № 4 – P.87-98
29. Gong, X. Review on powder-based electron beam additive manufacturing technology/ T. Anderson, K. Chou// Manufacturing Review. – 2014. – № 1. – P.2.
30. Liu, W. Fabrication of functionally graded TiC/Ti composites by Laser Engineered Net Shaping/ J.N. DuPont // Scr. Mater. – 2003. – № 48. – P.1334-1342.
31. A review on powder bed fusion technology of metal additive manufacturing / V. Bhavar et.al. //AM Handbook: Pr. Dev. Def. Ind. – 2017. – P.251-261.
32. Heigel, J.C. Thermo-mechanical model development and validation of directed energy deposition additive manufacturing of Ti–6Al–4V / P. Michaleris. E.W. Reutzel // AM. – 2015. – V.5. – P.9-19.
33. Gibson I. Sheet lamination processes / D. Rosen, B. Stucker // Ad. Manuf. Tech. – P.223-252.
34. Palka, K. Porous titanium implants: A Review / R. Pokrowiecki //Adv. Eng. Mater. – 2018. – V.20. – № 5.
35. Gokuldoss, P.K. Additive manufacturing processes: Selective laser melting, electron beam melting and binder jetting-selection guidelines / S. Kolla, J. Eckert // Materials – 2017. – V.10. – № 6. – P.1-12.
36. Binding mechanisms in selective laser sintering and selective laser melting / J.P. Kruth, P. Marcelis et.al. // Rap. Prototyp. J. – 2005. – V. 11. – № 1. – P. 26-36.
37. Selective laser sintering of bio-metal scaffold/F. H. Lui, R.T. Lee, W.H. Lin, Y.S. Liao// Procedia CIRP. – 2013. – № 5. – P. 83–87.
38. Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering/ J. Williams, A. Adewunmi, R. Schek et al. //Biomaterials. – 2005. – V.26 – № 23. – P.4817-4827.
39. Ultrahigh-strength titanium gyroid scaffolds manufactured by selective laser melting (SLM) for bone implant applications/A. Ataee, Y. Li, M. Brandt, C. Wen //Acta Mater. – 2018. – № 58. – P.354-368.
40. Optimisation of process parameters to address fundamental challenges during selective laser melting of Ti-6Al-4V: A review/ H. Shipley, D. McDonnell, M. Culleton et.al. // Inter. J. Mach.Tools. Manuf. – 2018. – № 128. – P.1-20.
41. Influence of the structural orientation on the mechanical properties of selective laser melted Ti6Al4V open-porous scaffold / V. Weibmann, R. Bader, H. Hansmann, N. Laufer // Mater. Des. – 2016. – № 95. – P. 188-197.
42. Edwards, P. Fatigue performance evaluation of selective laser melted Ti–6Al–4V / M. Ramulu //Mater. Sci. Eng. – 2014. – № 598. – P.327-337.

43. Effect of electron beam method on processing of titanium technogenic material/ Vutova K. et al. // Metals. – 2019. – V.6 – № 9. – P. 1-9.
44. Next-generation biomedical implants using additive manufacturing of complex, cellular and functional mesh arrays/ L.E. Murr et.al. //Phil. Trans. R. Soc. – 2010. – № 368. – P.1999-2032.
45. Direct metal fabrication of titanium implants with tailored materials and mechanical properties using electron beam melting technology/L.A.Harrysson et.al. //Mater. Sci. Eng. – 2008. – V.28 – №3. – P.366-373.
46. Zenou, M. Additive manufacturing of metallic materials/ L.Grainger // Additive Manufacturing. – 2018. – P.53-103.
47. Zhai, Y. Microstructure, static properties, and fatigue crack growth mechanisms in Ti-6Al-4V fabricated by additive manufacturing: LENS and EBM /H. Galarraga, D. Lados // Eng. Fail. Anal. – 2016. – № 69. – P.3-14.
48. Biomaterial surface modification of titanium and titanium alloys for medical applications/ M. Kulkarni, A. Mazare, P. Schmuki, A. Iglic// Nanomedice. – 2014. – № 111. – P.111-136.
