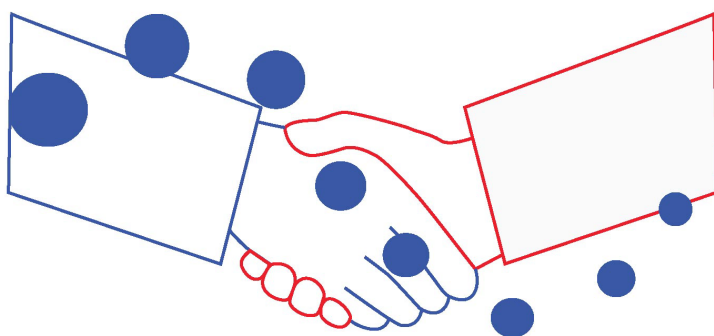


Knjiga apstrakata

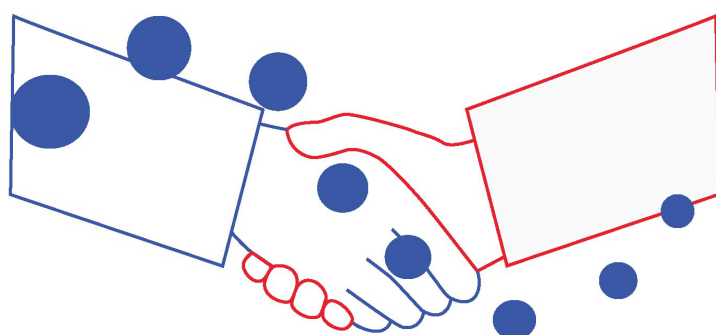
Naučni skup
Srpskog društva za ekstracelularne vezikule, SrbEV



27-28.10.2026. | Beograd, Republika Srbija

Abstract book

Scientific meeting of
Serbian Society for Extracellular Vesicles, SrbEVs



27-28.10.2026. | Belgrade, Republic of Serbia



Naučni skup Srpskog društva za ekstracelularne vezikule, SrbEV
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Naučni skup Srpskog društva za ekstracelularne vezikule, SrbEV
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Naučni skup Srpskog društva za ekstracelularne vezikule, SrbEV
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Zadovoljstvo mi je da, u ime Organizacionog odbora, predstavim Knjigu apstrakata Naučnog skupa Srpskog društva za ekstracelularne vezikule, SrbEVs2026.

Istraživanje ekstracelularnih vezikula (EV) danas je jedno od najbrže rastućih polja u biologiji i biomedicini. Taj tempo očit je i u Srbiji: sve je više grupa koje se bave ovim istraživanjima, širi se spektar tema a raste i broj istraživača koje ovo polje zanima. Srpsko društvo za ekstracelularne vezikule okuplja istraživače koji se ovom oblašću bave u zemlji i čini zajednički prostor za njihovo upoznavanje, povezivanje, razmenu znanja i uspostavljanje saradnji.

Četiri godine od osnivanja Društva i prvog nacionalnog skupa, SrbEVs2022, bile su za našu zajednicu vreme rasta članstva, umrežavanja, realizacije ciljeva i značajnog povećanja vidljivosti, kako samog Društva tako i njegovih članova. Kroz obuku mlađih istraživača u okviru Prve SrbEVs škole EV, podršku njihovom usavršavanju i ko-organizaciju međunarodnih skupova, među kojima se izdvajaju regionalni skup Small New World 2.0 u Gracu 2023. i 2nd MOVE Symposium u Beogradu 2024, Društvo je radilo na unapređenju istraživanja EV u Srbiji. Članovi su, uporedo s tim, svoje rezultate iznosili na brojnim međunarodnim i domaćim naučnim skupovima.

Sada je vreme da na jednom mestu sagledamo ta istraživanja: koja su pitanja postavljana, koji modeli i tehnike su korišćeni, koje su perspektive otvorene i gde su tačke preseka iz kojih mogu poticati novi pravci i zajednički projekti. Apstrakti sabrani u ovoj knjizi proizilaze iz sve četiri oblasti istraživanja EV: iz fundamentalnih istraživanja uloge i funkcije EV, iz ispitivanja njihovog biomarkerskog i terapijskog potencijala kao i iz razvoja novih metoda u ovom polju.

Nadam se da će ova Knjiga apstrakata ostati svedočanstvo jedne stepenice u razvoju EV polja u Srbiji i da će kolegama u zemlji i inostranstvu poslužiti da se upoznaju sa radom članova SrbEVs.

Velika nam je čast da ovom prilikom ugostimo kolege iz inostranstva i da sa njima uživo podelimo iskustva. Pre svega zahvaljujemo prof. dr Berndu Giebelu, svetski poznatom istraživaču EV, što je pristao da održi plenarno predavanje. Sa zadovoljstvom ćemo čuti i iskustva kolega iz Slovenije i Slovačke, sa kojima naši članovi već sarađuju.

Na kraju zahvaljujem svima koji su pomogli organizaciju skupa: ko-organizatorima, Institutu za primenu nuklearne energije (INEP) i Institutu za molekularnu genetiku i genetičko inženjerstvo (IMGGI); sponzorima, ne samo na finansijskoj pomoći već i na uvidu u spektar alata koji omogućavaju kvalitetnija istraživanja EV; autorima na priložima, jer razvoj svakog polja počiva na entuzijazmu istraživača; članovima Naučnog i Organizacionog odbora na uloženom vremenu i energiji, kao i svim učesnicima i gostima na tome što su skup učinili susretom koji ćemo pamtiti.

Beograd, oktobar 2026.

dr Maja Kosanović

Predsednik Srpskog društva za ekstracelularne vezikule



It is my pleasure to present, on behalf of the Organizing Committee, the Book of Abstracts of the Scientific Meeting of the Serbian Society for Extracellular Vesicles, SrbEVs2026.

Extracellular vesicle (EV) research is today one of the fastest-growing fields in biology and biomedicine. That pace is evident in Serbia as well: there is an increasing number of groups entering this field of research, the range of topics is widening, and so is the number of researchers drawn to the field. The Serbian Society for Extracellular Vesicles brings together researchers working in this area in the country and provides a common space for them to meet, connect, exchange knowledge and establish collaborations.

The four years since the founding of the Society and the first national meeting, SrbEVs2022, have been a time of growing membership, networking, the pursuit of our aims and a marked increase in visibility, both of the Society itself and of its members. Through the training of early-career researchers at the First SrbEVs EV School, support for their professional development, and the co-organization of international meetings, notably the regional meeting Small New World 2.0 in Graz in 2023 and the 2nd MOVE Symposium in Belgrade in 2024, the Society has worked to advance EV research in Serbia. In parallel, members have presented their results at numerous international and national scientific meetings.

The time has now come to offer an overview of that research in one place: which research questions have been asked, which models and techniques have been used, which perspectives have been opened, and where lines of research meet in ways that could spark new directions and joint projects. The abstracts collected in this book emerge from all four areas of EV research: fundamental studies of the roles and functions of EVs, investigation of their biomarker and therapeutic potential, and the development of new methods in the field.

I hope that this Book of Abstracts will remain a record of the current state of the EV field in Serbia, and that it will help colleagues at home and abroad become acquainted with the work of SrbEVs members.

It is a great honour for us to host colleagues from abroad on this occasion and to share experience with them in person. Above all, we thank Prof. Bernd Giebel, a world-renowned EV researcher, for agreeing to give the plenary lecture. We also look forward to hearing from colleagues from Slovenia and Slovakia, with whom our members already collaborate.

Finally, I wish to thank everyone who helped organize this meeting: the co-organizers, the Institute for the Application of Nuclear Energy (INEP) and the Institute of Molecular Genetics and Genetic Engineering (IMGGI); the sponsors, not only for their financial support but also for the insight they offer into the range of tools that make higher-quality EV research possible; the authors for their contributions, since the development of any field rests on the enthusiasm of its researchers; the members of the Scientific and Organizing Committees for the time and energy they have invested and all participants and guests for making this meeting an encounter we will remember.

Belgrade, October 2026.

Dr Maja Kosanović

President of Serbian Society for Extracellular Vesicles

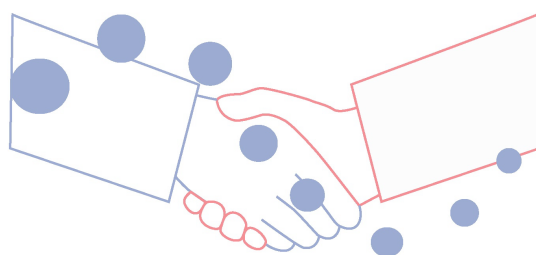
Program 27.10.2026.

