Annex 8
To Regulations for the Open Tender for 2024 Fundamental and Applied Research Projects
(15.03.2024)

Mid-term scientific project report form

Project name: **Advancing Immune Defense of Pancreatic β-Cells through Innovative Tissue Engineering Scaffolds**

1. Scientific excellence

Research Methodology and Progress

The project develops a tissue-engineered scaffold in which pancreatic β-cells and endothelial cells are embedded in a hydrogel and enclosed within a multilayer electrospun membrane whose outer surface is functionalized with 2D MXene nanolaminates to protect the transplanted cells from the recipient immune response while sustaining their viability and endocrine function. Work in the first half of the project has been organized into three work packages: WP1 (membrane fabrication, functionalization, and characterization, M1–M18), WP2 (scaffold assembly and biological assessment, started M12), and WP3 (management and dissemination).

During the reporting period the consortium has 1) established the electrospinning window for the individual polymer layers (PCL, PVA–sodium percarbonate, gelatin–VEGF liposomes), 2) selected and validated the MXene synthesis and delamination route to be used for surface functionalisation on the basis of a systematic biocompatibility study, 3) started multijet co-electrospinning of the multilayer membrane, and (iv) initiated the biocompatibility assessment of the individual monolayers and of the assembled membrane. Deliverables D1.1, D1.2, D1.3, and D2.1 have been submitted; D1.4 and D1.5 (due M19–M20) are in preparation, in line with the original Gantt chart.

The most significant scientific outcome of the period is the identification of the synthesis route that determines MXene biocompatibility. The following sections describe the scientific results for each work package.

Work Package 1 – Development, functionalization, and characterization of the electrospun membrane

Task 1.1 (PCL monolayer). Electrospun PCL mats were produced across a range of polymer concentrations, applied voltages, and collector distances. Fiber diameter, pore size, and porosity were quantified by SEM image analysis. PCL concentrations of 8–15 wt% were tested, yielding fiber diameters of 350–780 nm and pore sizes from 5 to 18 µm. Porosity ranged between 72% and 84%. The parameter set selected for the 2/3-thickness bulk layer was 12 wt% PCL, an applied voltage of 15 kV, and a collector distance of 15 cm.

Task 1.2 (gelatin fibers with VEGF liposomes). Liposomes were prepared and loaded with VEGF; the maximum loadable VEGF concentration and the liposome-to-gelatin ratio compatible with homogeneous, bead-free fiber formation at minimal flow rate were

determined. Release kinetics were measured in the monolayer. Liposome size was 135 nm (DLS, PDI < 11.2), with an encapsulation efficiency of 68% and a VEGF loading of 1.5 μg per mg of lipid. The cumulative release showed an 18% burst at 24 h, followed by sustained release of 65% at 7 d and 78% at 21 d, with ~15% retained bioactivity. A neutral, dipolar, aprotic cosolvent system was used to prevent acid-induced denaturation of the growth factor. Deliverable D1.2 (protocol) prepared in M16.

Task 1.3 (PVA–SPC layer). PVA–sodium percarbonate solutions were screened for spinnability and for oxygen-release performance. Oxygen generation was measured as a function of fiber diameter and SPC content, together with the release rate in culture medium and the maximum concentration tolerated by β-cells. SPC contents of 1–5 wt% were tested. The dissolved O_2 profile peaked at 48 h and provided sustained oxygen release for 12 days. The cytotoxicity threshold for β-cells was reached at >3 wt% SPC due to transient H_2O_2 accumulation. A 2 wt% SPC ratio was selected for optimal balance. Deliverable D1.3 prepared in M17.

Task 1.4 (multijet fabrication of the bilayer membrane). Co-electrospinning of PCL and gelatin-VEGF from separate needles, followed by the PVA–SPC stage, is being carried out at the target membrane thickness of 1–2 mm for the PCL bulk and 1/3 of the total thickness for the PVA-SPC layer. Layer adhesion shows excellent interface stability with no delamination after 72 h in aqueous media. Thickness reproducibility is ±14 % across standard batch areas, and FITC-gelatin fluorescence mapping confirms a homogeneous distribution of gelatin fibers throughout the membrane cross-section. D1.4 is due in M19 and is on schedule.

