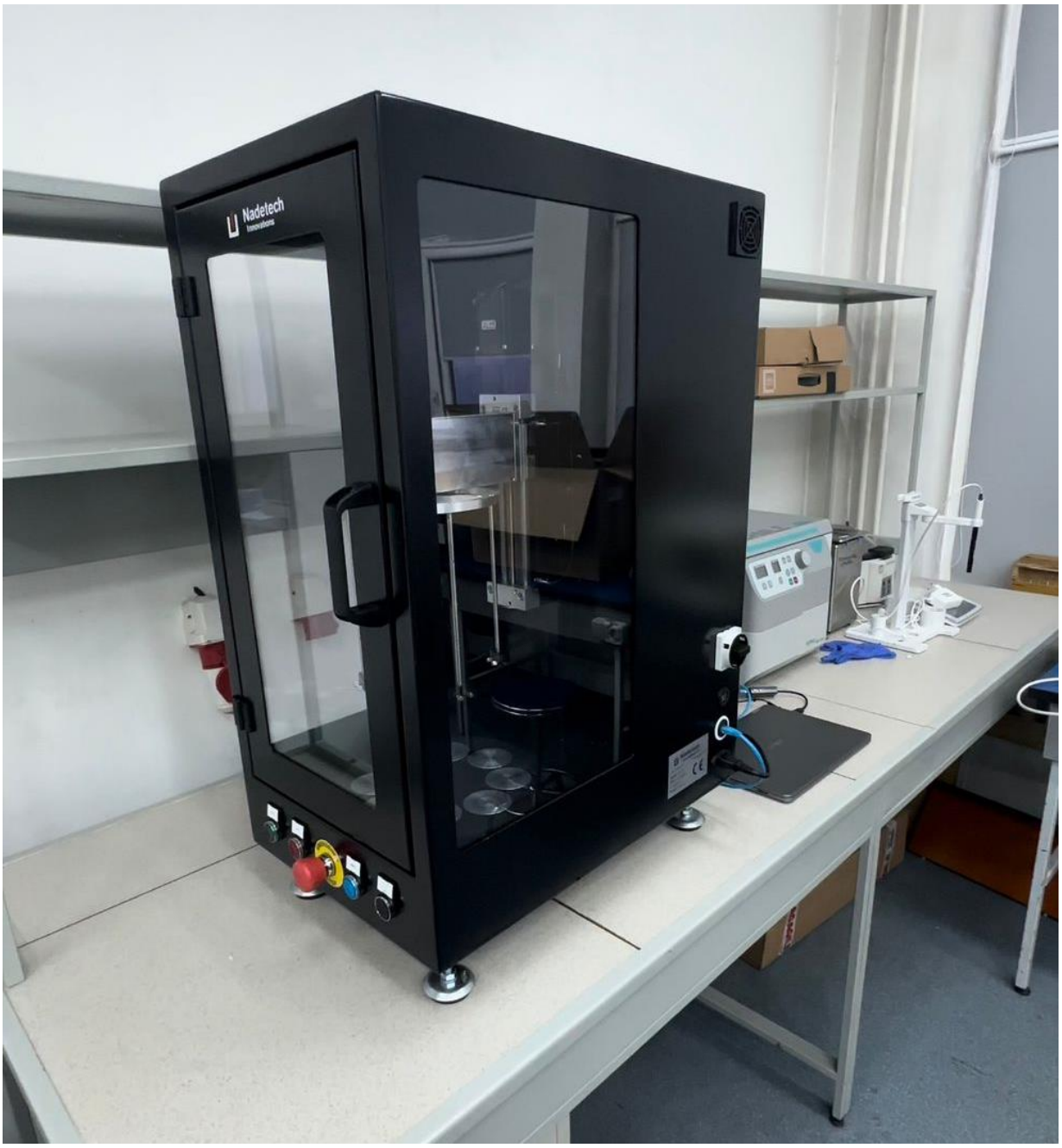
Brief information about the project

Name of the project	AP19577150 «Study of prolongation properties and cytotoxicity of antibacterial films for implantable products based on natural polysaccharides containing chlorhexidine and silver nanoparticles» Duration: Young Scientists Competition 2023-2025
Relevance	The relevance of the scientific project is to solve problems in practical traumatology and surgery, which are associated with the appearance of infections in the postoperative period, which require long-term antibiotic therapy, are accompanied by severe psychological trauma to patients, material costs, and sometimes even death. To exclude such consequences there is a necessity to apply antibacterial films on the surfaces of implanted products. These films can protect the surface, show significant antibacterial activity and find application in medicine.
