NAWA POLISH NATIONAL AGENCY FOR ACADEMIC EXCHANGE

MEDICAL UNIVERSITY OF WARSAW

REPORT ON RESEARCH AND OTHER ACTIVITIES OF THE PROJECT:

EVIRA – EVs FROM STEM CELLS IN TISSUE REGENERATION

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Duration of the project: 01.07.2022-30.06.2024

The main goal of the project was to establish a long-term, systemic scientific collaboration in the field of regenerative medicine and cell therapy between the Medical University of Warsaw (WUM) and strategic partners from Lithuania: the Lithuanian University of Health Sciences (LSMU), Kaunas University of Technology (KTU), and Kumamoto University (KU) in Japan. The result of this collaboration was the creation of a scientific consortium supported by business partners (Lithuanian biotech startups Kelifarma, Cumulatis) that was applying with additional scientific partners from the EU for projects within the Horizon Europe program for the years 2022-2024 in the field of regenerative medicine.

Numerous mutual trainings, internships, and study visits were conducted at the partner institutions and collaborating companies (Kelifarma, Cumulatis) to develop methods for the biotechnological cultivation of adult stem cells – including placenta-derived mesenchymal stem cells (PSCs) and induced pluripotents stem cells (iPS), their differentiation, as well as methods for the isolation, transport, and characterization of extracellular vesicles obtained from these cultures.

The project outcomes included the preparation of joint grant applications for research under the Horizon Europe program, the enhancement of the qualifications of WUM researchers in collaborating centers, and the implementation of innovative solutions in research and technology using GMP (Good Manufacturing Practice). The long-term goal of the project is to create innovative technology for the production of stem cells and functionally active extracellular vesicles under GMP conditions, with potential therapeutic applications.

SCIENTIFIC ACHIEVEMENTS:

1. Optimization of methods for culturing and differentiating adult stem cells, first of all MSCs in 2D and 3D cultures.

2. Optimization of the method for filtration and concentration of extracellular vesicles (EVs) from cell culture supernatants by Tangential Flow Filtration (TFF).

3. Implementation and optimization of a state-of-the-art research technique - fluorescence nanoparticle tracking analysis (FL-NTA) for quantitative evaluation and molecular phenotyping of EVs obtained from concentrated adult stem cell culture medium. We have shown that the composition and contamination of EVs samples has a significant impact on FL-NTA analysis, which should only be applied to contaminant-free samples. The culture medium used to isolate EVs must be free of fetal bovine serum (FBS) so that the EVs analyzed do not contain FBS-derived EVs.

5. Additional phenotypic analysis of EVs by classical EV characterization methods recommended by the International Society of Extracellular Vesicles (ISEV), such as Western Blot.

6. Functional potency *in vitro* tests of isolated EVs compared to the parental PSCs

7. Establishment of a protocol of nephron progenitor induction and expansion from iPS cells and its optimization towards future EV isolation

Results:

FL-NTA analysis

Background:

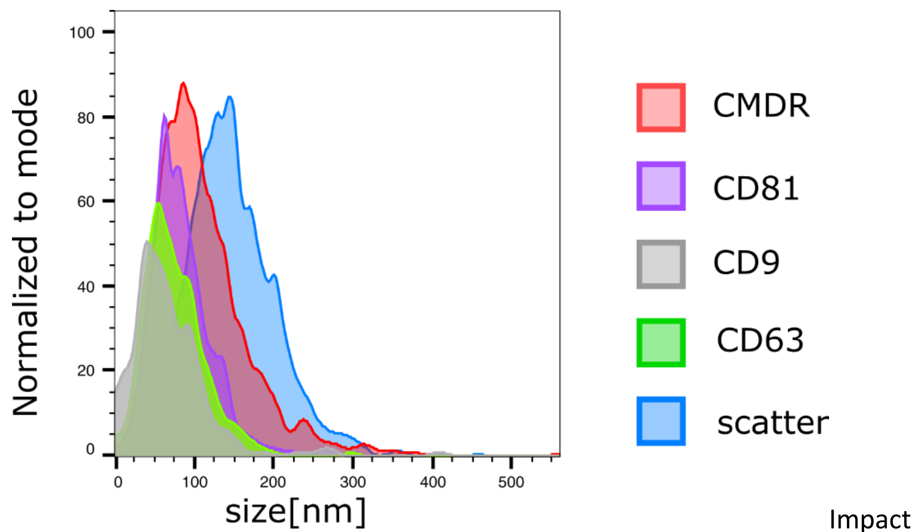
Placenta Stem Cells (PSCs) have huge regenerative potential but there are many challenges in their usage because of tumorigenic adverse effects. Extracellular Vesicles (EVs) derived from PSCs can potentially provide regenerative potential without the tumorigenic effects and could be used for future clinical applications.

Methods:

PSCs were cultured in the 2D environment in MEM or DMEM media with 10% FBS. The culture medium was collected at few different cell passages and centrifugated at 3000 x g for 30 min to remove cell debris. Then, Tangential Flow Filtration (TFF) was performed, using a 0.65 µm filter, a flow rate of 120 mL/min with a transmembrane pressure (TMP) of 5 psi to concentrate the cell secretome. The permeate was collected and concentrated using a 500 kDa cutoff filter (36 mL/min., 12 psi TMP). Then, EVs were characterized by fluorescent nanoparticle tracking analysis (FL-NTA).

Results:

FL-NTA showed that EVs derived from PSCs have a size range typical for small EVs. [Fig.1]. Medium size measured in scatter mode was 126.8 nm, in fluorescent mode after labelling with CMDR 128.6 nm. Particles positive for tetraspanins, as expected, were smaller: CD63+ 74.1 nm, CD9+ 61.5 nm, CD81+ 92.4 nm.



Impact

Figure 1 Distribution of particles in scatter and fluorescent mode after staining with tetraspanin markers and a membrane dye Cell Mask Deep Red (CMDR) on ZetaView 220 (Particle Metrix).

Medium concentration of EVs in scatter was 4.68 x 10^{10} particles/mL. We performed a comparison between EVs achieved from MEM and DMEM culture media. In case of CMDR staining, we observed a big background impacting the FL-NTA results, especially in samples from DMEM media. The concentration of particles from MEM media in scatter was significantly lower, although the concentration of CD81+ particles was significantly higher than from DMEM. [Fig.2]. The size of total particles (scatter) and CD81+ EVs was higher in DMEM. Only in case of CMDR the size of particles was significantly bigger in MEM. [Fig.3].

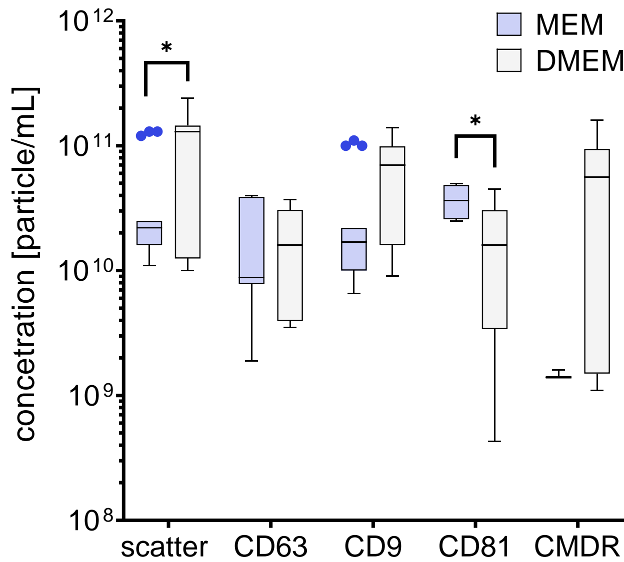


*Figure 2 Concentration of PSCs EVs from MEM and DMEM in scatter and after staining with tetraspanin markers or CMDR. * refers to p value ≤ 0.05 from t-test-unpaired comparison.*

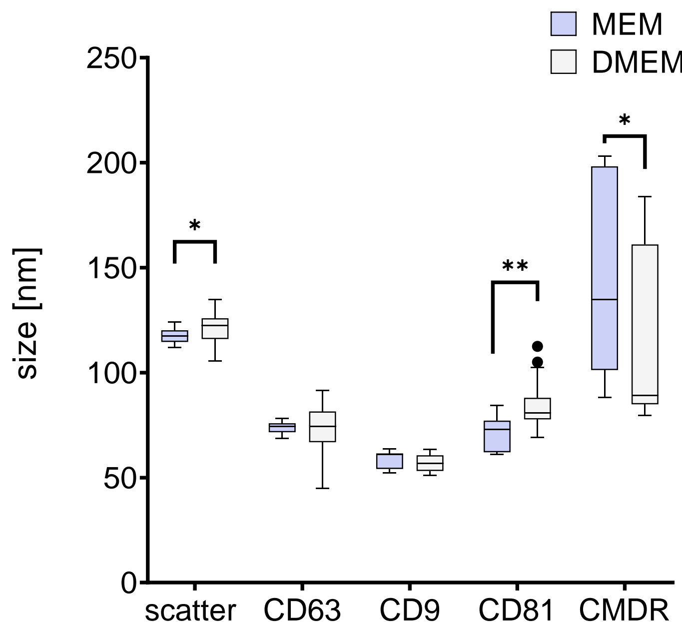


*Figure 3 Size of PSCs EVs from MEM and DMEM in scatter and after staining with tetraspanin markers or CMDR. ** refers to p value ≤ 0.002, * refers to p value ≤ 0.05 from t-test-unpaired comparison.*

We compared the percentage of tetraspanin and CMDR positive particles in MEM and DMEM, but we saw no statistically significant differences. [Fig.4]. The CD63+ were 31.3%, CD9+ 100%, CD81+ 42.4% and CMDR+ 46.5% of all particles visible in scatter.