9:00 -	Registracija / Registration	
09:30 - 09:45	Uvodna reč / Opening remarks	
09:45 - 10:00	Aleš Iglič	On the role of curved proteins and cytoskeleton forces in encapsulation of nanoparticles and vesicles
10:00 - 10:15	Veronika Kralj-Iglič	Curvature patch model - a statistical mechanical upgradation of the fluid mosaic model for anisotropic membranous nanostructures
10:15 - 10:30	Tamara Janković	Enzimi na membrani ekstracelularnih vezikula u kontekstu makroenzima
10:30 - 10:45	Aleksandra Korać	Komunikacija posredovana ekstracelularnim vezikulama tokom progresije tumora dojke
10:45 - 11:00	Ivan Jovanović	MikroRNK u ekstracelularnim vezikulama kod oboljenja CNS-a: od molekularnog profila do biološke funkcije
11:00 - 11:30	Kafe pauza / Coffee break	
11:30 - 12:15	Plenarno predavanje Bernd Giebel	Clinical potential of MSC-EVs and translational challenges
12:15 - 13:30	Ručak / Lunch	
13:30 - 13:45	Dragana Nikolić	Bakterijske ekstracelularne vezikule u komunikaciji između biljaka i korisnih rizosfernih i endofitnih bakterija
13:45 - 14:00	Nina Petrović	Ekstracelularne vezikule kao prediktori toksičnosti radioterapije: od molekularnih mehanizama do kliničke prakse
14:00 - 14:15	Miodrag Vuković	Poređenje metoda za izolaciju vezikula iz malignog pleuralnog izliva kod pacijenata sa NSCLC
14:15 - 14:30	Particle Metrix Bertrand Damart	ZetaView Evolution - NTA beyond size and concentration
14:30 - 14:45	Kafe pauza / Coffee break	
14:45 - 15:00	Sanja Goč	Heterogenost ekstracelularnih vezikula u malignitetima digestivnog trakta: analiza distribucije veličina i površinske glikozilacije
15:00 - 15:15	Jaroslav Katrlík	Glycosylation profiling of extracellular vesicles by lectin-based microarray and SPR
15:15 - 15:30	Jelena Janković Miljuš	Tiroidea-specifični biomarkeri u ekstracelularnim vezikulama: detekcija tiroglobulina i tirotropinskog receptora kao pomoć u monitoringu rekurentnog tiroidnog karcinoma

Program 28.10.2026.

12:00 - 13:00	Ručak / Lunch	
13:00 - 13:15	Milan Stefanović	Ispitivanje imunomodulatornih efekata izloženosti kondenzatu aerosola e-cigareta posredovanih EV in vitro i in vivo
13:15 - 13:30	Marko Prokić	Salivarne vanćelijske vezikule kao potencijalni biomarkeri u reumatoidnom artritisu
13:30 - 13:45	Maja Kosanović	Parazitske ekstracelularne vezikule kao prirodni imunomodulatori: mehanizmi delovanja i terapijski potencijal u respiratornim alergijama
13:45 - 14:00	Vicor / HansaBioMed Danilo Mladenović	At the frontiers of extracellular vesicle research
14:00 - 14:30	Kafe pauza / Coffee break	
14:30 - 14:45	Mihajlo Radmilović	Molecular and morphological characterization of extracellular vesicles from plasma using super-resolution microscopy
14:45 - 15:00	Vesna Spasovski	Ekstracelularne vezikule u osteoartritisu: terapija nove generacije bez primene ćelija
15:00 - 15:15	Marija Milivojević	Kinetika oslobađanja ADSC-EVs iz hibridnog PMAA/PEGDA/želatin/kolagen sistema
15:15 - 15:30	Miloš Lazarević	Kombinovanje probiotika i egzozoma poreklom iz matičnih ćelija u cilju poboljšanja regeneracije kosti
15:30 - 15:45	Pozdravna reč / Closing remarks	

Plenarno predavanje | Plenary lecture



Klinički potencijal MSC-EV i translacioni izazovi

Bernd Giebel

Institut za transfuzionu medicinu, Univerzitetska bolnica Essen, Univerzitet Duisburg-Essen

Ekstracelularne vezikule poreklom iz humanih mezenhimalnih stromalnih ćelija (MSC-EV) mogu da reprogramiraju proinflamatorne imunski odgovore ka regulatornim fenotipovima. Međutim, kliničku translaciju usporava funkcionalna varijabilnost između preparata - problem koji potiče od heterogenosti matičnih MSC. Prevazilaženje ove neujednačenosti zahteva i standardizovanu proizvodnju i napredne okvire kontrole kvaliteta (QC).

Kako bismo rešili varijabilnost između serija, uspostavili smo klonske linije MSC na nivou pojedinačne ćelije primenom strategije imortalizacije. EV dobijene iz ovih klonski ekspanovanih imortalizovanih MSC (ciMSC) zadržavaju pun imunomodulatorni potencijal i ispoljavaju terapijsku efikasnost *in vivo*. Radi rigorozne karakterizacije, konvencionalnu kontrolu kvaliteta dopunjujemo profilisanjem pojedinačnih EV pomoću protočne citometrije sa snimanjem (imaging flow cytometry).

Clinical Potential of MSC-EVs and Translational Challenges

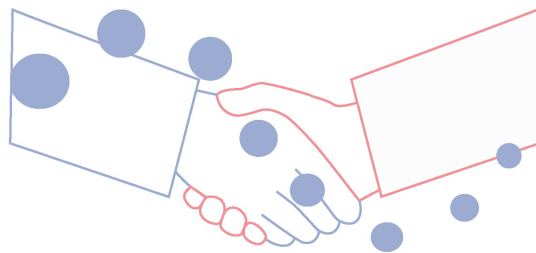
Bernd Giebel

Institute for Transfusion Medicine, University Hospital Essen, University Duisburg-Essen

Human mesenchymal stromal cell-derived extracellular vesicles (MSC-EVs) can reprogram pro-inflammatory immune responses toward regulatory phenotypes. However, clinical translation is bottlenecked by functional variability across preparations - a challenge inherited from parent MSC heterogeneity. Overcoming this inconsistency requires both standardized manufacturing and advanced quality control (QC) frameworks.

To resolve batch-to-batch variability, we established single-cell clonal MSC lines using an immortalization strategy. EVs derived from these clonally expanded immortalized MSCs (ciMSCs) retain full immunomodulatory potency and confer *in vivo* therapeutic efficacy. To ensure rigorous characterization, we complement conventional QC with single-EV profiling via imaging flow cytometry.

Usmene prezentacije | Oral presentations



Fundamentalna istraživanja EV

Fundamental EV research

Uloga zakrivljenih proteina i sila citoskeleta u enkapsulaciji nanočestica i vezikula

Aleš Iglič¹, Veronika Kralj-Iglič²

¹Univerzitet u Ljubljani, Fakultet elektrotehnike, Laboratorija za fiziku, Ljubljana, Slovenija (ales.iglic@fe.uni-lj.si);

²Univerzitet u Ljubljani, Fakultet zdravstvenih nauka, Laboratorija za kliničku biofiziku, Ljubljana, Slovenija (veronika.kralj-iglic@zf.uni-lj.si)

Enkapsulacija nanočestica i vezikula biološkom membranom predstavlja važnu fazu njihovog prenosa preko membrane. U ovom radu primenjene su Monte Carlo (MC) simulacije kako bi se ispitala sposobnost membrane da enkapsulira nanočestice i vezikule. Naš teorijski model opisuje vodeću ivicu membrane koju formiraju zakrivljeni proteini vezani za membranu, koji regrutuju protruzivne sile polimerizacije aktina, i identifikuje ulogu energija savijanja i adhezije. Između ostalog, utvrdili smo da je nesferične nanočestice teže enkapsulirati nego sferične. Kod nesferičnih nanočestica vreme enkapsulacije zavisi od početne orijentacije nanočestice. Predloženi mehanizam spontane samoorganizacije aktinskog citoskeleta na mestu invaginacije radi enkapsulacije u dobroj je saglasnosti sa nedavnim zapažanjima dobijenim eksperimentalnim tehnikama visoke rezolucije. Pošto je model opšti i uzima u obzir različitu krutost mete, može se primeniti na brojne tipove ćelija i raznovrsne mete, uključujući krute nanočestice i meke vezikule.

1. Sadhu, Barger, Penič, Iglič, Krendel, Gauthiere, Gov: A theoretical model of efficient phagocytosis driven by curved membrane proteins and active cytoskeleton forces, *Soft Matter*, 19, 31-43, 2023;

2. Mesarec, Gózdź, Kralj-Iglič, Kralj, Iglič, Coupling of nematic in-plane orientational ordering and equilibrium shapes of closed flexible nematic shells, *Scientific Reports*, 13:10663, 2023;

3. Sadhu, Luciano, Xi, Martinez-Torres, Schröder, Blum, Tarantola, Villa, Penič, Iglič, Beta, Steinbock, Bodenschatz, Ladoux, Gabriele, Gov, A minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge, *PNAS*, 121 (12): e2306818121, 2024;

4. Sadhu, Hernandez-Padilla, Eisenbach, Penič, Zhang, Vishwasrao, Behkam, Konstantopoulos, Shroff, Iglič, Peles, Nain, Gov, Experimental and theoretical model for the origin of coiling of cellular protrusions around fibers, *Nature Comm.*, 14: 5612, 2023.

5. Sadhukhan, Cornell, Sandhu, Peeters, Penič, Iglič, Fletcher, Jaumouille, Vorselen, Gov: Curved proteins drive both passive and actin-driven biting, pushing and engulfment of soft objects (in preparation).

Zahvalnica: zahvaljujemo se Slovenskoj agenciji za istraživanje i inovacije (ARIS) na grantovima P2-0232, P3-0388 and J3-60063.