49 Bioactive coatings for orthopaedic implants-recent trends in development of implant coatings/ B. Zhang, D. Myers, G. Wallace et.al //Inter. J. Molec. Sci. 2014. – V.15 – № 7. – P.11878-11921.
50. Cachinho, S.C.P., Titanium scaffolds for osteointegration: mechanical, in vitro and corrosion behavior/ R.N. Correia // J. Mater. Sci.: Mater. Med. – 2008. – №19. – P.451–457.
51. Vandrovcova, M. Adhesion, rowth and differentiation of osteoblasts on surface modified materials developed for bone implants /L. Bacakova // Physiol. Res. – 2011. – № 60. – P.403-417.
52. Sobieszczyk, S. Surface modifications of Ti and its alloys // Adv. Mater. Sci. – 2010. –V.10. – № 1. – P.29-42.
53. Puleo, D.A. Understanding and controlling the bone–implant interface/A. Nanci// Biomaterials. – 1999. – V.20 – № 23-24. – P.2311-2321.
54. Early bone formation around calcium-ion-implanted titanium inserted into rat tibia/ T. Hanawa, Y. Kamiura, S. Yamamoto et.al. //J. Biomed. Mater. Res. – 1997. – № 36. – P.131–136.
55. Evaluation in vitro and in vivo of biomimetic hydroxyapatite coated on titanium dental implants/ E.C.S. Rigo, A.O. Boschi, M. Yoshimoto et.al. //Mat. Sci. Eng. – 2004. – V.24. – № 5. – P.647-651.
56. Biological influence of Ca/P ratio on calcium phosphate coatings by sol-gel processing/ M. Catauro, F. Papale, L. Sapio, S. Naviglio // Mater. Sci. Eng. – 2016. – № 65. – P.188-193.
57. Corrosion behavior and mechanical properties of bioactive sol-gel coatings on titanium implants/ M. Catauro, F. Bollino, F. Papale et.al. // Mat. Sci. and Eng. – 2014. – № 125. – P.375-382.
58. Additive manufactured Ti6Al4V scaffolds with the RF magnetron sputter deposited hydroxyapatite coating/ E. Chudinova, M. Surmeneva, A. Koptioug et.al. // J. Phys. Conf. Ser. – 2016. – № 669 (1). – P.0-6.
59. Coating of titanium implants with collagen, RGD peptide and chondroitin sulfate/ S. Rammelt, T. Illert, S. Bierbaum, D. Scharnweber// Biomaterials. – 2006. – V.27 – № 32. – P.5561-55.
60. Effect of Collagen-Polycaprolactone Nanofibers Matrix Coating on the In Vitro Cytocompatibility and In Vivo Bone Responses of Titanium / M. Khandaker et.al. //B. Med. Biol. Eng. – 2018 – № 38. – P.197–210.
61. Regenerative potential of glycosaminoglycans for skin and bone /J.Salbach et.al. // J. Mol. Med. – 2012. – № 90 – P. 625-235.
62. Ku, Y. The effect of the surface modification of titanium using a recombinant fragment of fibronectin and vitronectin on cell behavior/C.P. Chung, J.H. Jang et.al. //Biomaterials. – 2005. – V.26 – №25. – P. 5153-5157.
63. A synthetic peptide containing the IKVAV sequence from the A chain of laminin mediates cell attachment, migration, and neurite outgrowth //K. Tashiro et.al. //J. Bio. Chem. – 1989. – № 264 – P.16174-16182.

64. D.S. Hwang Promotion of osteoblast proliferation on complex coacervation-based hyaluronic acid – recombinant mussel adhesive protein coatings on titanium / J.H. Waite, M. Tirrel //Biomaterials. – 2010. – P.1080-1084.
65. Covalently immobilised type I collagen facilitates osteoconduction and osseointegration of titanium coated implant/ H.Y. Ao et.al. //J. Orthop. Translation. – 2016. – № 5. – P.16.
66. Awad, N.K. A review of TiO2 NTs on Ti metal: Electrochemical synthesis, functionalization and potential use as bone implants / S.L. Edwards, Y.S. Morsi // Mater. Sci. Eng. C. Mater. Biol. Appl. – 2017. – V.1– № 76. – P.1401-1412.