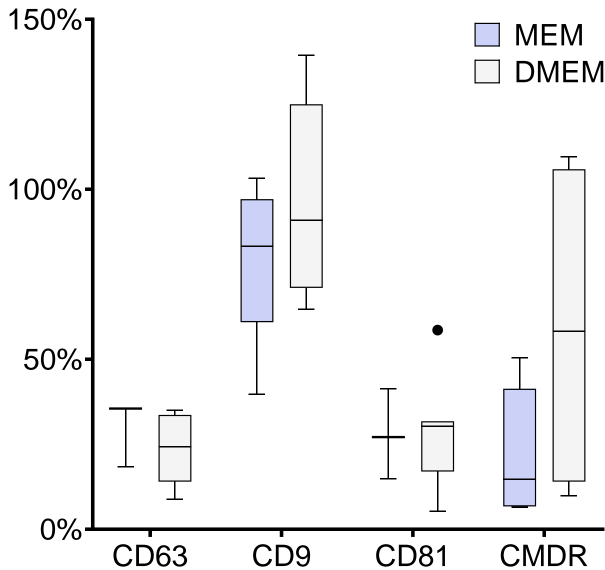


Figure 4 Percentage of tetraspanin or CMDR positive PSCs EVs from MEM and DMEM.

Conclusions:

EVs derived from PSCs show the presence of CD63, CD9 and CD81 markers. Concentration and size of particles seems to be slightly different depending on the culture media. Using DMEM leads to more particles (in total) with bigger size, but also with less CD81+ particles. Optimizing culture conditions for PSCs and analyzing PSCs EVs obtained in the same manner every time are needed to get highly reproducible results and a future final product that can be used in the clinic. Right now, we are only at the beginning of the road to obtaining that goal.

Western Blot analysis

Methods:

List of used antibodies and protein standard:

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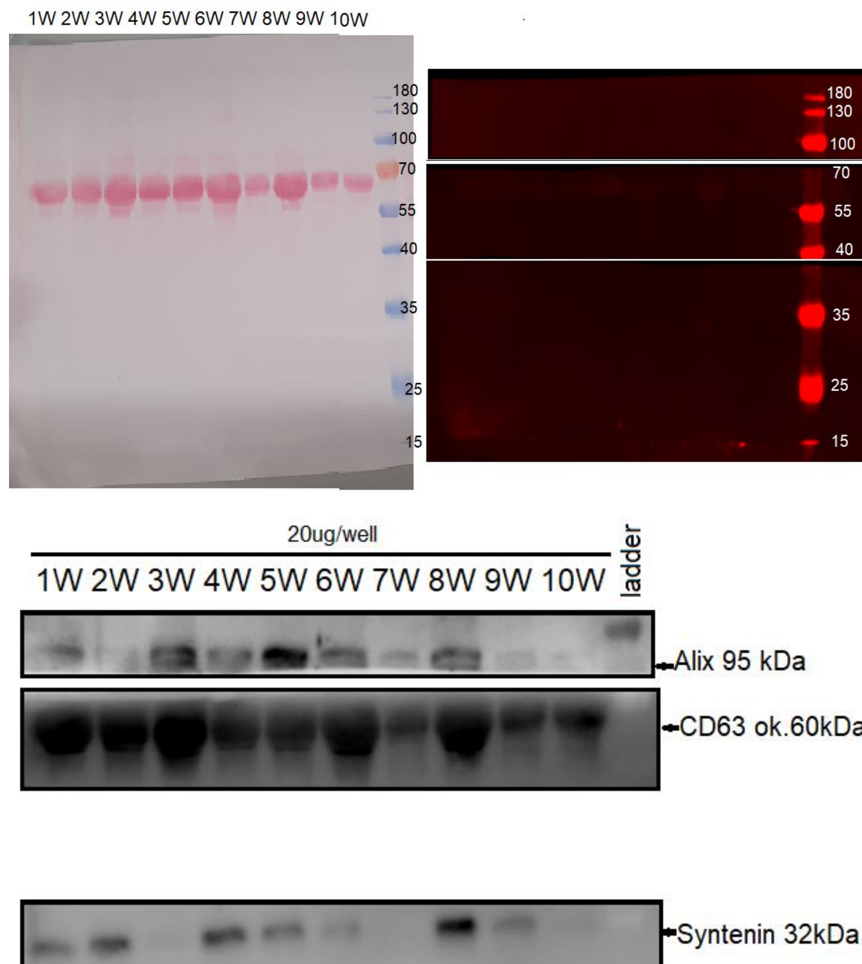
Rabbit Calnexin	ab13504 1:2000 1% milk	90kDa
Mouse Alix	ab117600 1:3000 1% milk	96kDa
Mouse HCAM	sc-7297 1:200 5% milk	90-95kDa
Mouse CD73	Sc-32299 1:200 5% milk	71kDa
Mouse CD63	Abcam lot? 1:400 1% milk	60-50kDa
Rabbit Arg I	GTX109242 1:2000 5% milk	43kDa
Rabbit Arg II	Abcam lot? 1:1000 5% milk	39kDa
Mouse GAPDH	Invitrogen 1:10000 5% milk	37kDa
Rabbit Syntenin	Ab19903 1:1000	32kDa

Thy1	Sc-53116 1:200 5% milk	25-37kDa
B-actin-HRP	Thermo lot? 1:40000	42kDa
Goat Anti-mouse HRP	A16078 1:5000	
Goat Anti-rabbit HRP	A16110 1:10000	
Incubation overnight 4 C		

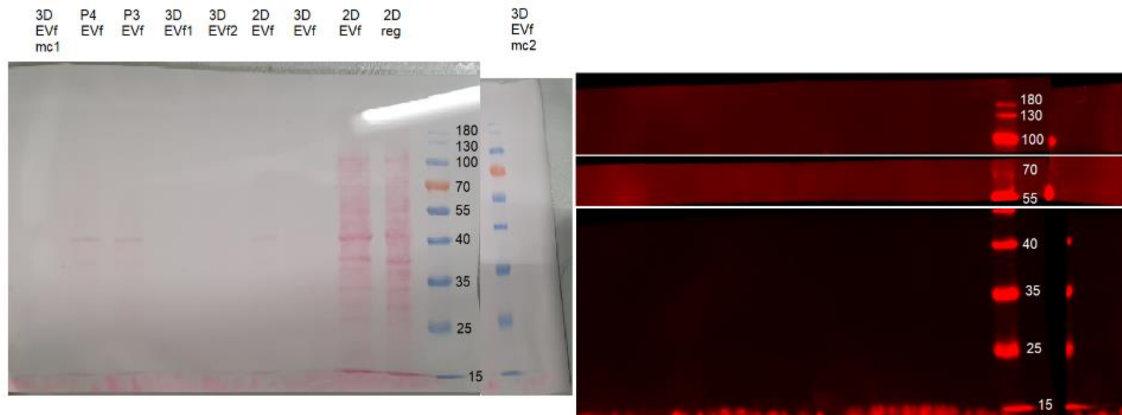
Composition of the SDS-PAGE electrophoresis gel and procedure details:

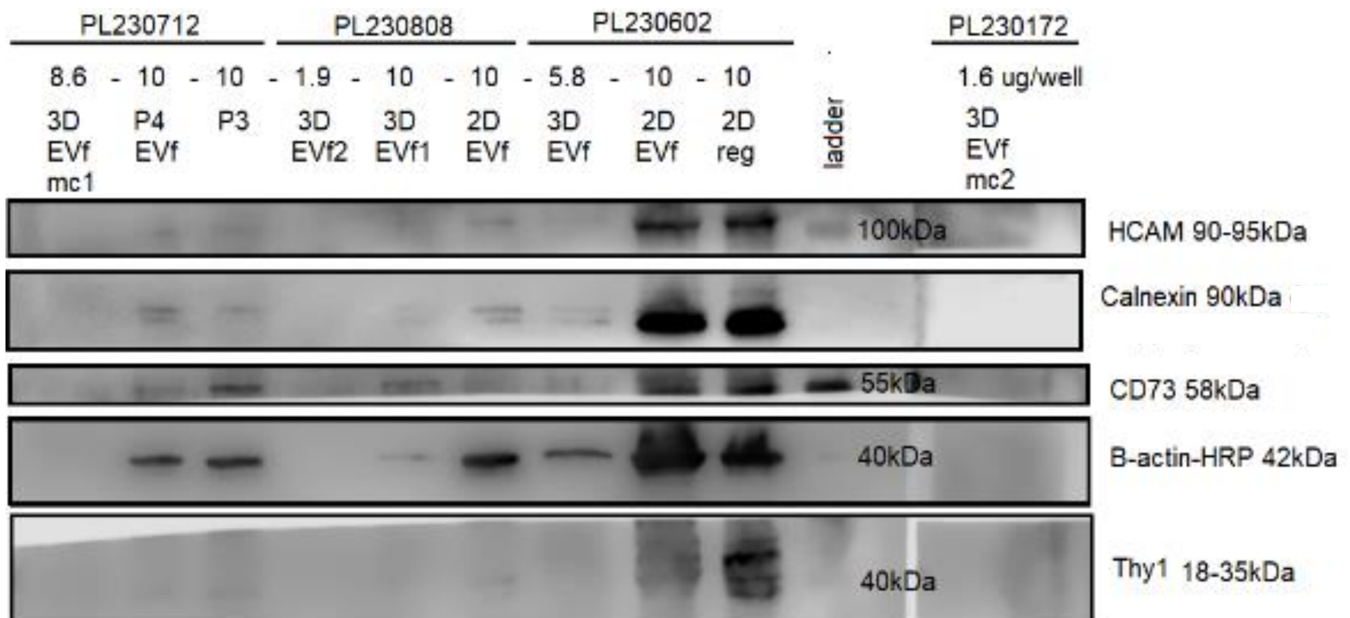
Gel	Stacking 4% (1 gel): H2O 3.01 ml 40% Acryl 0.5 ml 0,5M Tris pH 6,8 1.26 ml 10% SDS 125 ul 10% APS 100 ul TEMED 5 ul	Resolving 12% (1 gel): H2O 4.55 ml 40% Acryl 2.5 ml 1,5M Tris pH 8,8 2.5 ml 10% SDS 250 ul 10% APS 200 ul TEMED 5ul
electrophoresis	80V 30min, 110V	
Transfer	50min 1A 25V	
Imaging	Femto 2min, Fc Odyssey	
Protein concentration meaurement	microBCA	

Western Blot of PSC-derived EVs:



Western Blot of PSC cells





EVs were isolated from cell culture supernatants of PSCs obtained from 3 different placentas. The parental PSCs were of different passage number and were cultured in 2D and 3D conditions in DMEM medium supplemented with EV-free FBS. The presence of canonical EV markers like Alix, Tsg101 and syntenin was tested.

At the same time the parental PSCs were tested for the presence of selected stem cells marker: HCAM, CD73, Thy-1. Beta-actin and calnexin were evaluated as loading control. Because of difficulties in retrieving the PSC cells from the microcarriers after 3D culture, the cell number of the obtained 3D cell samples and the protein concentration of the obtained cell were much lower. Therefore, it was not possible to load the same protein amounts of all cell samples on the gel. This different sample loading is reflected by the very heterogenous signal strength of calnexin and beta-actin. The identified signals for the stem cell markers cannot be therefore be evaluated quantitatively, however it is evident that in samples with enough high protein content, like the 2D samples of cells from the third placenta, the detection of HCAM, CD73 and HCAM is possible.

Conlusions:

Typical exosomal proteins ALIX, CD63 and syntenin were visualized by WB, confirming that our isolates contain true EVs. The 2D and 3D culture of PSCs for EV isolation has to further optimized towards equal cell number at the time of EV harvest. The trypsinization procedure of the cells from the microcarriers in 3D culture hast to be optimized.

Functional assays of isolated PSC-derived EVs in comparison to parental cells:

- **Immunomodulatory effects of placenta MSC-derived EVs in a novel 3D, immune-competent in vitro human small airway model of cystic fibrosis and ARDS**

To check the immunomodulatory and regenerative potency of the isolated PSC-derived EVs in comparison to parental MSCs in inflammatory conditions, two innovative *in vitro* 3D disease models developed by the Epitjelix company were used: an cystic fibrosis model (small airway epithelial cells and fibroblasts obtained from a cystic fibrosis patients + airway macrophages from a healthy donor) and an ARDS (acute respiratory distress syndrome) model induced by LPS (small airway epithelial cells and fibroblasts + airway macrophages from a healthy donor).

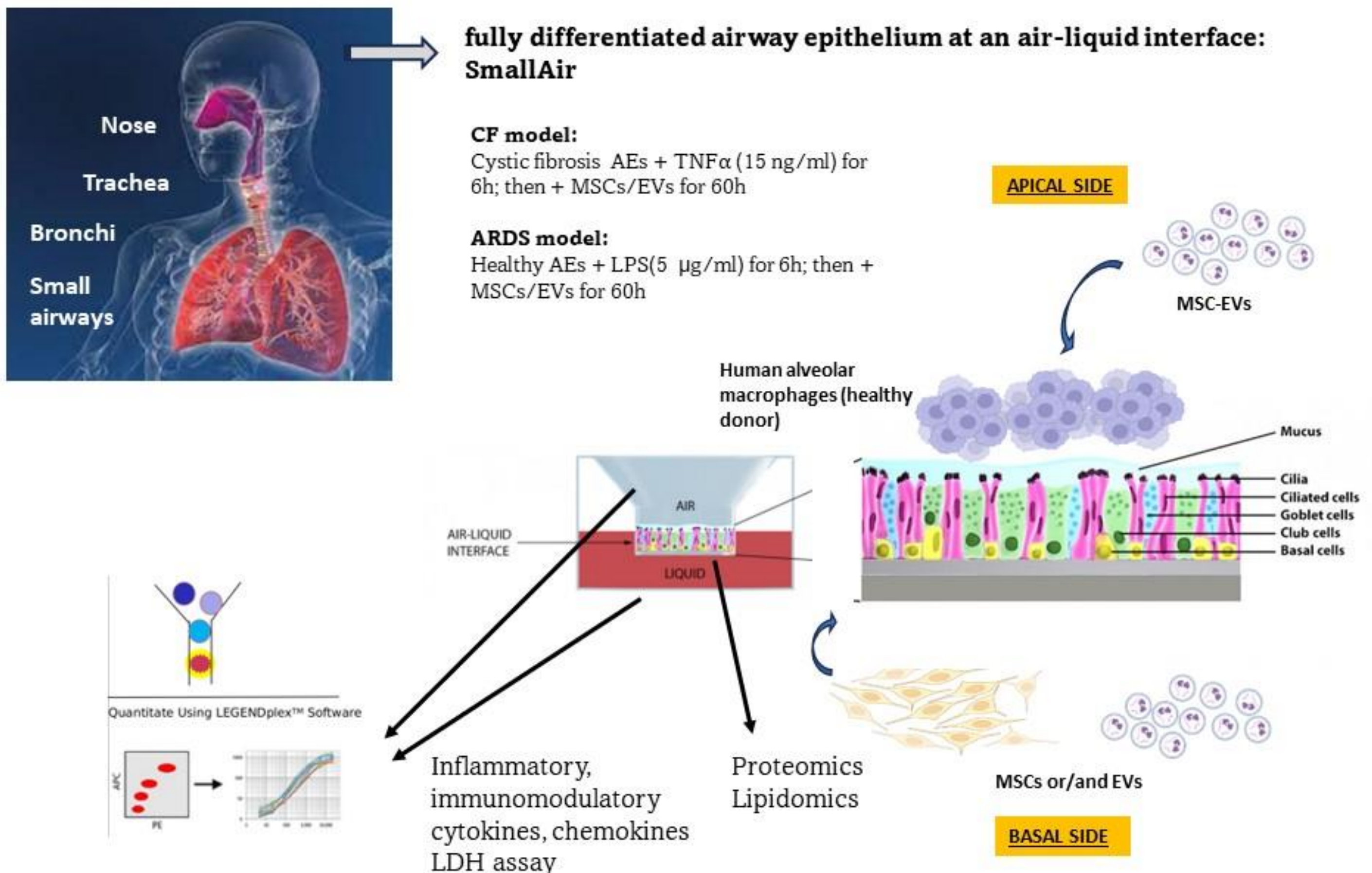


Figure 7: Schematic outline of the Epithelix 3D model for testing the functional potency of PSC-derived EVs

- Impact on epithelium cytotoxicity (LDH assay) and release of pro- and anti-inflammatory cytokines and chemokines at the apical side (mostly macrophage-derived) and basal side (mostly epithelial cell/fibroblasts-derived) using flow cytometry (Legendplex assay) were investigated. Epithelial cells and macrophages were collected for proteomic and lipidomic studies.