Purpose	Study of prolonging action and cytotoxicity of antibacterial nanofilms for implantable medical devices based on chitosan and polyacrylic acid, containing chlorhexidine and silver nanoparticles.
Objectives	1. Preparation of nanocoatings based on chitosan/PAA on prepared surfaces of implantable products using the LbL method at various pH values from 2 to 7. Selection of the optimal pH of the assembly for the introduction of chlorhexidine into nanocoatings. 2. Application of chlorhexidine into the structure of the resulting nanocoatings to obtain antibacterial coatings on the surface of implanted products. Establishment of the prolonging properties of the resulting film based on chitosan/PAA containing chlorhexidine. 3. Setting of conditions for the production of silver nanoparticles and their deposition into multilayers of nanofilms. Study of changes in the morphology and composition of coatings after the introduction of AgNPs into multilayers. 4. Establishment of the prolonging properties of the resulting film based on chitosan, polyacrylic acid, containing silver nanoparticles. Assessing properties of coatings such as stability/wear. 5. Study of the cytotoxicity of promising antibacterial nanocoatings based on chitosan and polyacrylic acid containing chlorhexidine. Determination in vitro of the cytotoxic effect of nanofilms based on chitosan and polyacrylic acid containing chlorhexidine based on cell culture survival using the generally accepted MTT assay. 6. Determination in vitro of the cytotoxic effect of nanofilms based on chitosan and polyacrylic acid containing AgNPs based on cell culture survival using the generally accepted MTT assay.

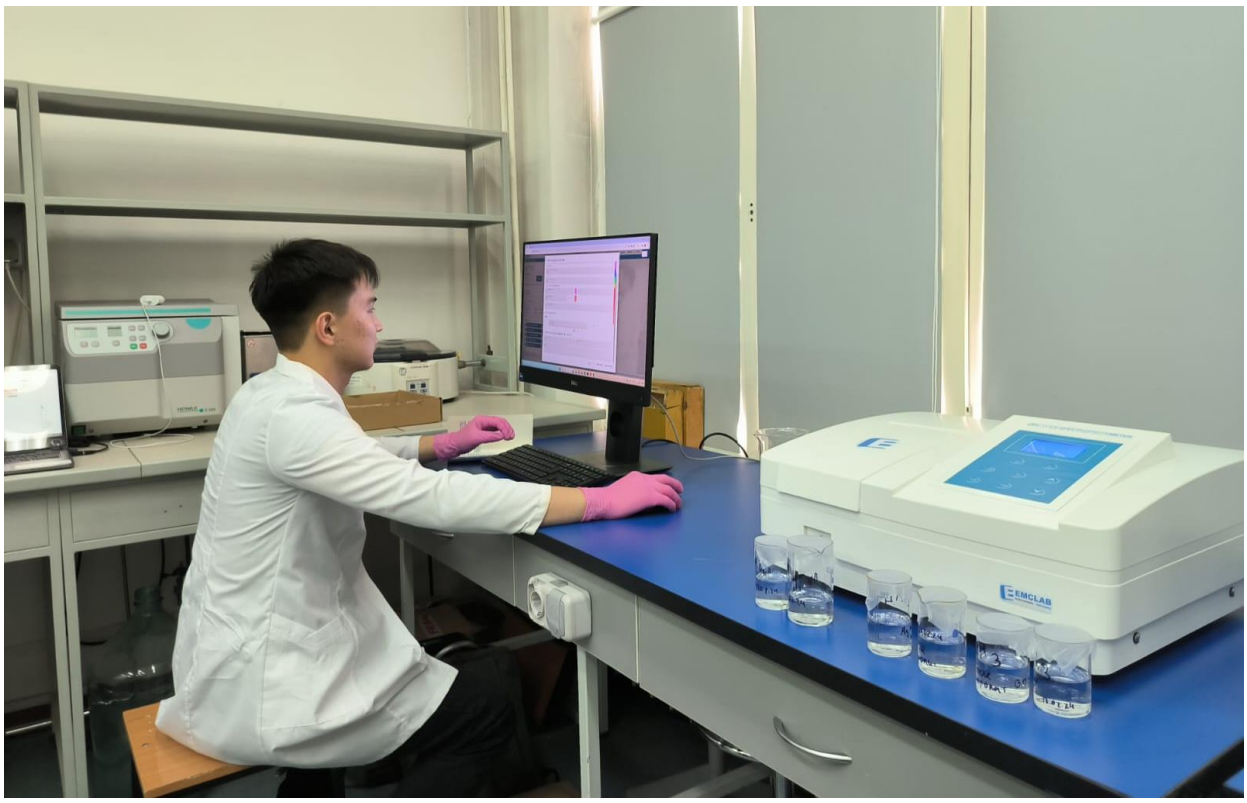
Expected and achieved results	**Expected results**: 1. Nanocoatings based on chitosan and polyacrylic acid (PAA) containing chlorhexidine will be obtained on the prepared surfaces of implanted substrates using the layer-by-layer method at various pH levels from 2 to 7. The morphology and surface roughness will be studied using SEM and AFM. The optimal pH of the assembly for the application of chlorhexidine will be selected. Chlorhexidine with a concentration of 0.05% will be introduced into multilayers by impregnation for 24–48 hours. The morphology and elemental composition of films after the introduction of an antibacterial drug will be studied using SEM and SEM EDX, and the antibacterial activity of the resulting coatings will be studied. 