67. Brammer, K.S. TiO 2 nanotubes for bone regeneration/ C.J. Frandsen, S.H. Jin // Tr. Biotech. – 2012. – V.30 – № 6 – P.315-322.
68. Modification of Titanium Implant and Titanium Dioxide for Bone Tissue Engineering / T.K. Ahn et.al //Nov. Biomater. Reg.Med. – 2018. – № 1077. – P.355-368.
69. Microstructure and deformation behavior of biocompatible TiO2 nanotubes on titanium substrate//G.A. Crala et.al. //Acta Biomater. – 2007. – V.3. – № 3. – P.359-367.
70. Osseointegration of titanium with an antimicrobial nanostructured noble metal coating/ S. Svensson, F. Suska, L. Emanuelsson et.al. // Nanomed: Nanotech. Bio. Med. – 2013. – № 9 (7). – P.1048-1056.
71. Osteoblast response and osseointegration of a Ti–6Al–4V alloy implant incorporating strontium / J.W. Park, H.K. Kim, Y.J. Kim et.al. //Acta Biomater. – 2010. – V.6 – № 7. – P.2843-2851.
72. Yang, L. Biological Responses to and Toxicity of Nanoscale Implant Materials / T.J. Webster. //Deg. Imp. Mater. – 2012. – P.481-508.
73. Polyelectrolyte multilayers and capsules: S-layer functionalization for improving stability and biocompatibility/ N. Habibi, L. Pastorino, G. Babolmorad et.al. // J. Dr. Deliver. Sci. Tech. – 2017. – V.38 – P.1-8.
74. Encapsulation of Basic Fibroblast Growth Factor by Polyelectrolyte Multilayer Microcapsules and Its Controlled Release for Enhancing Cell Proliferation/ Z. She, C. Wang, J. Li, G.B. Sukhorukov et.al. //Biomacromolecules. – 2012. – V.13 – № 7. – P.2174–2180.
75. Viability, adhesion, and bone phenotype of osteoblast-like cells on polyelectrolyte multilayer films / P. Tryoen-Toth et.al. // J. Biomed. Mater. Res. – 2002. – V.60 – № 4. 657-667.
76. Bioadhesive Microporous Architectures by Self-Assembling Polydopamine Microcapsules for Biomedical Applications / Z. Wang, C. Li, J. Xu, K. Wang et.al. // Chem. Mater. – 2015. – V.27 – № 3. – P.848–856.
77. Surface Modification of Nickel/Titanium Alloy and Titanium Surfaces via a Polyelectrolyte Multilayer/Calcium Phosphate Hybrid Coating / S. Schweizer, T. Schuster et.al. // Macromolec. Mater. Eng. – 2010. – V.295 – № 6. – P.535-543.
78. Growth factor release from polyelectrolyte-coated titanium for implant applications / Peterson et.al. // Appl. Mater. Interfaces. – 2014. – V.6 – № 3. – P.1866-1871.
79. The stability of BMP loaded polyelectrolyte multilayer coatings on titanium / R. Guillot, F. Gilde P. Becquart et.al. // Biomaterials. – 2013. – №34 (23). – P. 5737–5746.
80. Zhu, Q.L. Metal–organic framework composites /Q. Xu //Chem. Soc. Rev. – 2014. – № 16. – P. 5468-5512.
81. Metal–Organic Frameworks in Biomedicine / P. Horcajada, R. Gref, T. Baati et.al. //Chem. Rev. – 2012. – V.112 – № 2. – P.1232-1268.
82. Design and synthesis of an exceptionally stable and highly porous metal-organic framework/ H. Li, M. Eddaoudi, M.O'Keeffe, O.M. Yaghi // Nature. – 1999. – № 402. – P.276-279.
83. W. Chen Synthesis, functionalization, and applications of metal–organic frameworks in biomedicine / C. Wu //Dal. Trans. – 2018. – № 47. – P.2114-2133.
84. The Chemistry and Applications of Metal-Organic Frameworks /H. Furukawa, K. E. Cordova, M. O'Keeffe3, O. M. Yaghi // Science. – 2013. – V.341 – № 6149. – P.974-987.