Results:

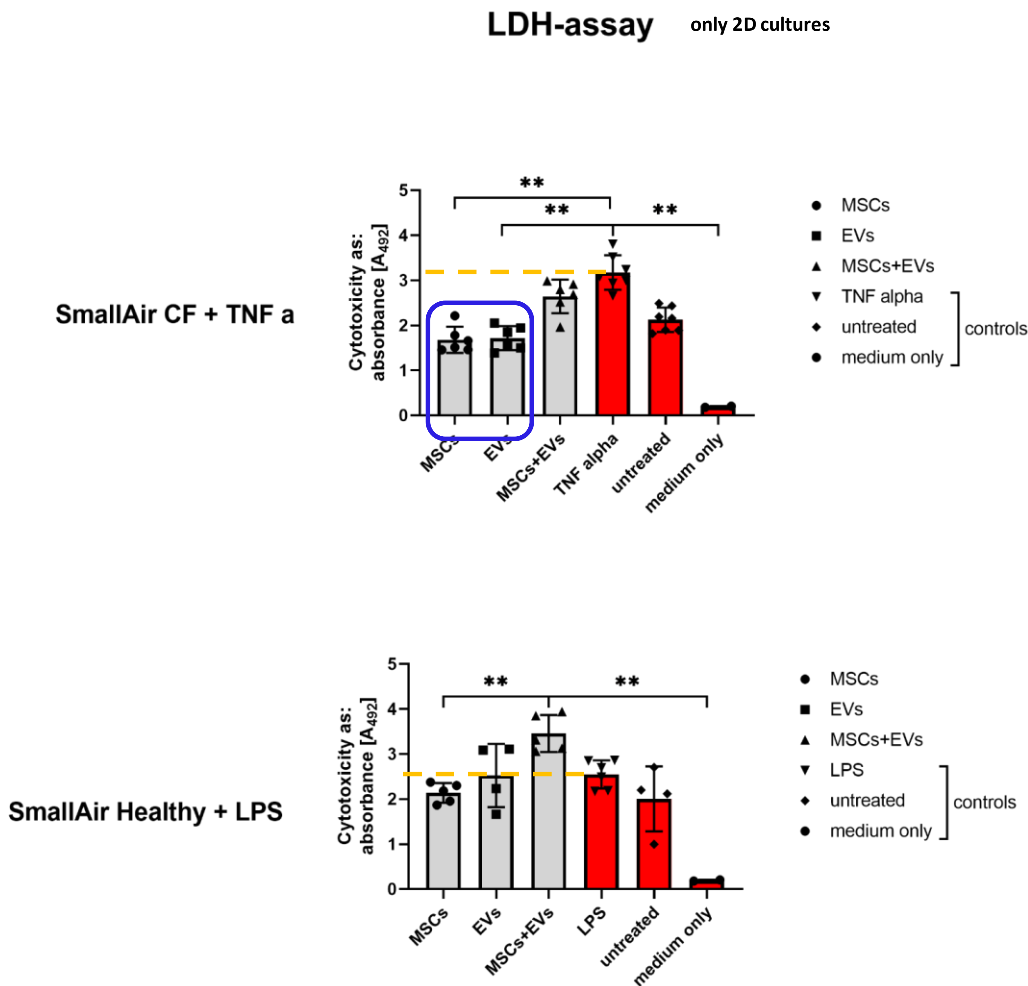


Figure 8: Impact on cell cytotoxicity of of MSC-derived EVs in comparison to parental MSCs in the cystic fibrosis (CF) model (upper panel) and ARDS model (lower panel)

In the CF model we observed in comparison to the TNFa-treated control a complete reversal of cellular cytotoxicity after treatment with MSCs and EVs alone, not so much for the combined treatment. In the ARDS model we noticed no significant reduction of cellular cytotoxicity in comparison to the LPS-treated control (for the combined treatment even an increase of cytotoxicity). However, the LPS-induced cytotoxic effect was in general lower than expected. For conclusive results the experiment should be repeated.

Cytokine levels in the basal medium (AECs, fibroblasts) in the CF model

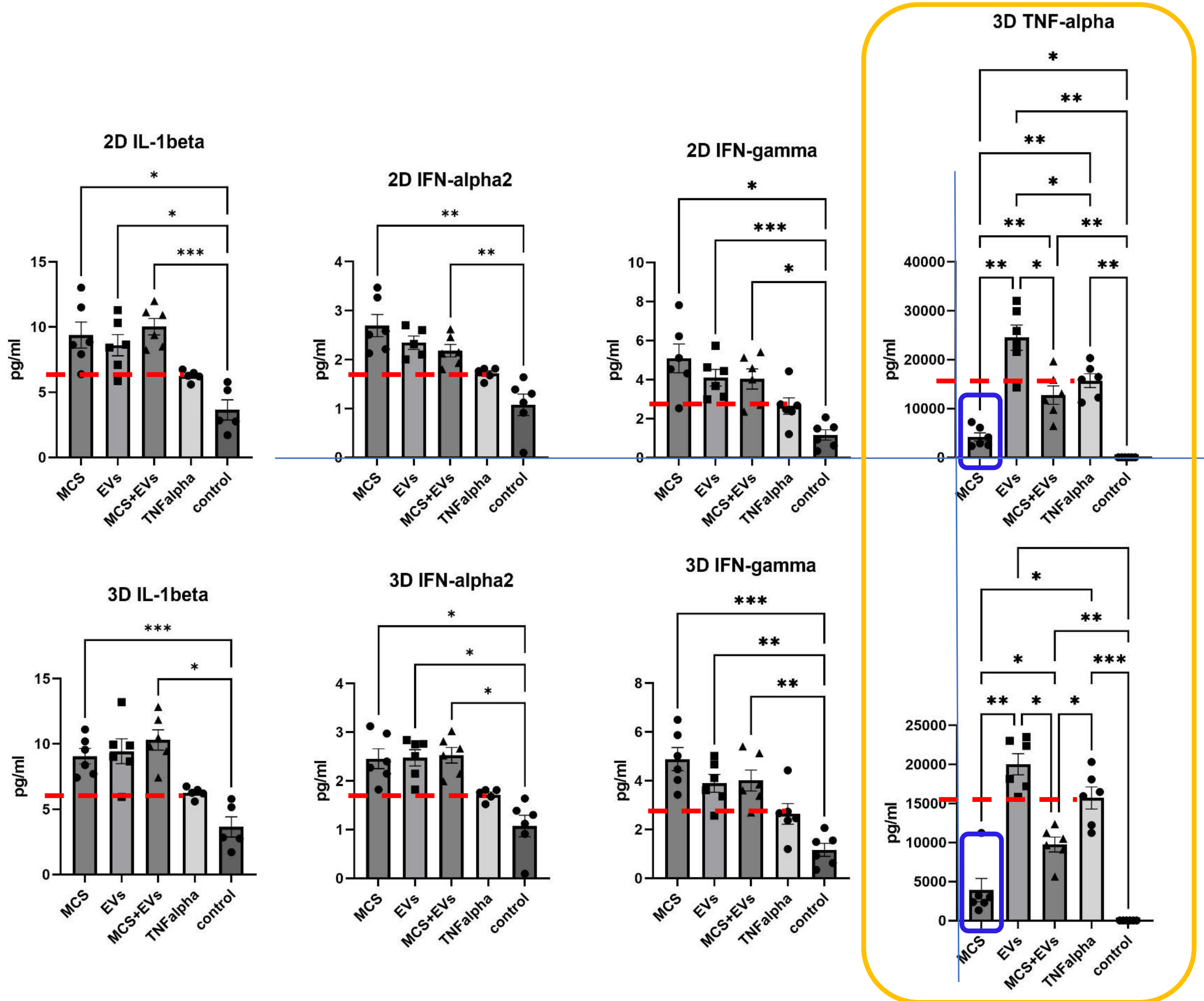


Figure 9: Levels of selected cytokines measured in the basal medium (corresponding to airway epithelial cells and fibroblasts) in the CF model upon co-incubation with MSCs and MSC-derived EVs

Generally, upon co-incubation with MSCs or EVs we observed, contrary to our expectations, an increase of pro-inflammatory cytokines. An outstanding exemption was the release of TNFa, were we noticed a drastic reduction of the TNFa levels upon treatment with MSCs alone and a less pronounced reduction in case of the MSC+EVs combination. However the treatment with EVs alone lead in contrary to an increase of the TNFa secretion. There was not much difference in cytokine secretion between the 2D and 3 D culture.

However strikingly, the same pattern of increase of cytokine secretion after co-incubation with MSCs and EVs were observed for some anti-inflammatory cytokines like IL-10 and IL-23.