On the role of curved proteins and cytoskeleton forces in encapsulation of nanoparticles and vesicles

Aleš Iglič¹, Veronika Kralj-Iglič²

¹University of Ljubljana, Faculty of Electrical Engineering, Laboratory of Physics, Ljubljana, Slovenia (ales.iglic@fe.uni-lj.si); ²University of Ljubljana, Faculty of Health Sciences, Laboratory of Clinical Biophysics, Ljubljana, Slovenia (veronika.kralj-iglic@zf.uni-lj.si)

Encapsulation of nanoparticles and vesicles by biological membrane is an important stage of their transfer across the membrane. In this work MC simulations are employed to investigate the ability of the membrane to encapsulate nanoparticles and vesicles. Our theoretical model describes the membrane leading-edge that are produced by curved membrane-attached proteins that recruit the protrusive forces of actin polymerization, and identifies the role of bending and adhesion energies. Among others we found that non-spherical nanoparticles are more difficult to encapsulate than spherical ones. For non-spherical nanoparticles encapsulation time depends on the initial orientation of the nanoparticles. The proposed mechanism for the spontaneous self-organization of the actin cytoskeleton at the encapsulation cup is in good agreement with recent high-resolution experimental observations. As our model is general and takes into account different target stiffness it may therefore be applied to many cell types and many types of the target, including rigid nanoparticles and soft vesicles.

1. Sadhu, Barger, Penič, Iglič, Krendel, Gauthiere, Gov: A theoretical model of efficient phagocytosis driven by curved membrane proteins and active cytoskeleton forces, *Soft Matter*, 19, 31-43, 2023;

2. Mesarec, Gózdź, Kralj-Iglič, Kralj, Iglič, Coupling of nematic in-plane orientational ordering and equilibrium shapes of closed flexible nematic shells, *Scientific Reports*, 13:10663, 2023;

3. Sadhu, Luciano, Xi, Martinez-Torres, Schröder, Blum, Tarantola, Villa, Penič, Iglič, Beta, Steinbock, Bodenschatz, Ladoux, Gabriele, Gov, A minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge, *PNAS*, 121 (12): e2306818121, 2024;

4. Sadhu, Hernandez-Padilla, Eisenbach, Penič, Zhang, Vishwasrao, Behkam, Konstantopoulos, Shroff, Iglič, Peles, Nain, Gov, Experimental and theoretical model for the origin of coiling of cellular protrusions around fibers, *Nature Comm.*, 14: 5612, 2023.

5. Sadhukhan, Cornell, Sandhu, Peeters, Penič, Iglič, Fletcher, Jaumouille, Vorselen, Gov: Curved proteins drive both passive and actin-driven biting, pushing and engulfment of soft objects (in preparation).

Acknowledgements: we thank the Slovenian Research and Innovation Agency (ARIS) for grants P2-0232, P3-0388 and J3-60063.

Enzimi na membrani ekstracelularnih vezikula u kontekstu makroenzima

Tamara Janković, Ninoslav Mitić, Sanja Goč, Filip Janjić, Miroslava Janković

Institut za primenu nuklearne energije, INEP, Univerzitet u Beogradu, Beograd, Srbija

Membrana ekstracelularnih vezikula (EV) predstavlja organizovan lipidni dvosloj čiji molekularni sastav odražava procese biogeneze i poreklo EV. Ona je izuzetno bogata enzimima koji zadržavaju svoju katalitičku aktivnost i uglavnom su orijentisani ka ekstracelularnom prostoru. Na osnovu lokalizacije, enzimi EV mogu biti integralni i periferni membranski proteini, ili proteini koji su usidreni preko GPI (glikozilfosfatidilinozitolskog) sidra. Na membrani EV je opisano prisustvo različitih klasa enzima: peptidaza, proteinaza i nukleotidaza, a neki od njih (poput 5'-nukleotidaze) su predloženi za markere specifičnih populacija EV. Pored njih, na membrani EV su prisutni i enzimi koji se rutinski određuju u kliničkoj biohemiji: gama-glutamil transferaza, alkalna fosfataza i kisela fosfataza. Enzimi na membrani EV ispoljavaju analitičke karakteristike makroenzima tipa 2. Klasični makroenzimi predstavljaju kompleks enzima sa imunoglobulinima (makroenzim tipa 1) ili sa lipoproteinima i fragmentima ćelijskih membrana (makroenzim tipa 2). Ove enzimске forme velike molekulske mase, prisutne su u serumu u fiziološkim i patofiziološkim stanjima, i iz seruma se izdvajaju metodama taloženja ili elektroforezom i detektuju merenjem enzimске aktivnosti. Enzimi na membrani EV su, takođe, prisutni u frakciji velike molekulske mase, što je od značaja za njihovo određivanje u kompleksnim biološkim uzorcima, jer tradicionalno merenje ukupne enzimске aktivnosti ne daje informacije o molekularnoj formi u kojoj je enzim prisutan. Zato je za njihovu detekciju potrebno razviti nove eseje koji povezuju enzimsku aktivnost sa karakteristikama EV populacije. Njihova primena bi uvela novi pravac u tačnoj biopsiji, u kojoj su EV već prepoznate kao analiti koji omogućavaju istovremeno određivanje različitih komponenti EV (proteine, nukleinske kiseline, lipide i metabolite). Na taj način bi se povećao biomarkerski potencijal rutinski određivanih enzima, pre svega u smislu diferencijalne dijagnostike patofizioloških stanja.

Zahvalnica: Ovaj rad je podržan od strane Ministarstva nauke, tehnološkog razvoja i inovacija Republike Srbije (ugovor br. 451-03-33/2026-03/200019). Istraživanje je u skladu sa Agendom 2030 – ciljem održivog razvoja Ujedinjenih nacija 3, koji promoviše dobro zdravlje i blagostanje.

Enzymes on the membrane of extracellular vesicles in the context of macroenzymes

Tamara Janković, Ninoslav Mitić, Sanja Goč, Filip Janjić, Miroslava Janković

Institute for the Application of Nuclear Energy, INEP, University of Belgrade, Belgrade, Serbia

The membrane of extracellular vesicles (EVs) is an organized lipid bilayer whose molecular composition reflects the processes of EV biogenesis and origin. It is particularly rich in enzymes that retain their catalytic activity and are predominantly oriented toward the extracellular space. Based on their localization, EVs-associated enzymes can be integral and peripheral membrane proteins, or proteins anchored through a glycosylphosphatidylinositol (GPI) anchor. The presence of different classes of enzymes has been described on the EVs membrane, including peptidases, proteinases, and nucleotidases, some of which, such as 5'-nucleotidase, have been proposed as markers of specific EV populations. In addition, the EVs membrane contains enzymes routinely measured in clinical biochemistry, including gamma-glutamyl transferase, alkaline phosphatase, and acid phosphatase. Enzymes associated with the EVs membrane exhibit analytical characteristics of type 2 macroenzymes. Classical macroenzymes are enzyme complexes formed with immunoglobulins (type 1 macroenzymes) or with lipoproteins and cell membrane fragments (type 2 macroenzymes). These high-molecular-mass enzyme forms are present in serum under both physiological and pathophysiological conditions and can be isolated from serum by precipitation methods or electrophoresis and detected by measuring enzymatic activity. EVs-associated enzymes are, likewise, present in the high-molecular-mass fraction, which is important for their determination in complex biological samples, since conventional measurement of total enzymatic activity does not provide information about the molecular form in which the enzyme is present. Therefore, their detection requires the development of new assays that link enzymatic activity with the characteristics of EVs populations. Their application could introduce a new direction in liquid biopsy, in which EVs are already recognized as analytes that enable the simultaneous determination of different EVs components, including proteins, nucleic acids, lipids, and metabolites. In this way, the biomarker potential of routinely measured enzymes could be increased, particularly in the context of the differential diagnosis of pathophysiological conditions.

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MikroRNK u ekstracelularnim vezikulama kod oboljenja CNS-a: od molekularnog profila do biološke funkcije

Ivan Jovanović, Aleksandra Stanković, Maja Živković

Laboratorija za radiobiologiju i molekularnu genetiku, Institut za nuklearne nauke Vinča, Institut on nacionalnog značaja za Republiku Srbiju, Univerzitet u Beogradu

Ekstracelularne vezikule (EV) predstavljaju važan oblik međucelijske komunikacije i potencijalni izvor molekularnih markera patoloških procesa. Njihova sposobnost prenosa regulatornih molekula, uključujući mikroRNK (miRNK), od posebnog je naučnog interesa u istraživanju oboljenja centralnog nervnog sistema (CNS), kod kojih je neposredan pristup obolelom tkivu ograničen. Međutim, izolacija, karakterizacija i molekularna analiza EV predstavljaju metodološki zahtevnu oblast, u kojoj pouzdana interpretacija rezultata u velikoj meri zavisi od pravilne primene različitih eksperimentalnih i analitičkih pristupa.

U istraživanjima koja ćemo predstaviti analizirali smo miRNK povezane sa ekstracelularnim vezikulama kod pacijenata sa glioblastomom i multiplom sklerozom, kao dva biološki različita modela oboljenja CNS-a. EV iz periferne krvi okarakterisane su kombinacijom morfoloških, biofizičkih i molekularnih metoda, dok su za određivanje nivoa miRNK primenjeni sekvenciranje malih RNK i ciljana kvantifikacija odabranih miRNK kandidata metodom qPCR.

Kod pacijenata sa glioblastomom rezultati ukazuju na povezanost nivoa određenih miRNK u cirkulišućim EV sa specifičnim obrascima genske ekspresije u mononuklearnim ćelijama periferne krvi. Uočene promene upućuju na složenu ravnotežu proinflamatornih i antiinflamatornih signala i mogu biti povezane sa ranim procesima sistemske imunomodulacije u glioblastomu. U multiploj sklerozi miRNK su analizirane u mononuklearnim ćelijama periferne krvi i vezikularnom kompartmentu kod pacijenata sa različitim kliničkim tokom bolesti. Integrativnim bioinformatičkim analizama odabrani su regulatorni miRNK kandidati, čiji je funkcionalni značaj dalje ispitivan eksperimentalnom promenom nivoa miRNK u kulturi ćelija i analizom odgovora ciljnih gena povezanih sa ferroptozom.