85. Enhanced Osseointegration of Porous Titanium Modified with Zeolitic Imidazolate Framework-8 / X. Zhang, J. Chen, X. Pei, J. Wang, Q. Wan et.al. // ACS Appl. Mater. Interfaces – 2017. – V.9. – № 30. – P.25171-25183.
86. Osteogenic activity and antibacterial effect of porous titanium modified with metal-organic framework films / J. Chen, X. Zhang, C. Huang //J. Biomed. Mater. Res. – 2017. – V.105 – № 3. – 834-846.
87. Fabrication of magnesium/zinc-metal organic framework on titanium implants to inhibit bacterial infection and promote bone regeneration / X. Shen, Y. Zhang, P. Ma et.al //Biomaterials. – 2019. – № 212. – P.1-16.
88. Controlled release of naringin in metal-organic framework-loaded mineralized collagen coating to simultaneously enhance osseointegration and antibacterial activity / M. Yu, D. You, J. Zhuang et.al. // ACS Appl. Mater. Interfaces. – 2017. – V.9. - № 23. – P.19698-19705.
89. Rational design of a stable, effective, and sustained dexamethasone delivery platform on a titanium implant: An innovative application of metal organic frameworks in bone implants / J. Ran, H. Zeng, J. Cai et.al. // Chem. Eng. J. – 2018. – V.333. – P.20-33.
90. Synthesis and characterization of CaSr-Metal Organic Frameworks for biodegradable orthopedic applications / J. Naomi, L. Harrison, K. Overholt et.al. //Sci. Rep. – 2019. – V.9. – № 1. – P.1-11.
91. Synthesis of Titanium Doped Iron Based Metal–Organic Frameworks and Investigation of Their Biological Activities / C. Gecgel, U. Simsek, M. Turabik, S. Ozdebir // J. Inorg. Org. Polym. Mater. – 2019. – V.30. – P.749-757.
92. A Comparison of the Biological Activities of Human Osteoblast hFOB1.19 Between Iron Excess and Iron Deficiency / G. Zhao et al. // Biol. Trace. Elem. Res. – 2012. – V.150. – № 1-3. – P.487-495.
93. Additive manufacturing for medical and biomedical applications: advances and challenges / Koptioug A. et al. // Materials science forum. Trans 2. Tech Publications. - 2014. – V.783. – P.1286-1291.
94. Surface modification of Ti6Al4V alloy scaffolds manufactured by electron beam melting / E. Chudinova, M. Surmeneva, A. Koptyug et al. // J. Physics: Conf. Ser. – 2019. – V.1145. – P.012030.
95. Klingvall E. R. Surface properties of implants manufactured using electron beam melting: Doctoral dissertation //Sweden: Mid Sweden University. -2016.
96. Unusually Stable, Versatile, and Pure Arenediazonium Tosylates: Their Preparation, Structures, and Synthetic Applicability / V.D. Filimonov, M.E. Trusova, P.S. Postnikov et al. // Organic letters. – 2008. – V.10. – №18. – P.3961-3964.
97. Smith, R.D. Voltammetric quantification of the spontaneous chemical modification of carbon black by diazonium coupling / P.G. Pickup // Electrochimica Acta. – 2009. – V.54 – №8. – P.2305-23.
98. Spectroscopic Studies of Structural Dynamics Induced by Heating and Hydration: A Case of Calcium-Terephthalate Metal−Organic Framework / M. Mazaj, G. Mali, M. Rangus et.al. //The jour. of Phys. Chem. – 2012. –V.117 – №15. – P. 7552-7564.
99. Surface energy and wettability of van der Waals structures / M. Annamalai, K. Gopinadhan, S. Han et al. // Nanoscale– 2016. V.8. – № 10. – P.5764-5770. http://www.rsc.org/suppdata/c5/nr/c5nr06705g/c5nr06705g1.pdf
100. Covalent modification of carbon surfaces by grafting of functionalized aryl radicals produced from electrochemical reduction of diazonium salts / M. Delamar, R. Hitmi, J. Pinson, J. Saveant // J. Am. Chem. Soc. – 2012. – V.114 – № 14. – P.5883-5884.
101. Modification of Ti6Al4V surface by diazonium compounds / M. Sandomierski, T. Buchwald, B. Strzemiecka, A. Voelkel //Spectrochim. Acta Part A: Mol. Biomol. Spectr. – 2017. – № 191. – P.27-35.