Task 1.5 (MXene functionalization). The decisive input of the reporting period came from the team's systematic study of MXene synthesis routes. Acidic (concentrated vs. diluted HF/HCl) etching and Li^+ vs. Na^+ intercalation were compared for Ti_3C_2Tx and $Ti_3C_{(1.5)}N_{(0.5)}Tx$: water-assisted etching with Na^+ intercalation increased hydroxylation, reduced -F terminations, and resulted in the lowest cytotoxicity, oxidative stress, and inflammatory signaling. This study defines the material specification currently applied to the outer PCL coating. Because V_4C_3 was more toxic and oxidized more quickly, it was decided to replace it with titanium carbide for the pilot experiment. Delaminated Ti_3C_2Tx nanosheets averaging 250–850 nm were tested at spray-coating concentrations of 50, 100, and 200 μg/cm^2. A 100 μg/cm^2 concentration yielded 94% coating uniformity with robust adhesion (<5% flake detachment after 48 h fluid shear testing).

Task 1.6 (characterization). SEM, AFM, and UV-Vis were applied to the nanofibre mats and to the MXene nanosheets; static and dynamic in vitro degradation and permeability of the final membrane are in progress. XPS surface composition confirmed a high O/F ratio for the MXene phase and prominent C-O/C=O peaks for the polymer matrix. XRD (002) peak position shifted to a lower angle $2\theta \sim 6.2^0$), confirming nanosheet intercalation. Results feed D1.5 (due M20).

Work Package 2 – Scaffold formation and biological assessment

Task 2.1 (biocompatibility of monolayers). Resazurin reduction assay and LDH assays were run on individual polymer monolayers with additives, and DCFH-dA was used to quantify ROS. INS-1E (β-cells) and HMEC-1 (human endothelial cells) lines were used. Viability at 24/72/168 h remained high across all substrates: PCL (96% / 94% / 91%), PVA-2% SPC (92% / 89% / 85%), gelatin-VEGF (98% / 106% / 115%, reflecting VEGF-stimulated proliferation), and the assembled bilayer membrane (95% / 97% / 94%). LDH release remained <5% for all groups compared to the lysis control, indicating intact cell membranes. ROS fold-change showed a transient 1.2-fold increase for the PVA-SPC layer at 48 h, confirming oxygen generation while remaining safely below the cytotoxic oxidative stress threshold. Deliverable D2.1 submitted in M18.

Task 2.2 (cell/gel ratio). Screening of β-cell: endothelial cell ratios and Matrigel volumes has started. At this stage, as a pilot experiment to achieve consistent results, it was decided to start with Matrigel. On the next step, spheroids will form with Amicagel. INS-1E-to-HMEC-1 ratios of 1:1, 5:1, and 10:1, tested across final Matrigel volumes of 50, 100, and 200 µL per well. Optimal spheroid formation, characterized by a uniform diameter of ~150 µm, a distinct β-cell core, and an endothelial shell, was achieved at the 5:1 ratio within 48 hours. Viability peaked at 94% in the 50 µL volume. At 100 and 200 µL volumes, restricted nutrient diffusion through the hydrogel resulted in minor core necrosis, reducing overall viability to 66%.

Secondment. The secondment to the University of Padua planned for M18 was replaced by a secondment to the Biofabics company (Porto, Portugal), supported by the MSCA-SE grant ESCULAPE project, which was successfully carried out. There were the development and optimization of a custom microfluidic system for dynamic membrane testing and continuous cell cultivation under physiological flow conditions.

2. Impact

2.1 Scientific results of the project

The findings of this project deliver significant advancements in tissue engineering, specifically addressing the critical hurdles of immune rejection and graft vascularization in cell-based diabetes therapies. The primary scientific impact is the successful development of a multilayered, electrospun biopolymer membrane (PCL, PVA, and gelatin) capable of simultaneous oxygen generation and sustained VEGF release. By functionalizing the outer PCL layer with MXene nanosheets, the project provides a theoretical and practical breakthrough in using 2D nanomaterials as local immunomodulatory barriers rather than relying solely on systemic immunosuppression. In the broader context of nanomedicine, validating the biocompatibility and immune-evasive properties of specific MXene synthesis routes establishes a new material specification for implantable bio-interfaces. The dissemination of these results has been carried out in accordance with open science principles, ensuring high visibility across both the materials science and clinical engineering communities.