Prikazani rezultati pokazuju da analiza miRNK u ekstracelularnim vezikulama može pružiti informacije koje prevazilaze identifikaciju pojedinačnih molekularnih markera i omogućiti povezivanje cirkulišućih miRNK obrazaca sa regulatornim mrežama i biološki relevantnim funkcionalnim procesima. Takav pristup otvara mogućnost razvoja EV-miRNK kao minimalno invazivnih pokazatelja prisustva ili toka bolesti, ali i prepoznavanja regulatornih miRNK i njima kontrolisanih molekularnih puteva kao potencijalnih terapijskih meta. Integracija vezikularnih, ćelijskih i funkcionalnih podataka stoga predstavlja perspektivan pravac ka preciznijoj molekularnoj dijagnostici i razvoju novih, mehanistički zasnovanih terapijskih strategija.

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MicroRNAs in extracellular vesicles in CNS diseases: from molecular profiling to biological function

Ivan Jovanović, Aleksandra Stanković, Maja Živković

Laboratory for Radiobiology and Molecular Genetics, Vinča Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade

Extracellular vesicles (EVs) are an important mechanism of intercellular communication and a potential source of molecular markers of pathological processes. Their ability to transfer regulatory molecules, including microRNAs (miRNAs), is of particular interest in research on central nervous system (CNS) diseases, where direct access to affected tissue is limited. However, EV isolation, characterization, and molecular analysis are methodologically demanding, and reliable interpretation of the results depends heavily on the appropriate use of complementary experimental and analytical approaches.

In the studies presented here, we analyzed EV-associated miRNAs in patients with glioblastoma and multiple sclerosis, two biologically distinct models of CNS disease. EVs from peripheral blood were characterized using a combination of morphological, biophysical, and molecular methods. MiRNA levels were assessed by small RNA sequencing and targeted quantification of selected miRNA candidates using qPCR.

In patients with glioblastoma, our results indicate an association between the levels of specific miRNAs in circulating EVs and distinct gene expression patterns in peripheral blood mononuclear cells. The observed changes point to a complex balance of pro-inflammatory and anti-inflammatory signals and may be linked to early systemic immunomodulation in glioblastoma. In multiple sclerosis, miRNAs were analyzed in peripheral blood mononuclear cells and the vesicular compartment of patients with different clinical disease courses. Integrative bioinformatic analyses identified candidate regulatory miRNAs, whose functional significance was further investigated by experimentally modulating miRNA levels in cultured cells and assessing the response of ferroptosis-related target genes.

These findings show that EV miRNA analysis can provide information beyond the identification of individual molecular markers, linking circulating miRNA patterns to regulatory networks and biologically relevant functional processes. This approach offers the potential to develop EV-associated miRNAs as minimally invasive disease indicators, while also identifying regulatory miRNAs and the molecular pathways they control as potential therapeutic targets. Integrating vesicular, cellular, and functional data thus represents a promising direction for more precise molecular diagnostics and the development of new mechanism-based therapeutic strategies.

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Komunikacija posredovana ekstracelularnim vezikulama tokom progresije tumora dojke

Aleksandra Korać¹, Sara Stojanović¹, Tamara Zakić², Igor Golić¹, Aleksandra Janković²

¹Biološki fakultet, Univerzitet u Beogradu, Beograd, Srbija; ²Institut za biološka istraživanja "Siniša Stanković"-Institut od nacionalnog značaja za Republiku Srbiju, Univerzitet u Beogradu, Beograd, Srbija

Tumor predstavlja složen ekosistem različitih tipova ćelija organizovanih u pseudoorgan. Njihovo udruživanje podržano je razmenom ekstracelularnih vezikula (EV) kao medijatorima ćelijske komunikacije. Na lokalnom nivou, EV igraju važnu ulogu u razvoju tumora, a specifične promene u njihovom molekulskom profilu smatraju se potencijalnim biomarkerima. Zato je od velike važnosti utvrditi identit ćelija koje proizvode/razmenjuju/komuniciraju putem EV tokom tumorigeneze. Ortotopski mišji model raka dojke iskorišćen je kao model sistem za analizu EV tokom razvoja tumora imunofluorescentnom i imunogold mikroskopijom, kao i Western blot analizom markera CD63, CD81 i TSG101. Specifičnost ekspresije unutar ćelijskih tipova tokom rane i kasne tumorigeneze biće razmatrana, kao i dinamička priroda komunikacije posredovane EV tokom progresije tumora.

Extracellular vesicle–mediated communication during breast cancer progression

Aleksandra Korać¹, Sara Stojanović¹, Tamara Zakić², Igor Golić¹, Aleksandra Janković²

¹Faculty of Biology, University of Belgrade, Belgrade, Serbia; ²Institute for Biological Research “Siniša Stanković” – National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

A tumor represents a complex ecosystem of different cell types organized into a pseudo-organ. Their association is supported by the exchange of extracellular vesicles (EVs) as mediators of cell–cell communication. At the local level, EVs play an important role in tumor development, and specific changes in their molecular profile are considered potential biomarkers. It is therefore of great importance to determine the identity of cells that produce, exchange, and communicate via EVs during tumorigenesis. An orthotopic mouse model of breast cancer was used as a model system to analyze EVs during tumor development by immunofluorescence and immunogold microscopy, as well as by Western blot analysis of the markers CD63, CD81, and TSG101. Cell type–specific expression during early and late tumorigenesis will be discussed, as will the dynamic nature of EV-mediated communication during tumor progression.

Model domena zakrivljenosti membrane - statističko-mehaničko unapređenje modela fluidnog mozaika za anizotropne membranske nanostrukture

Veronika Kralj-Iglič¹, Aleš Iglič²

¹Univerzitet u Ljubljani, Fakultet zdravstvenih nauka, Laboratorija za kliničku biofiziku, Ljubljana, Slovenija (veronika.kralj-iglic@zf.uni-lj.si) ² Univerzitet u Ljubljani, Fakultet elektrotehnike, Laboratorija za fiziku, Ljubljana, Slovenija (ales.iglic@fe.uni-lj.si)

Formiranje ekstracelularnih vezikula, uskih suženja i tunelskih nanocevi su procesi u kojima membrana podleže snažnom savijanju i, sa tim dosledno povezanoj, lateralnoj preraspodeli membranskih komponenata. Ovaj proces naziva se sortiranje prema zakrivljenosti i dovodi do toga da se sastav membrane nastalih struktura razlikuje od sastava matične membrane. Razumevanje ovih mehanizama od interesa je u oblasti ekstracelularnih vezikula, gde teorijski opis bioloških membrana predstavlja moćno oruđe. Priznati model različite elastičnosti površina (Area-Difference-Elasticity, ADE) za opis entiteta obavijenih membranom (Seifert U, Adv Phys 1997; 46:13) zasniva se na minimizaciji elastične energije membrane, pri čemu se membrana tretira kao izotropni kontinuum - u skladu sa modelom fluidnog mozaika plazma-membrane (Singer SJ, Nicolson GL, Science 1972; 175:720). Međutim, ovaj model ne može da objasni stabilnost snažno anizotropno zakrivljenih nanostrukture. Na osnovu teorijskih i eksperimentalnih dokaza predlažemo radikalno nov pristup teorijskom opisu, zasnovan na statističkoj fizici, koji nazivamo modelom domena zakrivljenosti (Curvature Patch Model). Membranu posmatramo kao sastavljenu od inkluzija koje karakterišu dve glavne intrinzične zakrivljenosti, čiji glavni pravci u opštem slučaju mogu biti rotirani u odnosu na glavne pravce stvarno zakrivljene membrane. Energija pojedinačne inkluzije izražava se invarijantama dvodimenzionalnog tenzora neslaganja zakrivljenosti, a inkluzije se tretiraju kao eksplicitno nezavisne - kako bi se zadovoljila statistika Boltzmannovog kanonskog ansambla iz koje sledi izraz za slobodnu energiju membrane. Model domena zakrivljenosti reprodukuje Helfrihovu izotropnu energiju savijanja ali daje i dodatni (negativni) član koji odgovara orijentacionom uređenju anizotropnih inkluzija u snažno anizotropno zakrivljenim oblastima membrane. Ovaj mehanizam, koji pokreće pupljenje membrane i prateće sortiranje prema zakrivljenosti, nespecifičan je u pogledu hemijskog sastava molekula; pre odražava oblik i lokalne interakcije molekula. Ipak, postoje indikacije da određeni tipovi lipida i proteina češće formiraju snažno zakrivljene sferične konfiguracije koje podležu fisiji i otpuštaju ekstracelularne vezikule - ili se izdužuju u invadopodije - kako bi se razgrađivale ili se širile u okolinu. U ovom prilogu predstavljamo model domena zakrivljenosti i ukazujemo na značaj sortiranja prema zakrivljenosti za razumevanje i usmeravanje ciljanih procesa koji će u budućnosti verovatno imati ključnu ulogu u nanomedicini.