2. The prolonging properties of antibacterial films based on chitosan/PAA/chlorhexidine will be investigated. Quantitative characteristics of the release of chlorhexidine from coatings in saline solution will be established depending on the time of contact (from 2–14 hours to several days). Processing and analysis of the kinetic dependencies of these processes will be carried out to predict the prolonged effect of the film in the human body. 3. Coatings based on chitosan and PAA containing silver nanoparticles (NPAg) will be obtained. The process of inclusion of Ag^+ ions with further "in situ" reduction in nanocoatings will be studied, the concentration and ratio of silver solution and reducing agent will be selected. The distribution of AgNPs on the surface will be determined using SEM, the quantitative content of silver and the form of silver in the layers using SEM EDX and X-ray diffraction. The antibacterial activity of the coatings will be tested. 4. The prolonging properties of the resulting film based on chitosan, polyacrylic acid, and AgNPs will be established. Quantitative characteristics of the release of silver particles from coatings in saline solution will be established depending on the contact time (from 2–14 hours to several days), and the kinetic dependencies of these processes will be analyzed to predict the prolonged effect of the film in the human body. The release of silver from the coatings after long-term storage (1 to 3 months) will be re-analyzed to evaluate the stability/wear properties of the coatings. 5. The cytotoxicity of antibacterial nanocoatings based on chitosan, polyacrylic acid containing chlorhexidine and chitosan/PAA nanofilms containing AgNPs will be investigated. The cytotoxic effect of nanofilms will be determined in vitro by cell culture survival using the generally accepted MTT assay. A sampling criterion at the mean cytotoxic concentration (CTC_{50}) will be performed. Quantification of cell death, cell growth inhibition, cell proliferation or colony formation, cytotoxicity assay. **Results achieved:**

	1. The optimal conditions for obtaining a nanofilm on the surface of implanted products, the selection of the optimal temperature, pH, concentration and the introduction of chlorhexidine have been established. Nanocoatings of the required structure and thickness based on chitosan and polyacrylic acid (PAA) were applied to the prepared surfaces of implanted substrates using the layer-by-layer method at various pH levels from 2 to 7. The morphology and surface roughness were studied using SEM and AFM, and the elemental and quantitative composition of the films was determined using SEM EDX. 2. Chlorhexidine with a concentration of 0.05% was introduced into multilayers by impregnation for 24–48 hours. The morphology, elemental composition of the films, as well as the antibacterial activity of the resulting coatings against gram-positive and gram-negative bacteria using the disk diffusion method were studied using SEM and SEM EDX. The prolonging properties of antibacterial films based on chitosan/PAA/chlorhexidine have been established. Quantitative characteristics of the release of chlorhexidine from coatings in saline solution have been established depending on the time of contact (from 2–14 hours to several days).