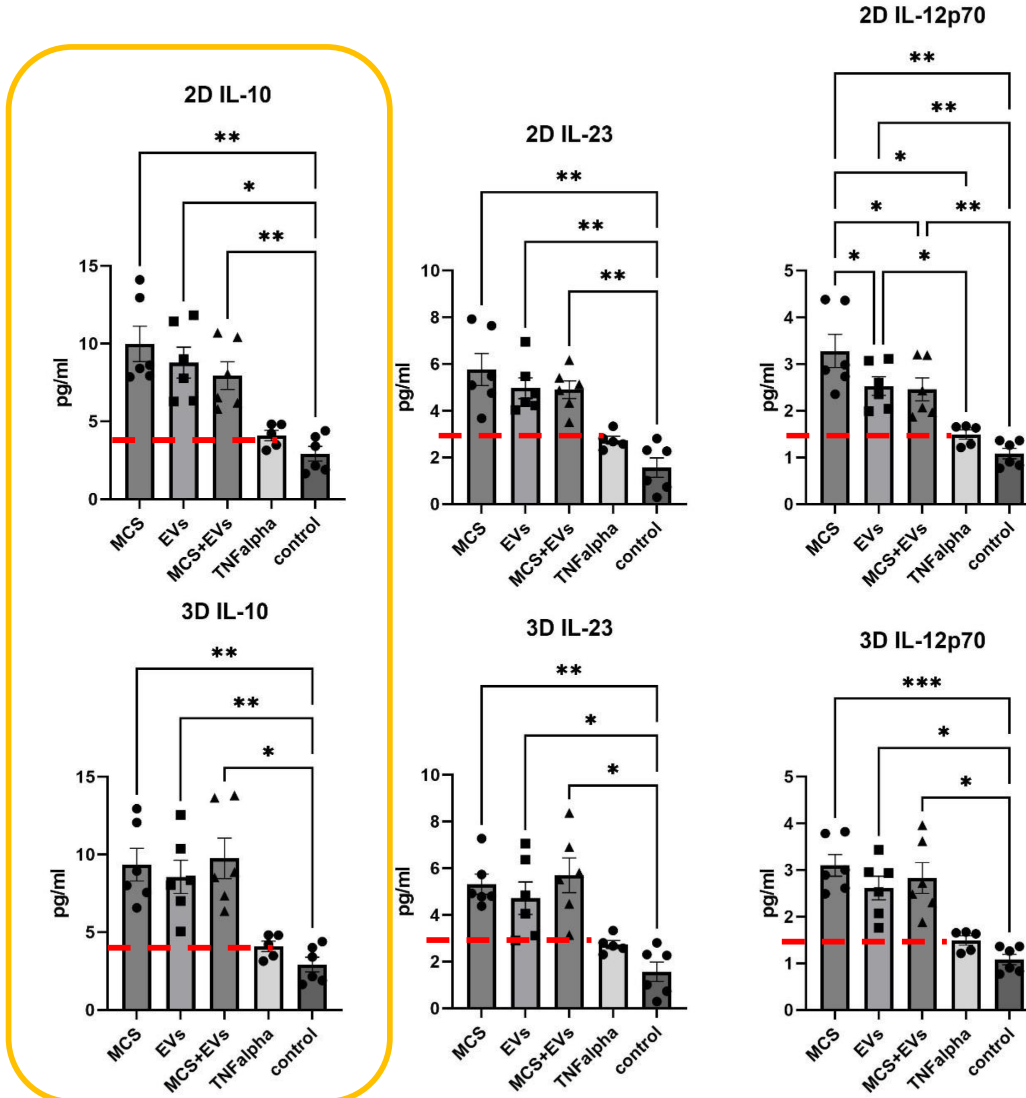


Figure 10: Levels of selected cytokines measured in the basal medium (corresponding to airway epithelial cells and fibroblasts) in the CF model upon co-incubation with MSCs and MSC-derived EVs - continuation

Looking at the cytokine secretion into the apical medium, which represents mostly macrophages, we observed a very drastic reduction of pro-inflammatory M1-type cytokines after treatment with MSCs but an increase after treatment with EVs or the combination. On the other hand, EVs and the combination but not the MSCs alone induced a very drastic increase in the production of anti-inflammatory cytokines of the M2-type like arginase 1, IL-4, TARC, etc. As above, similar results were obtained for the 2D and 3D cultures.

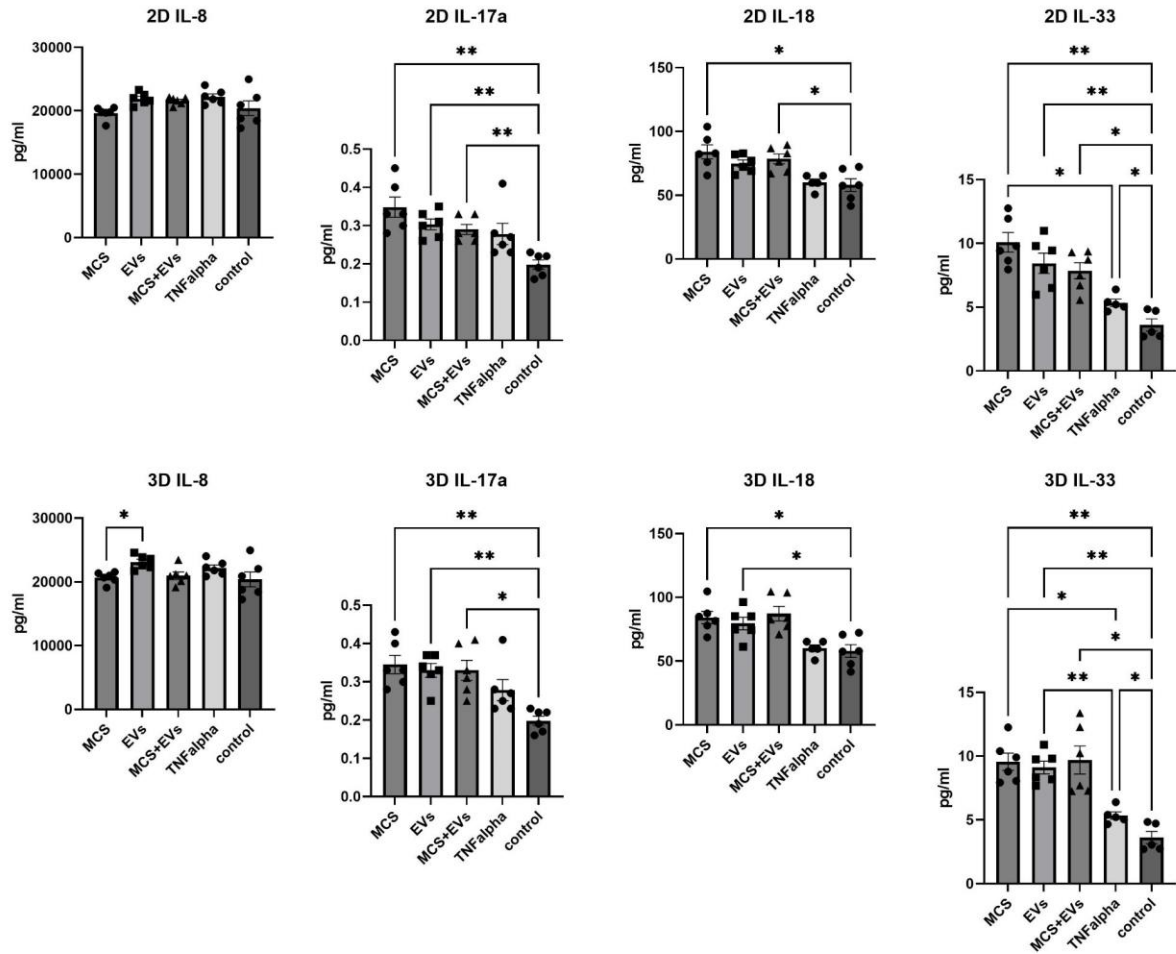


Figure 11: Levels of selected cytokines measured in the basal medium (corresponding to airway epithelial cells and fibroblasts) in the CF model upon co-incubation with MSCs and MSC-derived EVs – continuation

Cytokine levels in the apical medium (alveolar macrophages) in the CF model (only 2D shown)