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Curvature patch model

– a statistical mechanical upgradation of the fluid mosaic model for anisotropic membranous nanostructures

Veronika Kralj-Iglič¹, Aleš Iglič²

¹University of Ljubljana, Faculty of Health Sciences, Laboratory of Clinical Biophysics, Ljubljana, Slovenia (veronika.kralj-iglic@zf.uni-lj.si) ²University of Ljubljana, Faculty of Electrical Engineering, Laboratory of Physics, Ljubljana, Slovenia (ales.iglic@fe.uni-lj.si)

Formation of extracellular vesicles, narrow necks, and tunneling nanotubes are processes in which the membrane undergoes strong curving and consistently related lateral redistribution of membrane constituents. This process has been called curvature sorting, resulting in the membrane composition of the formed structures that differs from the composition of the mother membrane. Understanding these mechanisms is of interest in the field of extracellular vesicles, where the theoretical description of biological membranes provides a powerful tool. The acknowledged Area-Difference-Elasticity model for the description of membrane-enclosed entities (Seifert U, Adv Phys 1997; 46:13) is based on the minimization of the membrane elastic energy, with the membrane being treated as an isotropic continuum – in accordance with the Fluid Mosaic Model of the plasma membrane (Singer SJ, Nicolson GL, Science 1972; 175: 720). However, this model cannot explain the stability of strongly anisotropically curved nanostructures. Based on theoretical and experimental evidence, we propose a radically new approach in the theoretical description that is based on statistical physics, which we call the Curvature Patch Model. We consider the membrane composed of inclusions characterized by two intrinsic principal curvatures, which can in general have the principal directions rotated with respect to the principal directions of the actual curved membrane. The energy of a single inclusion is expressed by the invariants of the two-dimensional mismatch curvature tensor, and the inclusions are considered as explicitly independent – to comply with the Boltzmann canonical ensemble statistics leading to the expression of the membrane free energy. The Curvature Patch Model recovers the Helfrich isotropic bending energy but yields another (negative) term corresponding to orientational ordering of anisotropic inclusions in strongly anisotropically curved regions of the membrane. This mechanism that drives budding of the membrane and concomitant curvature sorting is non-specific as regards the chemical composition of the molecules. Rather, it reflects the shape and the local interactions of the molecules. However, evidences indicate that certain types of lipids and proteins are more likely to form strongly curved spherical configurations that undergo fission and shed extracellular vesicles - or elongate into the invadopodia - to destroy or proliferate into the environment. In this contribution, we present the Curvature Patch Model and indicate the effect of curvature sorting for understanding and manipulating targeted processes that will likely in the future play a key role in nanomedicine.

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Bakterijske ekstracelularne vezikule u komunikaciji između biljaka i korisnih rizosfernih i endofitnih bakterija

Sofija Nešić¹, Dragana Bosnić¹, Aleksandra Divac Rankov¹, Maja Kosanović², Jelena Samardžić¹, Igor Davidović¹, Bojana Banović Đeri¹ i Dragana Nikolić^{1*}

¹Institut za molekularnu genetiku i genetičko inženjerstvo, Univerzitet u Beogradu (IMGGI UB); ²Institut za primenu nuklearne energije INEP, Univerzitet u Beogradu; *(dragana.nikolic@imgge.bg.ac.rs)

Ekstracelularne vezikule (EV) imaju važnu ulogu u interakcijama između bakterija i animalnih i humanih ćelija, kao i između gljiva i biljaka, dok je njihova uloga u odnosima biljaka i bakterija znatno manje istražena. Vezikule spoljašnje membrane (Outer membrane vesicles, OMV), koje proizvode Gram-negativne bakterije, mogu da aktiviraju ili potisnu imunski odgovor biljaka, a direktna interakcija OMV sa membranama biljnih ćelija do sada je pokazana samo kod jednog fitopatogenog soja. Još manje se zna o njihovoj ulozi u simbiozi biljaka i korisnih bakterija.

Naš tim istražuje interakciju biljnih ćelija sa OMV koje proizvodi *Paraburkholderia phytofirmans* PsJN, bakterija poznata po stimulaciji rasta i povećanju otpornosti biljaka. Korišćenjem fluorescentno obeleženih OMV i konfokalne mikroskopije pokazali smo da PsJN-OMV direktno stupaju u kontakt sa korenskim dlačicama i epidermalnim ćelijama *Arabidopsis thaliana* i paradajza. Upotrebom samoutišavajuće fluorescentne boje rodamin R18, pokazali smo i da se PsJN-OMV fuzionišu sa membranama biljnih ćelija.

Analizirali smo i RNK asocirane sa PsJN-OMV. Sekvenciranjem RNK iz OMV i bakterijskih ćelija utvrđene su značajne razlike u dužini RNK i regionima genoma sa kojih potiču. Rezultati ukazuju da se RNK ne pakuju u OMV nasumično, već da dolazi do selektivnog obogaćivanja određenih RNK molekula u vezikulama, dok se druge zadržavaju u ćelijama.

Ovi rezultati predstavljaju značajan korak ka razumevanju uloge OMV u ranim fazama kolonizacije korena i uspostavljanja simbiotskih odnosa između biljaka i korisnih bakterija.

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Bacterial extracellular vesicles in communication between plants and beneficial rhizospheric and endophytic bacteria

Sofija Nešić¹, Dragana Bosnić¹, Aleksandra Divac Rankov¹, Maja Kosanović², Jelena Samardžić¹, Igor Davidović¹, Bojana Banović Đeri¹ i [Dragana Nikolić^{1*}](#)

¹University of Belgrade, Institute of Molecular Genetics and Genetic Engineering (IMGGE UB); ²University of Belgrade, Institute for the Application of Nuclear Energy (INEP); *(dragana.nikolic@imgge.bg.ac.rs)

Extracellular vesicles (EVs) play an important role in interactions between bacteria and animal and human cells, as well as between fungi and plants, while their role in plant-bacteria relationships is much less studied. Outer membrane vesicles (OMVs), produced by Gram-negative bacteria, can activate or suppress the plant immune response, but direct interaction of OMVs with plant cell membranes has so far been shown for only one phytopathogenic strain. Even less is known about their role in the symbiosis between plants and beneficial bacteria.

Our team investigates the interaction of plant cells with OMVs produced by *Paraburkholderia phytofirmans* PsJN, a bacterium known to stimulate plant growth and increase plant resistance. Using fluorescently labeled OMVs and confocal microscopy, we demonstrated that PsJN-OMVs directly contact root hairs and epidermal cells of *Arabidopsis thaliana* and tomato. By using the self-quenching fluorescent dye rhodamine R18, we also showed that PsJN-OMVs fuse with plant cell membranes.

We also analyzed RNA associated with PsJN-OMVs. Sequencing RNA from OMVs and bacterial cells revealed significant differences in RNA molecules length and the genome regions from which they originate. The results suggest that RNA is not randomly packaged into OMVs; certain RNA molecules are selectively enriched in the vesicles, while others remain in the cells.

These results represent an important step toward understanding the role of OMVs in the early stages of root colonization and the establishment of symbiotic relationships between plants and beneficial bacteria.

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Ispitivanje imunomodulatornih efekata izloženosti kondenzatu aerosola e-cigareta posredovanih EV *in vitro* i *in vivo*

Milan Stefanović*, Anđela Stevanović, Nevena Vezmar, Bojan Ilić, Aleksandra Divac Rankov, Mila Ljujić

Institut za molekularnu genetiku i genetičko inženjerstvo, Univerzitet u Beogradu, Grupa za funkcionalnu genomiku i ćelijsku toksikologiju, Beograd, Srbija, *(milan.stefanovic@imgge.bg.ac.rs)

Upotreba elektronskih cigareta (e-cigareta) beleži kontinuiran porast, naročito među mladima, iako dugoročni efekti njihove upotrebe još uvek nisu u potpunosti razjašnjeni.

Ekstracelularne vezikule (EV) predstavljaju važne medijatore međućelijske komunikacije i učestvuju u regulaciji različitih fizioloških i patoloških procesa. Njihova uloga je opisana u progresiji brojnih plućnih oboljenja, uključujući astmu, hroničnu opstruktivnu bolest pluća i karcinom pluća. Zebrica (*Danio rerio*) se sve češće koristi kao model-organizam u biomedicinskim istraživanjima zahvaljujući visokom fekunditetu, providnosti embriona, sličnostima urođenog imunog sistema sa humanim i dostupnosti brojnih reporterskih linija.

Cilj ovog istraživanja bio je da se ispita imunomodulatorni potencijal EV izolovanih iz BEAS-2B ćelija nakon izlaganja kondenzatu aerosola e-cigareta, koristeći Tg(mpx:GFP) reportersku liniju zebrića čiji neutrofili ekspimiraju zeleni fluorescentni protein i THP-1 ćelijsku liniju. Larve zebrića stare 48h su podvrgute amputaciji repa, zatim tretirane EV poreklom od BEAS-2B i slikane nakon 4h tretmana. U zebrićama tretiranim EV, izolovanim iz netretiranih, kao i tretiranih ćelija, zabeležen je manji broj neutrofila u regionu povrede u poređenju sa kontrolnom grupom tretiranom samo elucionim puferom, dok između grupa tretiranih EV nije uočena razlika. Rezultati qPCR analize pokazali su da tretman THP-1 ćelija EV poreklom iz BEAS-2B ćelija nije doveo do promene ekspresije gena za interleukin 6 (IL-6) u odnosu na kontrolnu grupu.

Dobijeni rezultati predstavljaju početnu fazu ispitivanja imunomodulatornih efekata izloženosti kondenzatu aerosola e-cigareta posredovanih EV. Istovremeno, oni pružaju osnovu za dalja istraživanja promena u molekularnom sastavu EV i njihove potencijalne uloge u regulaciji odgovora epitelih ćelija pluća na izlaganje aerosolima e-cigareta.