Research team members with their identifiers (Scopus Author ID, Researcher ID, ORCID, if available) and links to relevant profiles	1. Savdenbekova Balzhan Yesaimkhankyzy, PhD project leader, al-Farabi Kazakh National University, teacher, h-index-2, https://orcid.org/0000-0001-8812-5809, ScopusID=57190410653. 2. Jumagaziyeva Ardak Bisenbaevna, PhD, SR, JSC "Scientific Center of Anti-Infective Drugs", acting. Head of the Microbiology Laboratory. h-index-3, https://orcid.org/0000-0002-8610-7321, ScopusID=57210255995. 3. Bekissanova Zhanar Bolatovna, Master of Technical Sciences, Researcher, al-Farabi Kazakh National University, Lecturer, Department of Physical Chemistry of Catalysis and Petrochemistry h-index-3, https://orcid.org/0000-0001-6142-0963, ScopusID = 57218598280. 4. Rakhmatullayeva Dilafruz Talgatkyzy, Master of al-Farabi Kazakh National University, JR, 1st year doctoral student of the Department of Physical Chemistry of Catalysis and Petrochemistry https://orcid.org/0000-0002-8096-1068. 5. Seydulayeva Ayazhan Alpeiskyzy, bachelor, specialist of the department of physical chemistry, catalysis and petrochemistry of KazNU named after al-Farabi, https://orcid.org/0000-0002-7972-9624. 6. Sailau Aruzhan Galymkyzy, bachelor, specialist of the department of physical chemistry, catalysis and petrochemistry of KazNU named after al-Farabi, https://orcid.org/0000-0002-6174-5431.

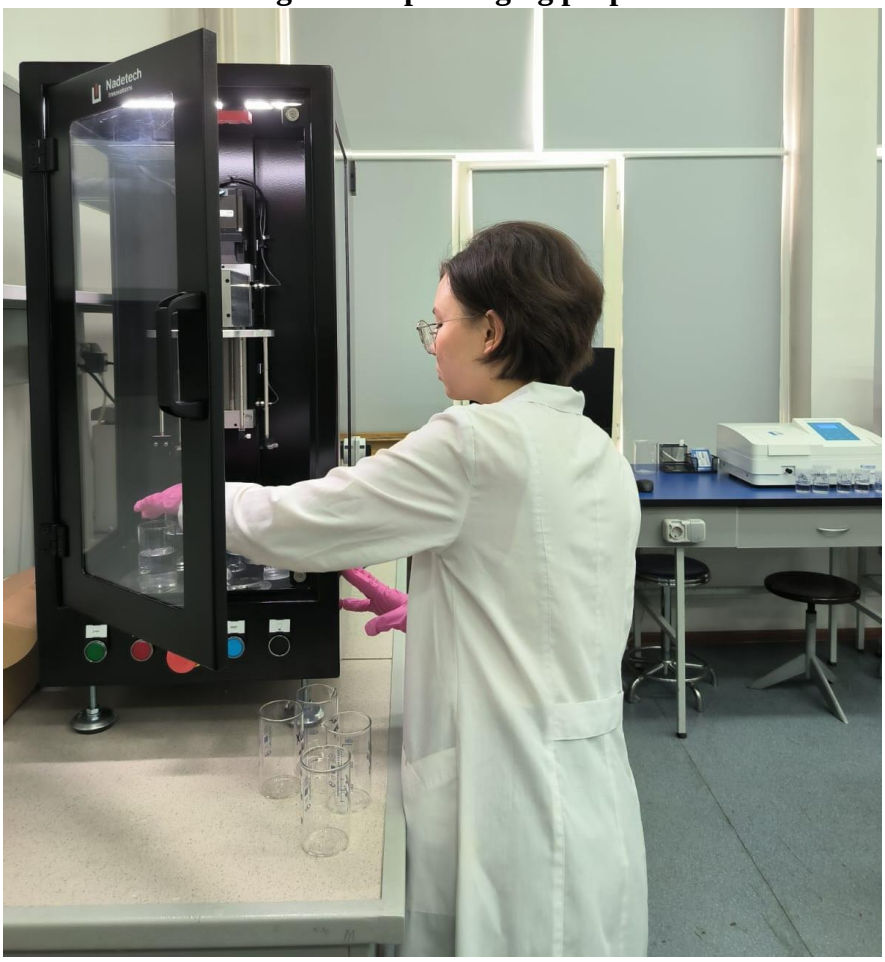
List of publications with links to them	1. B.E. Savdenbekova, D.T. Rakhmatullayeva, Zh.B. Bekissanova. Obtaining of antibacterial coating with silver nanoparticles on a titanium implant, Savdenbekova, B., & Bekissanova, Zh. (2023). Scientific journal "Reports of the National Academy of Sciences of the Republic of Kazakhstan", 346(2), 153-165. https://doi.org/10.32014/2023.2518-1483.217.