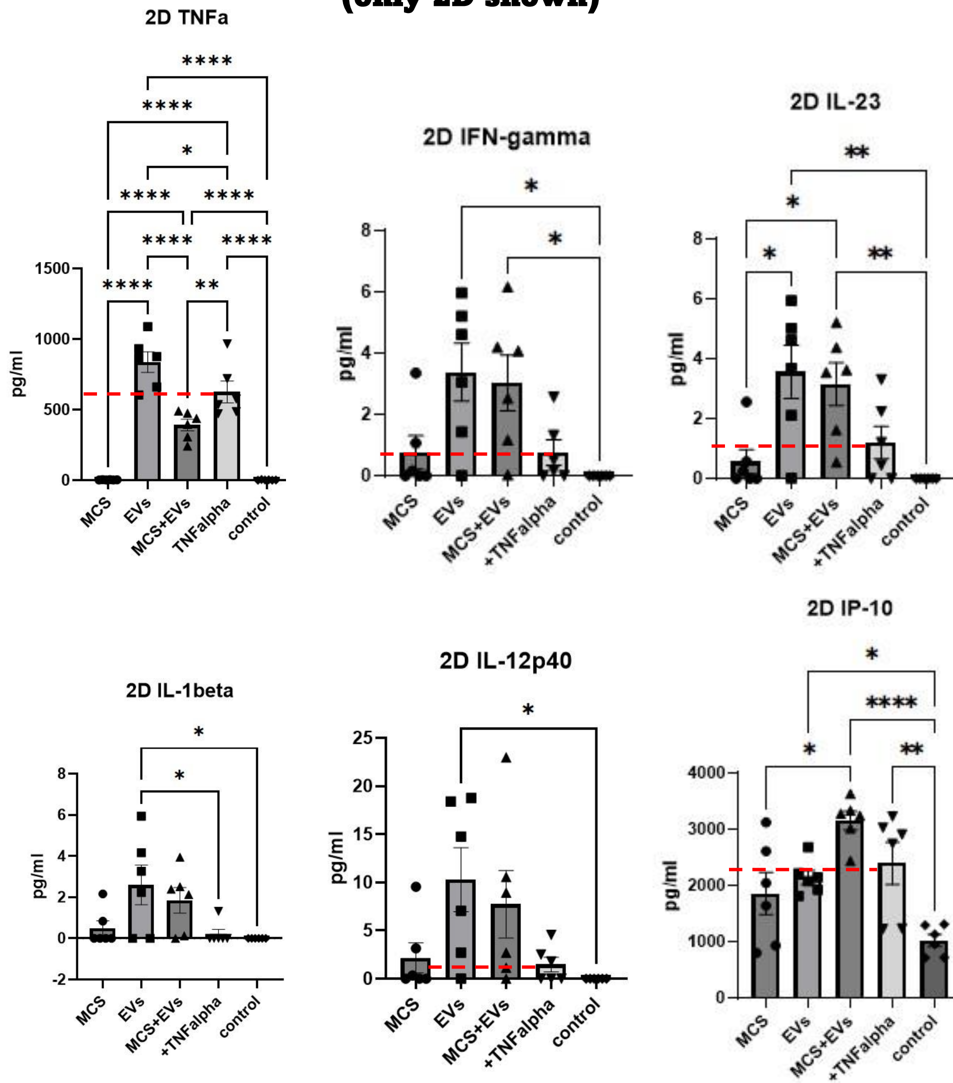


Figure 12: Levels of selected cytokines measured in the apical medium (corresponding to airway macrophages) in the CF model upon co-incubation with MSCs and MSC-derived EVs

Cytokine levels in the apical medium (alveolar macrophages) in the CF model (only 2D shown)

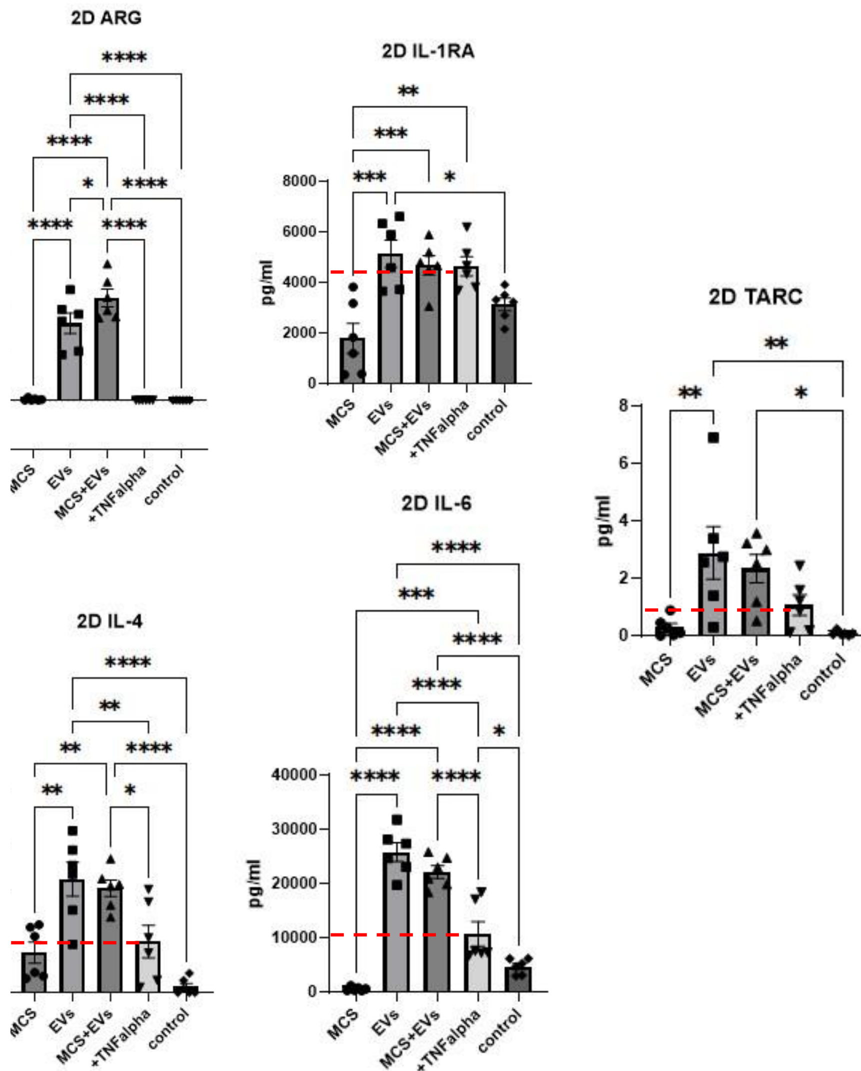


Figure 13: Levels of selected cytokines measured in the apical medium (corresponding to airway macrophages) in the CF model upon co-incubation with MSCs and MSC-derived EVs - continuation

Concerning the ARDS model, we have a contrary situation in the case of the basal medium in comparison to the CF model. Here. the treatment with MSCs alone or the combination increased the levels of pro-inflammatory cytokines, and the treatment with EVs reduced their levels. Again the exemption is the secretion of TNFa, where we have an opposite effect. In the apical medium representing the macrophages we have a strong induction of M2-type cytokines upon treatment with EVs or the combination with the exemption of TNFa, where EVs are up-regulating its secretion.

Cytokine levels in the basal medium (AECs, fibroblasts) in the ARDS model (healthy AECs + LPS) – only 2D samples used

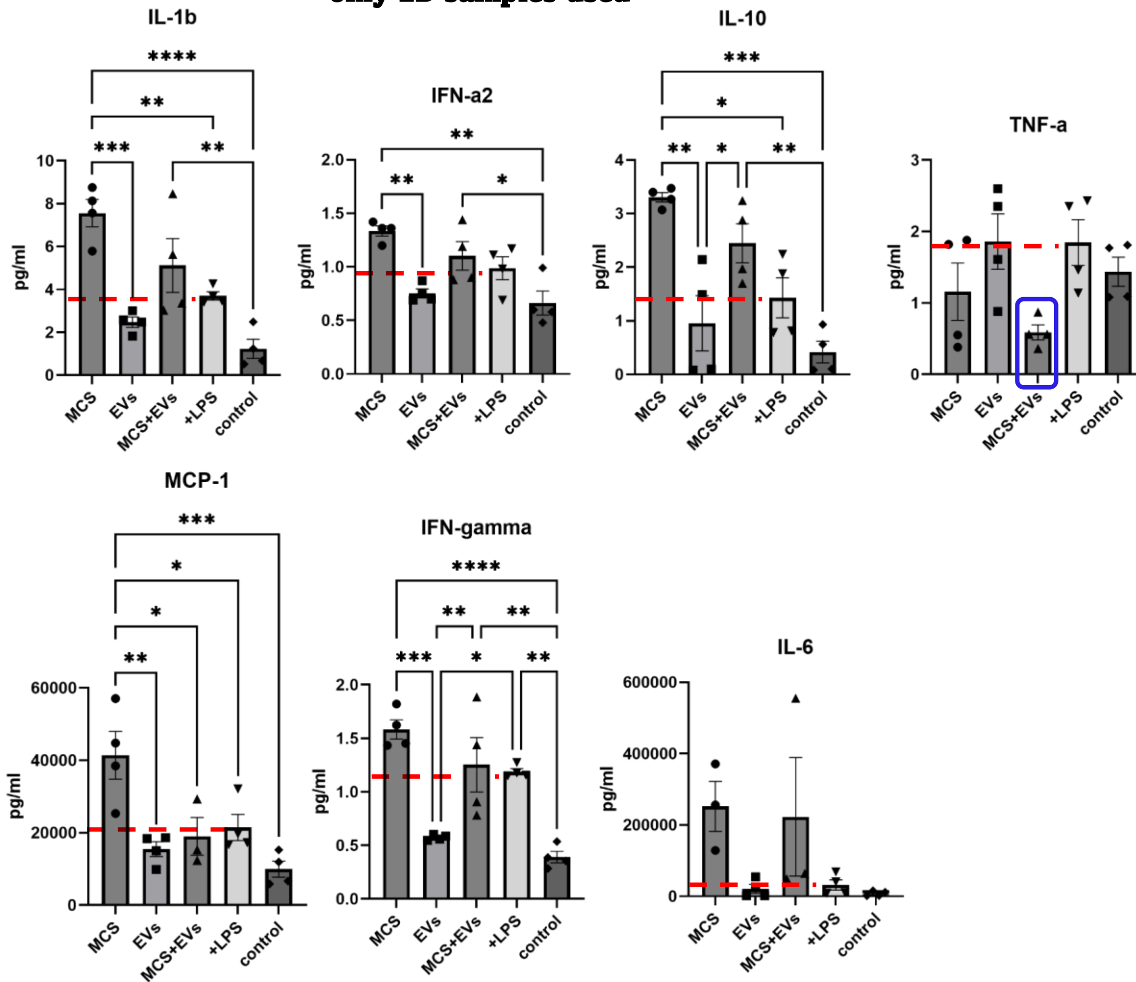


Figure 14: Levels of selected cytokines measured in the apical medium (corresponding to airway macrophages) in the ARDS model upon co-incubation with MSCs and MSC-derived EVs - continuation