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Investigating the role of extracellular vesicles in immune responses to e-cigarette aerosol condensate *in vitro* and *in vivo*

Milan Stefanović*, Anđela Stevanović, Nevena Vezmar, Bojan Ilić, Aleksandra Divac Rankov, Mila Ljujić

Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Group for Functional Genomics and Cellular Toxicology, Belgrade, Serbia; *(milan.stefanovic@imgge.bg.ac.rs)

The use of electronic cigarettes (e-cigarettes) is continuously increasing, particularly among young people, despite the limited understanding of their health effects. This study aimed to evaluate whether extracellular vesicles (EVs) released by BEAS-2B bronchial epithelial cells following exposure to e-cigarette aerosol condensate modulate immune response.

EVs were isolated from BEAS-2B cells cultured under control conditions or following exposure to e-cigarette aerosol condensate. Their effects on inflammation and immune cell recruitment were evaluated *in vivo* using Tg(mpx:GFP) zebrafish larvae and *in vitro* using the human THP-1 cell line. Three-day-old zebrafish larvae underwent tail amputation and were subsequently treated with BEAS-2B-derived EVs. Neutrophil recruitment to the injury site was assessed by fluorescence imaging 4 h after treatment. THP-1 cells were treated with the same EVs, and interleukin-6 (IL-6) gene expression was assessed by RT-qPCR. T

reatment of zebrafish larvae with EVs derived from both control and exposed BEAS-2B cells resulted in a reduced number of neutrophils at the injury site compared to larvae treated with elution buffer alone. However, no significant difference in neutrophil recruitment was observed between the two groups treated with EVs from control and exposed cells. Treatment with EVs did not significantly alter IL-6 gene expression compared with the control group in THP-1 cells.

These findings suggest that BEAS-2B-derived EVs can influence neutrophil recruitment following tissue injury. These results represent an initial characterization of EV-mediated responses following e-cigarette aerosol exposure and support further investigation into the role of epithelial cell-derived EVs as potential mediators of lung–immune cell communication following e-cigarette use.

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Biomarkerski potencijal EV
Biomarker potential of EVs

Heterogenost ekstracelularnih vezikula u malignitetima digestivnog trakta: analiza distribucije veličina i površinske glikozilacije

Sanja Goč¹, Filip Janjić¹, Ninoslav Mitić¹, Tamara Janković¹, Jovana Rosić Stojković², Jasna Bjelanović³, Goran Barišić^{2,4}, Milan Jovanović^{5,6}, Zoran Rujanovski^{5,6}, Srđan Petković⁷, Miroslava Janković¹

¹Institut za primenu nuklearne energije, INEP, Univerzitet u Beogradu, Odeljenje za imunohemiju i glikobiologiju, Beograd, Srbija, ²Medicinski fakultet, Univerzitet u Beogradu, Beograd, Srbija, ³Univerzitetski klinički centar Srbije, Centar za medicinsku biohemiju, Beograd, Srbija, ⁴Univerzitetski klinički centar Srbije, Klinika za digestivnu hirurgiju - Prva hirurška, Beograd, Srbija, ⁵Vojnomedicinska akademija, Klinika za opštu hirurgiju, Beograd, Srbija, ⁶Medicinski fakultet Vojnomedicinske akademije, Univerzitet odbrane, Beograd, Srbija, ⁷Vojna bolnica Novi Sad, Odeljenje opšte hirurgije, Novi Sad, Srbija

Ekstracelularne vezikule (EV) predstavljaju heterogenu populaciju membranskih nanočestica čija veličina i molekularni sastav odražavaju njihovo ćelijsko poreklo, biološku funkciju i promene povezane sa (pato)fiziološkim stanjima. Kod malignih bolesti, promene u distribuciji veličina i površinskoj glikozilaciji EV mogu odražavati karakteristike tumora. Za analizu površinske glikozilacije korišćeni su lektini Wheat germ agglutinin (WGA), koji vezuje N-acetilglukozamin i sijalinske kiseline, i Sambucus nigra agglutinin (SNA), specifičan za α 2,6-vezanu sijalinsku kiselinu. Cilj istraživanja bio je uporediti distribuciju veličina ukupnih EV i WGA- i SNA-reaktivnih subpopulacija kod pacijenata sa malignitetima digestivnog trakta i zdravih ispitanika. EV su izolovane ultracentrifugiranjem iz seruma pacijenata sa lokalno uznapredovalim karcinomom rektuma (LARC, n=6), hepatocelularnim karcinomom (HCC, n=6), karcinomom pankreasa (PC, n=10) i zdravih ispitanika (HD, n=10). Distribucija veličina analizirana je metodom praćenja nanočestica (NTA), a lektin-reaktivne subpopulacije fluorescentnim NTA. Statistička analiza sprovedena je Kruskal-Wallis i Mann-Whitney testom. Medijana veličine ukupnih EV bila je veća kod HCC (147,1 nm; p=0,0002) i PC (155,5 nm; p<0,0001) nego kod HD (126,4 nm), dok kod LARC nije bilo razlike (128,6 nm; p=0,9364). Udeo EV >200 nm bio je veći u svim malignim grupama nego kod HD (LARC 19,8%, HCC 25,6%, PC 31,3% prema 12,5%). Kod WGA-reaktivnih EV medijana veličine bila je manja kod PC (122,0 nm; p=0,002), a veća kod LARC (169,9 nm; p=0,031) u odnosu na HD (150,6 nm), dok kod HCC nije bilo značajne razlike. LARC je imao najveći udeo WGA-reaktivnih EV >200 nm (43%), a PC najmanji (20%). Kod SNA-reaktivnih EV nisu utvrđene značajne razlike između HCC (164,8 nm), PC (174,7 nm) i HD (176,1 nm), dok je kod LARC detektovan veoma mali broj čestica. HCC i PC karakterisalo je pomeranje ukupne EV populacije ka većim veličinama, dok su WGA-reaktivne EV pokazale različite obrasce među malignitetima. Kombinovana analiza distribucije veličina i lektin-reaktivnih subpopulacija može doprineti karakterizaciji cirkulišućih EV i identifikaciji potencijalnih biomarkera za tečnu biopsiju.

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Heterogeneity of extracellular vesicles in digestive tract malignancies: analysis of size distribution and surface glycosylation

Sanja Goč¹, Filip Janjić¹, Ninoslav Mitić¹, Tamara Janković¹, Jovana Rosić Stojković², Jasna Bjelanović³, Goran Barišić^{2,4}, Milan Jovanović^{5,6}, Zoran Rujanovski^{5,6}, Srđan Petković⁷, Miroslava Janković¹

¹Institute for the Application of Nuclear Energy, INEP, University of Belgrade, Department for Immunochemistry and Glycobiology, Belgrade, Serbia, ²Faculty of Medicine, University of Belgrade, Belgrade, Serbia, ³University Clinical Centre of Serbia, Centre for Medical Biochemistry, Belgrade, Serbia, ⁴University Clinical Centre of Serbia, Clinic for Digestive Surgery - First Surgical Clinic, Belgrade, Serbia, ⁵Military Medical Academy, Clinic for General Surgery, Belgrade, Serbia, ⁶Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia, ⁷Military Hospital Novi Sad, Department of General Surgery, Novi Sad, Serbia

Extracellular vesicles (EVs) represent a heterogeneous population of membranous nanoparticles whose size, biogenesis and molecular composition reflect their origin and biological function, but also changes associated with (patho)physiological conditions. In malignant diseases, including carcinomas of the digestive tract, changes in the size distribution and surface glycosylation of EVs may reflect tumour characteristics. For the analysis of surface glycosylation, the following lectins were used: Wheat germ agglutinin (WGA), which binds N-acetylglucosamine and sialic acid, and Sambucus nigra agglutinin (SNA), specific for α 2,6-linked sialic acid. The aim was to compare the size distributions of total EVs and WGA- and SNA-reactive subpopulations in patients with malignancies of the digestive tract and healthy donors. EVs were isolated by ultracentrifugation from the sera of patients with locally advanced rectal cancer (LARC, n=6), hepatocellular carcinoma (HCC, n=6), pancreatic cancer (PC, n=10), and healthy donors (HD, n=10). The size distribution of total EVs was analysed by nanoparticle tracking analysis (NTA), while WGA- and SNA-reactive subpopulations were analysed by fluorescence NTA. Statistical analysis was performed using Kruskal-Wallis and Mann-Whitney tests in GraphPad Prism 8.0. The median size of total EVs was higher in HCC (147.1 nm; p=0.0002) and PC (155.5 nm; p<0.0001) than in HD (126.4 nm), whereas no difference was observed in LARC (128.6 nm; p=0.9364). The proportion of EVs >200 nm was higher in all malignant groups than in HD (LARC 19.8%, HCC 25.6%, PC 31.3% vs. 12.5%). WGA-reactive EVs were smaller in PC (122.0 nm; p=0.002) and larger in LARC (169.9 nm; p=0.031) than in HD (150.6 nm), with no significant difference in HCC. LARC showed the highest proportion of WGA-reactive EVs >200 nm (43%), while PC showed the lowest (20%). No significant differences in SNA-reactive EV size were observed between HCC, PC, and HD, while very few particles were detected in LARC. Overall, HCC and PC showed a shift toward larger total EVs, whereas WGA-reactive EVs displayed distinct patterns across malignancies. Combined analysis of EV size and lectin reactivity may support EV characterization and biomarker discovery for liquid biopsy.