Published and Submitted Papers:

The dissemination strategy prioritized high-impact, open-access journals. The following articles detail the foundational nanomaterial and biological interactions driving the projectЖ

No.	Reference	Quartile/ status
1	Diedkova K., Roslyk I., Kanas N., Grine L., Deineka V., Blacha-Grzechnik A., Boroduskis M., et al., Gogotsi Y., Pogorielov M. Effects of Etching and Delamination on Biocompatibility of Ti-Based MXenes. ACS Appl. Mater. Interfaces, 2025, 17(34), 47919–47937. DOI: 10.1021/acsami.5c08807	Q1, published
2	Volodymyr Deineka, Yevheniia Husak, Marks Truhins, Anastasia Konieva, Irina Kube-Golovin, Gunther Wennemuth, et al., Acelya Yilmazer, Maksym Pogorielov; Biodistribution of tumor-targeted MXene-antibody conjugates. Biomater. Sci. 2026;	Q1, published
3	Sulaieva O., Kobyliak N., Panko I., Meged T., Gaidamak O., Pogorielov M., Deineka V. Biological and Therapeutic Potentials of MXenes in Tumor Microenvironment-Driven Oncology. ACS Appl. Bio Mater., 2025, 8(11), 9542–9556. DOI: 10.1021/acsabm.5c00982	Q1, review

Project results have been actively presented at leading international scientific events to secure feedback and build future consortia:

Event, place, date	Presenter	Title / type
15th IEEE International Conference on Nanomaterials: Applications & Properties (NAP-2025), September 7-12, Bratislava, Slovakia.	V. Deineka	Oral
17th International Conference on Nanomaterials – Research & Application (NANOCON 2025), October 15 – 17, Brno, Czech Republic.	V. Deineka	Oral
The International Scientific Conference on Medicine and Health Sciences of the University of Latvia 2026, March 26, Riga, Latvia.	P. Shubin	Poster
84th International Scientific Conference of the University of Latvia, April 14–16, 2026, Riga, Latvia	P. Shubin	Poster

2.2 Research Development Opportunities:

The project's interdisciplinary methodology has successfully fortified our international research networks and catalyzed significant new collaborative opportunities. Within the core consortium, the collaboration with Drexel University (USA) played a pivotal role in optimizing our MXene synthesis routes, while the partnership with the University of Padua (Italy) was instrumental in evaluating the immune response to the tissue-engineered constructs. To ensure the highest standards of material quality and rigorous analysis, this foundational network was further strengthened by two key entities. Adam Mickiewicz University (Poland) provided crucial expertise and advanced protocols for characterizing complex nanomaterials and their surface modifications, and the Material Research Center served as the primary producer and supplier of high-quality MXenes for the experimental phases. Furthermore, the successful execution and growing visibility of the current project served as a powerful stepping stone for expanding our global footprint. Building on our initial momentum, the team identified and integrated a diverse array of new strategic partners, bringing together the University of Liverpool (United Kingdom), Ankara University (Turkey), Inserm (France), the SME Yoursciencebe Srl (Belgium), and the Auckland University of Technology (New Zealand). Gathering this highly complementary, multi-sectoral consortium was the direct driver behind the successful acquisition of the newly awarded (July 2026) Horizon Europe MSCA-SE BioMIX project. Coordinated by the University of Latvia, this new grant enables expanded international secondments, deepens cross-border knowledge transfer, and secures the sustained funding necessary to advance these novel tissue-engineered technologies toward preclinical development.

Within the reporting period, we submitted the following proposals to international and local calls:

1. HORIZON-MSCA-2026-SE-01-01 – “Biomimetic MXene Nanoplatforms for Targeted Combination Therapy of Triple-Negative Breast Cancer” (**funded**, LU coordinator)

3. M-ERA.NET Call 2026 – “Sequentially Activated Coatings for Mg-Based Orthopedic Implants (MAGOSTAC)” (under evaluation).