102. Grafting polymers to titania nanoparticles by radical polymerization initiated by diazonium salt/ A. Mesnage, M.A. Magied, P. Simon et al. // J. Mater. Sci. – 2011. – № 46. - P. 6332-6338.

103. G. Zeb, P. Viel, S. Palacinb, X.T. Le, On the chemical grafting of titanium nitride by diazonium chemistry, RSC Adv. 5 (2015) 50298–50305.
104. Functionalization of titanium surfaces with a simple electrochemical strategy / D. Quinton, A. Galtayries, F. Prima, S. Griveau // Sur. Coat. Tech. – 2011. – № 206. – P.2302-2307.
105. Spontaneous Grafting of Diazonium Salts: Chemical Mechanism on Metallic Surfaces / A. Mesnage, X. Lefevre, P. Jegou et.al. //Amer. Chem. Soc. – 2012. – V.28. – № 32. – P.11767-11778.
106. Aryldiazonium tosylates as new efficient agents for covalent grafting of aromatic groups on carbon coatings of metal nanoparticles/ P.S. Postnikov, M.E. Trusova, T.A. Fedushchak et.al. //Nanotech. Rus. – 2010. – V.5 – № 7. – P.446.
107. Surface modification of Au and Ag plasmonic thin films via diazonium chemistry: Evaluation of structure and properties / O. Guselnikova, P. Postnikov, R. Elashnikov, M. Trusova et.al. // Coll. Sur. Physicochem. Eng. Asp. – 2017. – № 516. – P.274-285.
108. Belengar, D. Electrografting: a powerful method for surface modification / J. Pinson // Chem. Soc. Rev. – 2011. – V.40. – P.3995-4048.
109. Sensitized Photografting of Diazonium Salts by Visible Light / M. Bouriga et al. // Chem. Mater. – 2013. – V.25. – №1. – P.90-97.
110. Study of the spontaneous formation of organic layers on carbon and metal surfaces from diazonium salts / A. Adenier et al. //Surf. Sci. – 2006. – V.600. – № 2.1. – P.4801-4812.
111. Influence of surface wettability on competitive protein adsorption and initial attachment of osteoblasts /J. Wei, T. Igarashi, N. Okumori et.al //Biomed. Mater. – 2009. – V.4 – № 4. – P.45002.
112. Enhancing surface free energy and hydrophilicity through chemical modification of microstructured titanium implant surfaces /F. Rupp, L. Sheideler, N. Olshanska et.al. //J. Bio. Mater. Res. – 2005. – V.75A. – № 2. – P.323-334.
113. Relationship between surface properties (roughness, wettability) of titanium and titanium alloys and cell behaviour / L. Ponsonnet, K. Reybier, N.Jaffrezie et.al. // Mater. Sci. Eng. – 2003. – № 23. – P.551-560.
114. The influence of implant surface properties on cell adhesion and proliferation / V. Pesakova, D. Kubies, H. Hulejova, L. Himmlova //J. Mater. Sci.: Mater. Med. – 2007. – V.18. – P.465-473.
115. Metal-organic framework (MOF-5) coated SERS active gold gratings: A platform for the selective detection of organic contaminants in soil/ O. Guselnikova, P. Postnikov, R. Elashnikov et.al. // Anal. Chim. Acta, 2019. –№ 1068. – P.70-79.
116.https://www.fishersci.ca/shop/msdsproxy?productName=AC216225000&productDescription=tert-butyl-nitrite-90-pure-acros-organics-3
117. http://metr-k.ru/support/gases/diethylether
118. Безопасность жизнедеятельности: практикум / Ю.В. Бородин, М.В. Василевский, А.Г. Дашковский, О.Б. Назаренко, Ю.Ф. Свиридов, Н.А. Чулков, Ю.М. Федорчук. – Томск: Изд. Томского политехнического университета, 2009. – Р.101.
119. Безопасность труда при работе с химическими веществами: инструкция МОТ Женева, Международное бюро труда. - 2001. 13.04.2 ISBN 92-2-108006-4.
120. http://toxi.dyndns.org/Spravki/medical_help/med_help/Medical.pdf