Conclusions

In general we observed significant differences between CF and ARDS model as well as between epithelial cells/fibroblasts and macrophages regarding the impact of MSCs and MSC-derived EVs. In many cases we noticed an opposite effect of MSCs and EVs, especially in the case of macrophages. In the CF model EVs and EVs/MSCs induced obviously a polarization switch of macrophages from M1 towards M2, evident by the strong up-regulation of anti-inflammatory, M2-type cytokines, especially of ARG1 (also IL-4, IL-6, TARC). This is in contradiction to the also observed general induction of other pro-inflammatory cytokines. In summary, the results are all in all not conclusive and the experiment has to be repeated. The results for the ARDS model are more conclusive and indicate an anti-inflammatory effect of MSC-derived EVs, and not so much of the MSCs alone, on the epithelial cells. This anti-inflammatory effect is even more pronounced in the case of macrophages, where also the combination of MSC

and EVs shows anti-inflammatory properties. However, on the other hand, an increase of the level of pro-inflammatory TNFa was observed.

Cytokine levels in the apical medium (alveolar macrophages) in the ARDS model (healthy AECs + LPS)

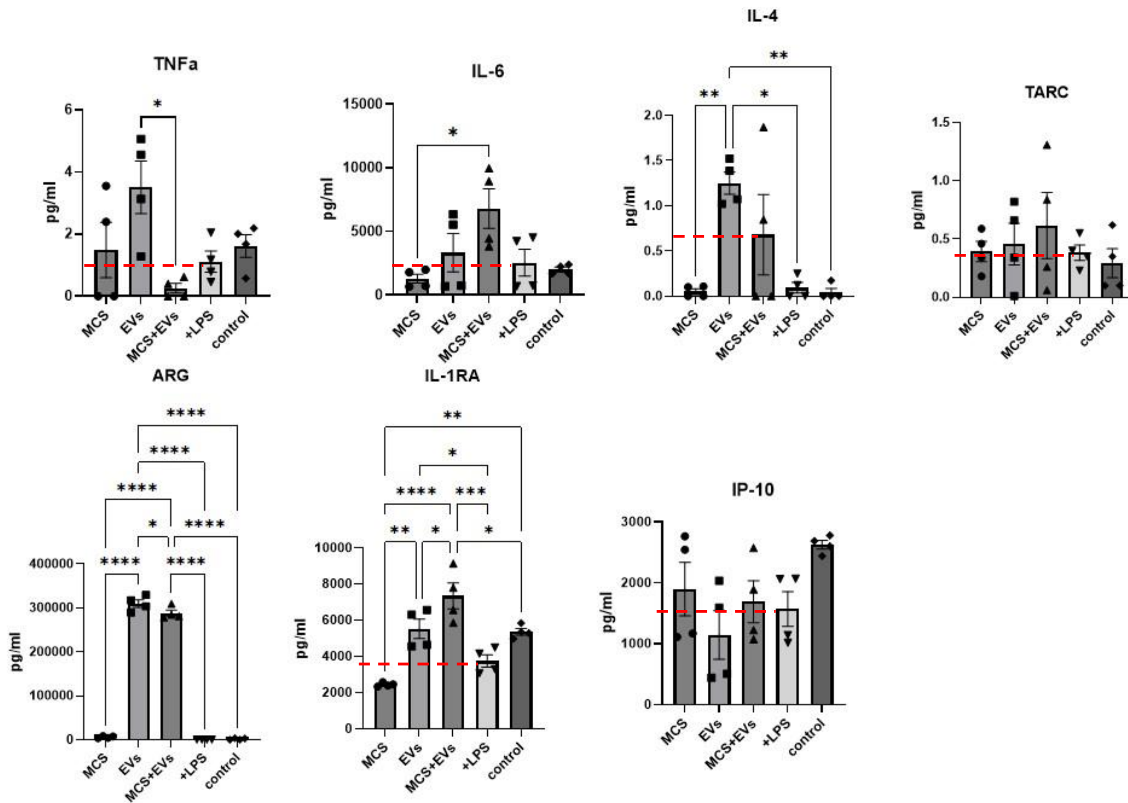


Figure 15: Levels of selected cytokines measured in the apical medium (corresponding to airway macrophages) in the ARDS model upon co-incubation with MSCs and MSC-derived EVs - continuation

Nephron progenitors induction and expansion from iPS cells

During the internships at KU, researchers from WUM, LSMU and KTU learned and performed nephron progenitor induction and differentiation towards kidney organoids according to the protocol established by the NAWA20 Partner, prof. Nishinakamura (optimized protocol from Morizane *et al.*, doi:10.1038/nprot.2016.170). The obtained kidney organoid system will provide a platform for studies of the impact of EVs on nephrotoxicity and kidney regeneration. Furthermore, a nephron progenitor expansion protocol was tested to obtain larger cells numbers as a future source of EVs with potential regenerative properties. Future development towards high-scale culture in 3D bioreactors for EV isolations, analogical to the MSC cell culture, is anticipated.

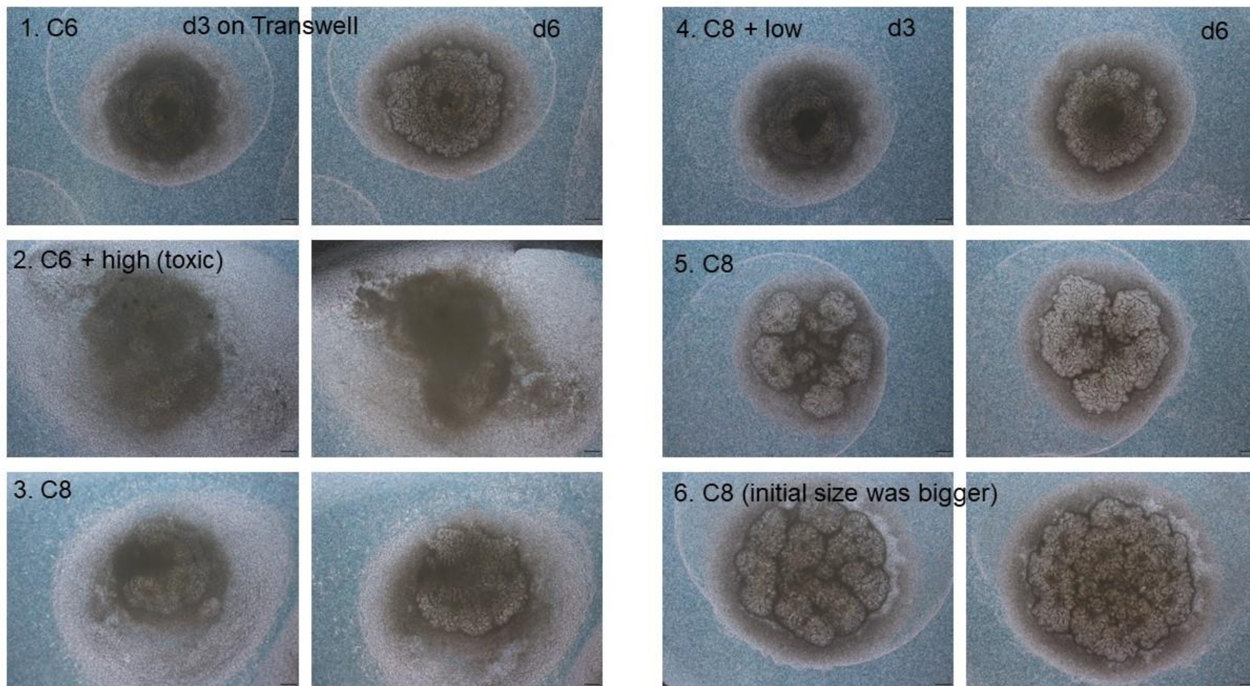


Figure 16: Generation of nephron progenitor cells and kidney organoids from human iPS cells – morphology of kidney organoids after nephron progenitor cell induction and 3D culture-protocol (21 day of culture).Typical nephron structures like tubules are visible.