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Tiroidea-specifični biomarkeri u ekstracelularnim vezikulama: detekcija tiroglobulina i tirotropinskog receptora kao pomoć u monitoringu rekurentnog tiroidnog karcinoma

Nevena Bobar¹, Ninoslav Mitić¹, Maja Kosanović¹, Sonja Šelemetjev¹, Tijana Išić Denčić¹, Katarina Taušanović^{2,3} i [Jelena Janković Miljuš¹](#)

¹Institut za primenu nuklearne energije – INEP, Univerzitet u Beogradu, Beograd, Srbija; ²Klinika za endokrinu hirurgiju, Univerzitetski klinički centar Srbije, Beograd, Srbija; ³Medicinski fakultet, Univerzitet u Beogradu, Beograd, Srbija

Tiroidni karcinom je najčešći endokrini malignitet, a diferencijacija benignih od malignih nodusa standardnim citološkim analizama ostaje nepouzdana u oko 30% slučajeva, što često vodi nepotrebnim hirurškim intervencijama. Kod pacijenata sa diferentovanim karcinomom tiroidne žlezde, i pored uspešnog inicijalnog lečenja, rekurenca se javlja u 10–30% slučajeva. Postoperativni monitoring pacijenata zasniva se na merenju tiroglobulina (Tg), ali prisustvo anti-Tg (Tg-At) i heterofilnih antitela onemogućava pouzdanu detekciju kod trećine pacijenata, što ukazuje na potrebu za novim, pouzdanijim biomarkerima. Ekstracelularne vezikule (EVs) predstavljaju potencijalni izvor ćelijski specifičnih biomarkera, primarno zahvaljujući svojoj stabilnosti u telesnim tečnostima. Sa druge strane, molekularni sadržaj unutar i na površini vezikula odražava poreklo i stanje ćelije koja ih sekretuje, čineći EVs atraktivnim kandidatom za neinvazivnu dijagnostiku i prognostiku putem tečne biopsije. Površinski proteini specifični za ćeliju porekla dodatno omogućavaju tkivno-specifičnu izolaciju vezikula.

Cilj ove studije bio je da se dodatno istraži sadržaj EVs poreklom iz tiroidnog tkiva, radi ispitivanja potencijalnih meta za imunoafinitetnu izolaciju, kao i tiroidno-specifičnih biomarkera bolesti. Istraženo je prisustvo receptora za tirostimulišući hormon (TSHR) na površini EVs tiroidnog porekla, kao pogodne mete za metode imuno-izolacije vezikula. Sadržaj vezikula ispitan je na prisustvo Tg, kao potencijalnog surogat-markera za praćenje rekurencije kod Tg-At pozitivnih pacijenata. Hipoteze su testirane prvo na humanim tiroidnim ćelijskim linijama, a zatim na EVs izolovanim iz plazme pacijenata sa benignim i malignim tiroidnim nodusima, uključujući i rekurentne slučajeve. Nakon toga hipoteze su testirane kod pacijenata u kompletnoj remisiji. Za izolaciju i karakterizaciju vezikula je korišćeno diferencijalno ultracentrifugiranje i NTA, dok su markeri ispitivani Western imunoblotom. Intravezikularni Tg je dodatno detektovan metodom imunoradiometrijskog merenja.

TSHR je detektovan u EV-obogaćenim preparatima iz plazme pacijenata sa tiroidnim nodusima i rekurentnim karcinomom, čime se otvara mogućnost njegove upotrebe kao mete za imunoizolaciju EVs. Elektroforetski profili TSHR i Tg u plazma EVs razlikovali su se od profila u tkivu, što ukazuje na moguće specifično sortiranje proteina u vezikule. Intravezikularni Tg detektovan je i kod pacijenata sa nodusima i kod rekurentnih slučajeva sa prisutnim serumskim Tg-At, podržavajući hipotezu da je intravezikularni Tg manje dostupan interferenciji antitela i time pogodniji za detekciju. Zbog eksploratornog dizajna i malog broja rekurentnih slučajeva, nalazi su preliminarni i zahtevaju validaciju na većim prospektivnim studijama.

Thyroid-specific biomarkers in extracellular vesicles: detection of thyroglobulin and thyrotropin receptor as an aid in monitoring recurrent thyroid carcinoma

Nevena Bobar¹, Ninoslav Mitić¹, Maja Kosanović¹, Sonja Šelemetjev¹, Tijana Išić Denčić¹,
Katarina Taušanović^{2,3} i Jelena Janković Miljuš¹

¹ Institute for the Application of Nuclear Energy – INEP, University of Belgrade, Belgrade, Serbia; ² Clinic for Endocrine Surgery, University Clinical Centre of Serbia, Belgrade, Serbia; ³ Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Thyroid carcinoma is the most common endocrine malignancy, and the differentiation of benign from malignant nodules by standard cytological analysis remains unreliable in approximately 30% of cases, frequently leading to unnecessary surgical interventions. In patients with differentiated thyroid carcinoma, recurrence occurs in 10–30% of cases despite successful initial treatment. Postoperative monitoring is based on thyroglobulin (Tg) measurement, but the presence of anti-Tg (Tg-Ab) and heterophilic antibodies unables reliable detection in one third of patients, highlighting the need for new and more reliable biomarkers. Extracellular vesicles (EVs) represent a potential source of cell-specific biomarkers, primarily due to their stability in body fluids. Furthermore, the molecular content within and on the surface of vesicles reflects the origin and state of the secreting cell, making EVs an attractive candidate for non-invasive diagnostics and prognostics through liquid biopsy. Surface proteins specific to the cell of origin additionally enable tissue-specific isolation of vesicles.

The aim of this study was to further investigate the content of EVs of thyroid tissue origin, in order to examine potential targets for immunoaffinity isolation as well as thyroid-specific disease biomarkers. The presence of thyrotropin receptor (TSHR) on the surface of thyroid-derived EVs was explored as a suitable target for vesicle immuno-isolation methods. The vesicle content was examined for the presence of Tg as a potential surrogate marker for monitoring recurrence in Tg-Ab-positive patients. The hypotheses were first tested in human thyroid cell lines, and subsequently in EVs isolated from the plasma of patients with benign and malignant thyroid nodules, including recurrent cases. The hypotheses were thereafter tested in patients in complete remission. Differential ultracentrifugation and NTA were used for vesicle isolation and characterisation, while the markers were examined by Western immunoblot. Intravesicular Tg was additionally detected by immunoradiometric assay.

TSHR was detected in EV-enriched preparations from the plasma of patients with thyroid nodules and recurrent carcinoma, opening the possibility of its use as a target for EV immuno-isolation. The electrophoretic profiles of TSHR and Tg in plasma EVs differed from those in tissue, suggesting a possibility for specific sorting of proteins into vesicles. Intravesicular Tg was detected both in patients with nodules and in recurrent cases with serum Tg-Ab present, supporting the hypothesis that intravesicular Tg is less accessible to antibody interference and therefore more suitable for detection. Due to the exploratory design and the small number of recurrent cases, the findings are preliminary and require validation in larger prospective studies.

Ekstracelularne vezikule kao prediktori toksičnosti radioterapije: od molekularnih mehanizama do kliničke prakse

Nina Petrović, Tatjana Stanojković

Institut za onkologiju i radiologiju Srbije, Beograd, Srbija

Ekstracelularne vezikule (EV) predstavljaju jedne od glavnih medijatora međucelijske komunikacije. Sama činjenica da se ove strukture obavijene lipidnim dvoslojem stvaraju u svim domenima od arhea preko bakterija, biljaka i gljiva, pa sve do životinja i čoveka, ukazuje na njihov značaj i povezanost sa svim biološkim procesima. EV menjaju svoj sadržaj i brojnost nakon ekspozicije zračenju. Radioterapija, pored hemio, imuno i/ili hormonske terapije je i danas jedan od glavnih modaliteta za lečenje solidnih malignih tumora. Osnovni mehanizam radioterapije je upravo to da se isporuči ciljna doza zračenja visokoproliferišućim maligno transformisanim ćelijama, a da istovremeno maligno neizmenjeno tkivo bude pošteđeno. U praksi je taj slučaj gotovo nemoguć, te neizmenjeno, uslovno rečeno, "normalno" tkivo uvek primi određenu dozu zračenja, pri čemu mogu nastati različiti neželjeni efekti. Neželjeni efekti mogu biti akutni ili kasni, odnosno hronični. Pojava neželjenih efekata značajno može uticati na kvalitet života, kao i na tok terapije, ukoliko se zračenje odlaže ili prekida. Dosadašnja istraživanja naše grupe bila su usmerena na identifikaciju biomarkera individualne radiosenzitivnosti i predikciju akutne i kasne radiotoksičnosti normalnog tkiva. Integracijom genomskih, transkriptomskih, proteomskih, metabolomskih i epigenetskih analiza pokazali smo da su promene ekspresije mikro RNK (miR-21, miR-146a, miR-155, miR-10b i miR-34a), promenjeni nivoi inflamatornih medijatora IL-6 i TGF- β , kao i polimorfizmi gena uključenih u odgovor na oštećenje DNK i inflamaciju (TGFB1, XRCC1 i XRCC3), povezani sa razvojem radiotoksičnosti. Međutim, naši rezultati, kao i nalazi drugih istraživačkih grupa, ukazuju na to da nijedan pojedinačni biomarker nema dovoljnu prediktivnu snagu za kliničku primenu. Zbog toga se pažnja sve više usmerava ka integrativnim biomarkerima, među kojima EV predstavljaju posebno perspektivan izvor informacija. Njihov molekularni sadržaj objedinjuje različite omičke nivoe i odražava kompleksnu međucelijsku komunikaciju nakon zračenja, što ih pozicionira kao logičan nastavak naših dosadašnjih istraživanja i perspektivnu platformu za razvoj prediktora individualne radiosenzitivnosti i radiotoksičnosti. Istraživanja su pokazala da su nivoi ekspresije mikro RNK izolovanih iz EV povezani sa pojavom neželjenih efekata koji prate radioterapiju, kao i to da se broj EV razlikuje između ispitanika koji imaju i nemaju neželjene efekte RT. Osim mogućnosti predikcije radijacione toksičnosti, EV bi se mogle koristiti i za modifikaciju odgovora ne samo na terapiju zračenjem, već i ostalih modaliteta lečenja pacijenata sa malignim bolestima, a zatim i kao dodatni agensi za lečenje u kombinaciji sa drugim standardnim pristupima.