Patents	-



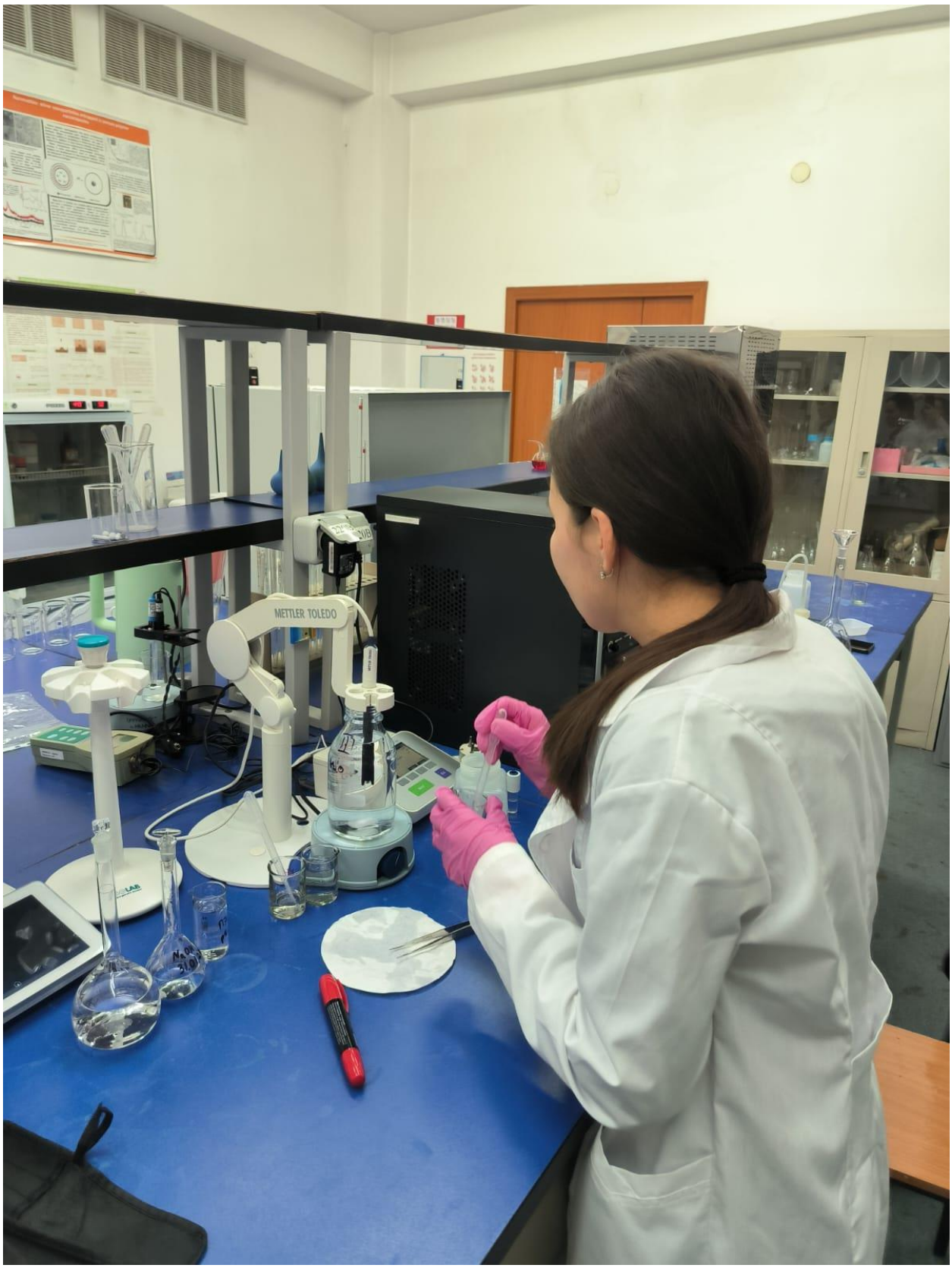
Installation for obtaining coatings by immersion



Investigation of prolonging properties



The process of obtaining coatings on the surface of implants