4. LUKE Joint Call 2026 – “Hybrid Biomimetic Cellular Signaling MXene Nanocarriers for Modulating Immune Evasion in Targeted Treatment of Triple-Negative Breast Cancer” (under evaluation).

5. LZP FLPP project 2026 – “Chitosan–birch bark-derived hybrid nanofibers for wound healing” (under evaluation).

6. LZP FLPP project 2026 – “Bladder cancer-on-a-chip model using patient-derived organoids for photothermal therapy” (under evaluation)

2.3 Socio-economic impact of results

The ultimate socio-economic goal of this project is to alleviate the massive healthcare burden imposed by diabetes and its severe secondary complications (e.g., angiopathy, nephropathy, neuropathy). The IDF estimates that diabetes-related expenses in Europe exceed €104 billion annually. By developing a self-regulating, tissue-engineered graft that can survive without systemic cytostatic therapy, this project lays the groundwork for reducing patient dependence on daily insulin injections and continuous clinical monitoring. From a clinical and surgical translation perspective, the ability to implant a robust, prevascularized cellular construct without necessitating lifelong immunosuppression significantly reduces postoperative complication rates and patient morbidity. To accelerate the transition from the bench to the medical market, the consortium is collaborating with SMEs, such as Linari Engineering (Italy), to scale up the multijet electrospinning process to meet reproducible, biomedical-grade manufacturing standards.

2.4 Publicity and Communication

Communication and dissemination activities were carried out throughout the reporting period to increase the project's visibility among both the scientific community and the general public, while promoting awareness of the societal relevance of tissue engineering and biomaterials research.

Public outreach. The research team actively participated in the University of Latvia's annual Researchers' Night event, where interactive demonstrations of electrospinning technology, biomaterial fabrication, and cell culture techniques were presented to high school students, university applicants, and members of the public. These activities provided hands-on insight into modern biomedical research and highlighted its potential applications in regenerative medicine and healthcare.

The project was also featured during the Shadow Day initiative, which enabled secondary school students to spend a day in the laboratory, observe ongoing research activities, interact with researchers, and gain a better understanding of career opportunities in science, biotechnology, and biomedical engineering.

Online communication. Updates on project milestones, scientific publications, conference presentations, and other achievements were disseminated through social media platforms, including LinkedIn and X (formerly Twitter), to reach the broader scientific community, biotechnology stakeholders, and patient advocacy networks:

-https://x.com/V_Deineka/status/2079944625326846369?s=20

-https://www.linkedin.com/posts/volodymyr-deineka-42a53a307_ieeenapconf-msca4ukraine-activity-7373255575112953856-XTdt?utm_source=share&utm_medium=member_desktop&rcm=ACoAAE42JOIB-kAW-GtwmDZbwCpKcVduaNoJ8Wc

-https://www.linkedin.com/posts/volodymyr-deineka-42a53a307_i-was-pleased-to-attend-the-17th-international-activity-7386027528991260672-

29hu?utm_source=share&utm_medium=member_desktop&rcm=ACoAAE42JOIB-kAW-GtwmDZbwCpKcVduaNoJ8Wc

-https://x.com/V_Deineka/status/1967491395763265610?s=20

-https://x.com/V_Deineka/status/1980263466247090617?s=20

These activities increased the project's visibility, fostered public engagement, and promoted knowledge exchange beyond the academic community.

2.5 Contribution to capacity building of the scientific team and study environment

The project operates on the principle of “learning through research”, integrating early-career researchers and students directly into the experimental workflow and fostering the development of advanced scientific competencies. The research activities actively involved students at different academic levels, including Anastasiia Haidai, a Bachelor's student from the Faculty of Medicine, and Mark Truhins, a Master's student from the Faculty of Biology at the University of Latvia, providing them with valuable hands-on experience in biomedical research and interdisciplinary laboratory practices.

Through their involvement in the project, students gained practical expertise in advanced techniques, including imaging flow cytometry, multijet electrospinning, quantitative molecular assays, and 2D nanomaterial functionalization. This direct participation in ongoing research strengthened their understanding of the connections among fundamental biological questions, materials engineering, and translational biomedical applications.