Achievements regarding joint project applications for European funding:

The main goal of the project was to establish long-term, systemic scientific cooperation in the field of regenerative medicine and cell therapy between WUM and strategic partners from Lithuania: LSMU, KTU and KU in Japan. The result of this cooperation was the creation of a scientific consortium, which applied for 3 projects under Horizon Europe (HE) in 2022-2024:

1. A grant application was submitted by LSMU, KTU, WUM in the EIC Pathfinder Challenges 2022 competition under the acronym ARCTURO (No.101115597) on 19.10.2022 entitled: „Breakthrough platform for pilot scale generation of highly potent gene-engineered biological drug prototype for ischemic stroke treatment". KU could not participate in this project. Unfortunately, the project was rejected.

2. Under the EIC Pathfinder OPEN 2023 competition under the acronym NEXUS (nr.101131038) entitled: Development of Next-Generation Hypo-Immunogenic and Tissue Specific Gene Engineering Platform for Inflammasome Related Diseases. 07.03.2023 grant application was submitted by LSMU, KTU, WUM. Additional partners were Lithuanian companies Kelifarma and CasZyme, Vilnius University, and Aarhus University (Denmark). KU could not take part in this project due to the rules of the competition. On 17.05.23 the reviewers' preliminary positive opinions were received and by 25.05.23 their numerous comments were responded to. Unfortunately, the project was rejected, however it received a Seal of Excellence:

SEAL OF EXCELLENCE

European Commission

European Commission

Horizon Europe

The project proposal

101131038 — NEXUS

"Development of Next-Generation Hypo-Immunogenic and Tissue Specific Gene Engineering Platform for Inflammasome Related Diseases"

submitted under the Horizon Europe EIC call

HORIZON-EIC-2023-PATHFINDEROPEN-01-01 — EIC Pathfinder Open

by

Kelifarma and other participants

following evaluation by an international panel of independent experts

was recognised as a high-quality project proposal in a highly competitive evaluation process

but could not receive funding due to budgetary constraints and is therefore recommended by the European Commission for funding by other sources.

Mariya Gabriel,
Commissioner for Innovation, Research, Culture, Education and Youth

Elisa Ferreira,
Commissioner for Cohesion and Reforms

HORIZON EUROPE

Brussels, 01/08/2023

SEAL OF EXCELLENCE

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HORIZON EUROPE

3. Under the EIC Pathfinder OPEN 2024 competition under the acronym GENOREN (No. 101187825) entitled: Preclinical development of a next-generation cell engineering platform for chronic disease treatment. 07.03.2024. Additional partners include Lithuanian companies Kelifarma and CasZyme, Vilnius University, Vytautas Magnus University in Kaunas, and Aarhus University (Denmark). KU was unable to participate in this project.

The long-term outcome of the project will be, among other things, the creation of an innovative technology for the production of stem cells and functionally active extracellular vesicles under GMP conditions, with potential therapeutic applications.

Activities regarding exchange of knowledge:

Participation in conferences:

- Small New World GSEV/ASEV (28-29.10.2022, Salzburg, Austria) - 3 persons, presentation of 2 posters (from WUM)
- ISEVxTech (16-18.11.2022, Honolulu, USA) - 3 people, presentation of 2 posters (from WUM)
- ISEV 2023 (17.-21.05.2023, Seattle, USA), presentation of 2 posters (from WUM)
- International Society of Stem Cell Research (ISSCR) Annual Meeting (14.17.06, Boston USA). 1 person (from LSMU)

- 5th Congress of Polish Biosciences BIO2023, (13-16.09.2023, Szczecin) - 2 oral presentations (Dr. Małgorzata Czystowska-Kuźmicz, M.Sc. Karolina Soroczyńska, from WUM)
- 1st MOVE Symposium, (23-29.10.2023, Malaga Spain)- 1 oral presentation (Dr. hab. Małgorzata Czystowska-Kuźmicz), 1 poster (Dr. Alicja Głuszko) from WUM
- Advanced Therapies Congress Terrapinn (18-21.03, 2024 London, United Kingdom), 3 persons (from LSMU)
- International Society for Extracellular Vesicles Annual Meeting 2024 ISEV2024 (9-13.05.2024, Melbourne, Australia) – 3 posters (from WUM, 1 person from LSMU
- MakeLearn, TIM&PIConf 2024 (23.-25.05.2024, Lublin, Poland) – 1 oral presentation (from KTU)
- IEEE Conference on Artificial Intelligence - IEEE CAI 2024 (23.-28.06.2024), Singapore) – 1 person (from KTU)

Internships:

The project included internships at partner units where knowledge was exchanged on specific specialties and knowledge gained at conferences and workshops. Training topics included:

Researchers from LSMU and KTU trained at WUM:
14.-16.02.2024 – 3 persons from LSMU, 16.-18.06.2024 – 2 persons from LSMU, 1 person from KTU
Topics:
- methods for isolation and characterization of extracellular vesicles from stem cell cultures
- in vitro functional testing of extracellular vesicles from stem cell cultures
- in vivo testing of the regenerative potential of stem cells and their secretome (extracellular vesicles)

Researchers from WUM trained LSMU and KTU
18.-20.10.2023 and 22.-27.06.2024 – 1 person from WUM
Topics:
- Methods for culture and differentiation of adult stem cells in 2D and 3D cultures in GMP system
- Methods for filtration and concentration of extracellular vesicles by Tangential Flow Filtration (TFF) method
- Biotechnology process and continuous culture monitoring in practice

Researchers from WUM, LSMU and KTU trained at KU
19.02-01.03.2024 – 1 person from LSMU, 17.02-03.03 – 1 person fom KTU, 04.-21.04.2024 – 2 persons from WUM
Topics:
- Advanced techniques used in regenerative medicine: iPSC culture and differentiation
- renal organoid culture

- Trainings and Workshops:

- Hands-on training 2022 (03-04.11.2022, Geneva, Switzerland): 3D cell culture for functional assays - 1 person (from WUM)
- EMBL Course: Whole Transcriptome data analysis (26-31.05.2024, Heidelberg, Germany) – 1 person from WUM

Organizing of a international conference

An international conference "Clinical Application of Stem Cells in Kidney Transplantation and Nephrology" was prepared and held on 19.10.2023 at the Radisson Hotel in Kaunas.
There was a series of lectures on regenerative medicine and clinical application of stem cells and their secretion of extracellular vesicles and also on nephrology. Participants included a partner from Japan (Prof. Ryuichi Nishinakamura), partners from Lithuania (Prof. Romaldas Mačiulaitis, Dr. Justinas Mačiulaitis and their research

teams) and a representative from WUM (Magdalena Długołęcka, M.D.). International speakers included Prof. Petra Reinke (Berlin, Germany), and Prof. Paul Harden (Oxford, UK).

LITHUANIAN UNIVERSITY OF HEALTH SCIENCES · KAUNO KLINIKOS

19 OCTOBER 2023

Clinical Application of Stem Cells in Kidney Transplantation and Nephrology

Time	Programme
10:00 – 11:00	Registration of conference
11:00 – 11:15	Opening speech
11:15 – 15:00	PART I Moderators: **prof. Romaldas Mačiulaitis, prof. Ryuichi Nishinakamura**
11:15 – 11:45	Challenges translating promissing animal cell therapy results to humans **Prof. Romaldas Mačiulaitis**, Kaunas, Lithuania
11:45 – 11:50	Questions / Answers
11:50 – 12:30	Towards an optimized process of clinical cell therapy manufacturing **Prof. Petra Reinke**, Berlin, Germany
12:30 – 12:35	Questions / Answers
12:35 – 13:05	Coffee break
13:05 – 13:40	Round table discussion with audience "My challenges and lessons learned" Panelists and quality experts
13:40 – 14:20	Reconstructing kidney from human IPS cells **Prof. Ryuichi Nishinakamura**, Kumamoto, Japan
14:20 – 14:25	Questions / Answers
14:25 – 14:55	Perinatal stem cell efficacy in the prevention of acute kidney injury. Preclinical model **PhD student Agnė Gryguc**, Kaunas, Lithuania
14:55 – 15:00	Questions / Answers
15:00 – 16:00	Lunch break
16:00 – 17:50	PART II Moderators: **prof. Inga Arūnė Bumblytė, prof. Paul Harden**
16:00 – 16:30	Unmet needs in nephrology and transplantology **Prof. Inga Arūnė Bumblytė**, Kaunas, Lithuania
16:30 – 16:35	Questions / Answers
16:35 – 17:05	Experience of applying mesenchymal stem cell therapy in kidney injury **Dr. Justinas Mačiulaitis, PhD student Rūta Insodaitė**, Kaunas, Lithuania
17:05 – 17:10	Questions / Answers
17:10 – 17:40	Regulatory cell therapy in kidney transplantation **Prof. Paul Harden**, Oxford, United Kingdom
17:40 – 17:45	Questions / Answers
17:45 – 17:55	Best poster presentation **Paulius Valiukevičius**, Kaunas, Lithuania
17:55 – 18:00	End of conference and summary

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During the conference, the Partner from Japan (Prof. Ryuichi Nishinakamura), partners from Lithuania (Prof. Romaldas Mačiulaitis, Dr. Justinas Mačiulaitis and others) and a representative from WUM (Magdalena Długołęcka, M.Sc.) held a series of meetings on progress in cooperation and further plans on 18-20.10.2023.