using the dip coater installation



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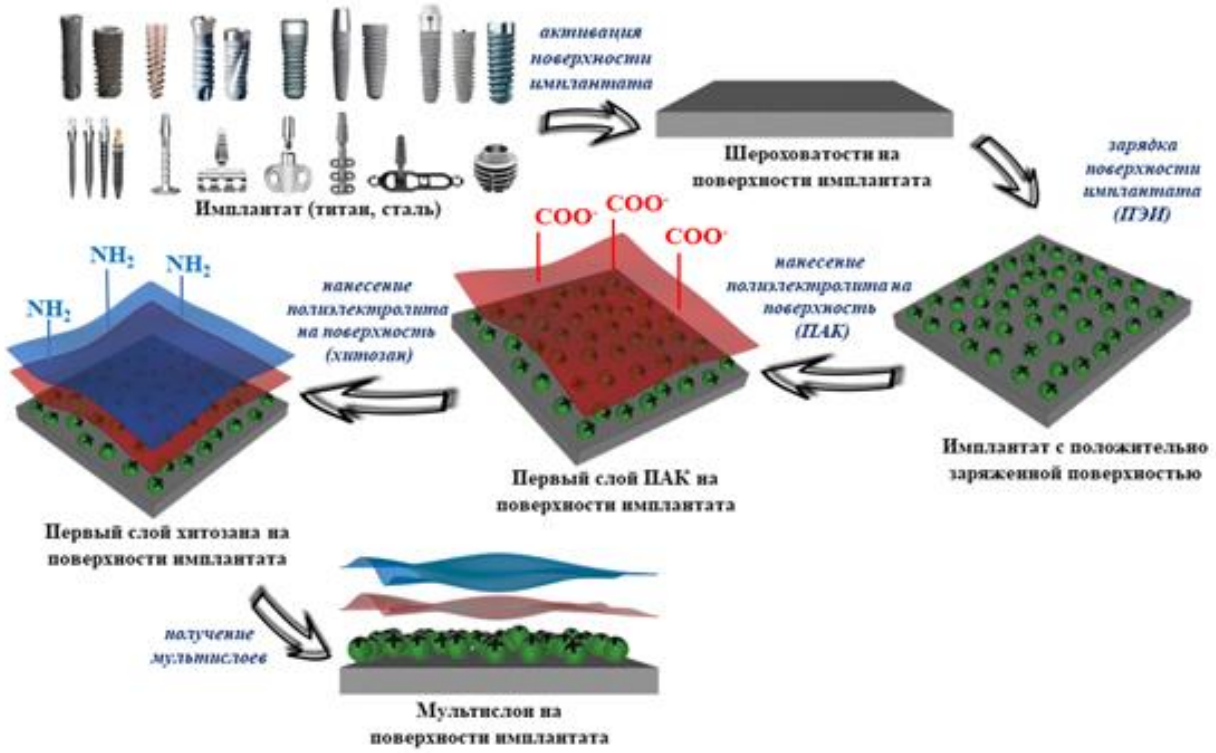

The scheme of obtaining nanofilms on the surface of implantable products