The successful cooperation with MSCA-SE projects ESCULAPE and MX-MAP through the secondments to the Biofabics (Porto, Portugal) and Essen University Hospital (Essen, Germany), (RESPILON, Prague, Czech Republic), extended the ability of using microfluidic systems, increased the experiences in different type of electrospinning processes, and further diversified the team's engineering capabilities and expanded opportunities for knowledge exchange within the European research environment. By cross-training biologists in polymer chemistry and materials scientists in cellular and molecular biology approaches, the project significantly enhanced the interdisciplinary capacity of the research team at the University of Latvia.

Overall, the project strengthened the local scientific environment by developing a new generation of researchers with multidisciplinary skills, increasing their readiness for future academic and industrial careers, and enhancing the competitiveness of graduates and researchers within the European biotechnology sector.

3. Implementation

Progress in Fulfilling the Work Plan

The project is progressing in accordance with the initial work plan and methodology set out in the project application. By the mid-term reporting period, the core tasks in Work Package 1 and the initial tasks in Work Package 2 have been successfully executed without critical delays. In WP1 (Development, functionalization, and characterization of the electrospun membrane),

the consortium successfully established the electrospinning window for the individual polymer layers, including PCL, PVA-sodium percarbonate, and gelatin-VEGF liposomes. A critical milestone was achieved by identifying the most biologically safe MXene synthesis and delamination route (water-assisted etching with Na^+ intercalation) for surface functionalization. Deliverables D1.1, D1.2, and D1.3 have been completed and submitted on schedule. Multijet co-electrospinning of the multilayer membrane is currently underway, keeping deliverables D1.4 and D1.5 strictly on track for their respective M19 and M20 deadlines. In WP2 (Scaffold formation and biological assessment), cytocompatibility studies (Resazurin reduction, LDH assays, and ROS quantification) demonstrated high cell viability across all substrates, effectively fulfilling Deliverable D2.1 (submitted in M18). Task 2.2 screening for optimal cell-to-hydrogel ratios has successfully commenced. As a strategic risk-mitigation step to ensure consistent preliminary results, Matrigel was used as a pilot hydrogel before transitioning to Amikagel. This yielded optimal spheroid formation at a 5:1 β-cell-to-endothelial-cell ratio in a 50 μL volume.

An adaptive and highly beneficial change to the implementation plan involved the planned M18 secondment. Instead of the University of Padua, a secondment to the Biofabics company (Porto, Portugal) was successfully executed with co-financing from the MSCA-SE ESCULAPE project. This strategic pivot directly resulted in the development and optimization of a custom microfluidic system for dynamic membrane testing and continuous cell cultivation under physiological flow conditions. The risk assessment and management plan outlined in the proposal has been actively monitored. Technical challenges, such as identifying the cytotoxicity threshold of SPC-generated H_2O_2 (capped at >3 wt% SPC) and mitigating core necrosis at higher hydrogel volumes, were efficiently managed through systematic optimization, ensuring the project remains well within its projected timeline and budget.

Research Data Management Plan (DMP)

The project's Data Management Plan has been structured to guarantee that all generated data adhere strictly to FAIR principles (Findable, Accessible, Interoperable, and Reusable).

Data generation and collection: All experimental protocols, electrospinning parameters, material characterization data (SEM, XRD, XPS), and biological evaluation results are systematically documented and standardized.

Storage and backup: Primary datasets are stored on secure University of Latvia servers with regular backups to ensure data integrity and prevent loss.

Open access and FAIR data: In alignment with the project's exploitation strategy, key datasets supporting peer-reviewed publications will be made publicly available.

Metadata and interoperability: Datasets are structured with appropriate metadata to ensure they remain accessible and interpretable for the global scientific community and future cross-disciplinary research endeavors.

There have been no significant changes to the project's management structure during the reporting period. The project continues to be coordinated and scientifically led by Dr.

Volodymyr Deineka (Institute of Atomic Physics and Spectroscopy, University of Latvia). The University of Latvia's Research, Human Resources, Finance, and Procurement Departments provide administrative management, financial monitoring, procurement procedures, and legal compliance, ensuring that all project activities are implemented in accordance with the University's internal regulations and the requirements of the Latvian Council of Science.