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Extracellular vesicles as predictors of radiotherapy toxicity: from molecular mechanisms to clinical practice

Nina Petrović, Tatjana Stanojković

¹Institute of Oncology and Radiology of Serbia, Belgrade, Serbia

Extracellular vesicles (EVs) are mediators of intercellular communication. EVs as lipid bilayer subcellular structures are present in all domains from archaea to bacteria, plants and fungi, all the way to animals and humans indicates their involvement in every biological process. EVs change their content and abundance in response to radiation. Radiotherapy, in addition to chemo, immuno and/or hormonal therapy, is still one of the mainstream modalities for the treatment of solid malignant tumors. The basic mechanism of radiotherapy is to deliver the dose precisely to the highly proliferating malignantly transformed cells, and at the same time to spare surrounding tissue as much as possible. In practice, this case is almost impossible, and surrounding tissue always receives a certain dose of radiation, which can cause various adverse effects. Adverse effects can be acute or late (chronic). Adverse effects, known as radiation toxicity can significantly affect the quality of life, as well as the course of therapy, if radiation is delayed or interrupted. Our group's previous research was focused on the identification of biomarkers of individual radiosensitivity and the prediction of acute and late radiotoxicity of normal tissue. By integrating genomic, transcriptomic, proteomic, metabolomic and epigenetic analyses, we showed that changes in the expression of micro RNAs (miR-21, miR-146a, miR-155, miR-10b and miR-34a), altered levels of the inflammatory mediators IL-6 and TGF- β , as well as polymorphisms of genes involved in the response to DNA damage and inflammation (TGFB1, XRCC1 and XRCC3), are associated with the development of radiotoxicity. However, our results, as well as the findings of other research groups, indicate that no single biomarker has sufficient predictive power for clinical application. Therefore, attention is increasingly directed towards integrative biomarkers, among which EVs have emerged as potential predictors of radiotoxicity, as well. Their molecular content and number can change after the radiation exposure. EV cargo and abundance in response to RT represents logical continuation of our previous research and a promising platform for the development of predictors of individual radiosensitivity and radiotoxicity. Research has shown that the expression levels of microRNAs extracted from EVs is associated with the occurrence of side effects that follow radiotherapy. Besides the possibility of predicting radiation toxicity, EVs could be used to modify the response not only to radiation therapy, but also to other treatment modalities of patients with malignant diseases, and then as additional treatment agents in combination with standard therapeutical approaches.

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Salivarne ekstracelularne vezikule kao potencijalni biomarkeri u reumatoidnom artritisu

Marko Prokić^{1*}, Ninoslav Mitić¹, Jovana Mihailović², Mirjana Šefik Bukilica², Žanka Bojić-Trbojević¹

¹Institut za Primenu Nuklearne Energije - INEP, Beograd, Srbija; ²Institut za Reumatologiju, Beograd, Srbija

Autoimunska oboljenja predstavljaju hronična inflamatorna stanja čiji nastanak zavisi od kompleksnog međudejstva genetičkih faktora, faktora životne sredine i životnih navika. Njihova rastuća prevalencija i potreba za ranom dijagnostikom ukazuju na značaj identifikacije novih, specifičnijih biomarkera. Postojeći biomarkeri, poput anti-CCP antitela i reumatoidnog faktora (RF) u reumatoidnom artritisu (RA), imaju ograničenu specifičnost, jer se mogu detektovati i kod drugih autoimunskih oboljenja.

Saliva predstavlja heterogenu smešu sekreta salivarnih žlezda, sistemskog transudata poreklom iz cirkulacije i produkata mikrobiote, čije je oralna duplja glavno stanište. Za razliku od telesnih tečnosti poput krvi, sinovijalne i cerebrospinalne tečnosti, uzorkovanje salive je jednostavno, neinvazivno i bezbolno. Međutim, prisustvo različitih kontaminanata u salivi može otežati detekciju rastvorenih biomarkera. Izolacija i analiza ekstracelularnih vezikula (EV) predstavljaju potencijalni pristup za prevazilaženje ovog problema.

Salivarne EV imaju značajan potencijal za detekciju i praćenje oralnih oboljenja, s obzirom na njihovu direktnu interakciju sa oralnim okruženjem. Anatomska blizina salivarnih žlezda usnoj duplji omogućava da salivarni EV predstavljaju bogat i dostupan izvor biomarkera koji odražavaju lokalna patofiziološka stanja. Pored toga, slično molekulima koji putem cirkulacije mogu dospeti u salivu iz distalnih delova organizma, i EV mogu dospeti u salivu putem sistemske cirkulacije. Ovo svojstvo ukazuje na njihov potencijal za ranu detekciju i praćenje sistemskih oboljenja, uključujući metaboličke poremećaje, autoimunska oboljenja i maligne bolesti.

U okviru ovog istraživanja izvršena je izolacija i karakterizacija EV iz salive osoba obolelih od RA koje su bile podvrgnute biološkoj ili konvencionalnoj, nebiološkoj terapiji, kao i njihovo poređenje sa EV izolovanim iz salive zdravih osoba koje su predstavljale kontrolnu grupu. Dobijeni rezultati ukazuju na postojanje značajnih razlika u karakteristikama EV između ispitivanih grupa. Uočene razlike najverovatnije predstavljaju posledicu kompleksnog međudejstva više faktora, uključujući dužinu trajanja bolesti, mehanizme njenog nastanka i održavanja, kao i tip primenjene terapije. Dalja istraživanja su neophodna kako bi se utvrdile potencijalne razlike u obrascima glikozilacije EV između ispitivanih grupa i dodatno procenio njihov potencijal kao biomarkera za dijagnostiku i praćenje RA.

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Salivary extracellular vesicles as potential biomarkers in rheumatoid arthritis

Marko Prokić^{1*}, Ninoslav Mitić¹, Jovana Mihailović², Mirjana Šefik Bukilica², Žanka Bojić-Trbojević¹

¹Institute for the Application of Nuclear Energy – INEP, Belgrade, Serbia; ²Institute of Rheumatology, Belgrade, Serbia

Autoimmune diseases are chronic inflammatory conditions whose onset depends on a complex interplay of genetic factors, environmental factors, and lifestyle. Their rising prevalence and the need for early diagnosis highlight the importance of identifying new, more specific biomarkers. Existing biomarkers, such as anti-CCP antibodies and rheumatoid factor (RF) in rheumatoid arthritis (RA), have limited specificity, as they can also be detected in other autoimmune diseases.

Saliva is a heterogeneous mixture of secretions from the salivary glands, systemic transudate originating from the circulation, and products of the microbiota whose primary habitat is the oral cavity. Unlike body fluids such as blood, synovial fluid, and cerebrospinal fluid, saliva sampling is simple, noninvasive, and painless. However, the presence of various contaminants in saliva can hamper detection of soluble biomarkers. Isolation and analysis of extracellular vesicles (EVs) represent a potential approach to overcoming this limitation.

Salivary EVs have considerable potential for detection and monitoring of oral diseases, given their direct interaction with the oral environment. The anatomical proximity of the salivary glands to the oral cavity enables salivary EVs to constitute a rich and accessible source of biomarkers that reflect local pathophysiological states. In addition, similarly to molecules that can reach saliva from distal parts of the body via the circulation, EVs may also enter saliva through the systemic circulation. This property indicates their potential for early detection and monitoring of systemic diseases, including metabolic disorders, autoimmune diseases, and malignancies.

In this study, EVs were isolated and characterized from the saliva of patients with RA who were receiving biological or conventional non-biological therapy, and compared with EVs isolated from the saliva of healthy individuals serving as the control group. The results indicate significant differences in EV characteristics among the groups examined. The observed differences most likely result from a complex interplay of multiple factors, including disease duration, mechanisms of disease onset and maintenance, and the type of therapy applied. Further research is needed to determine potential differences in EV glycosylation patterns among the groups and to further assess their potential as biomarkers for the diagnosis and monitoring of RA.

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Metode u istraživanju EV

Methods in EV research

Profilisanje glikozilacije ekstracelularnih vezikula lektinskim mikroerejem i SPR-om

Jaroslav Katrlík*, Lucia Pažitná, Patrícia Dudoňová, Muhammad Umair Khan, Jana Žilová

Institut za hemiju, Slovačka akademija nauka, Bratislava, Slovačka; *(katrlík@yahoo.com)

Ekstracelularne vezikule (EV) nose složene molekulske informacije, uključujući karakteristične obrasce površinske glikozilacije koji mogu odražavati njihovo ćelijsko poreklo i fiziološko ili patološko stanje. Mi razvijamo afinitetne pristupe za profilisanje glikozilacije EV, zasnovane na lektinima, na dve komplementarne platforme: lektinskim mikroerejima i površinskoj plazmonskoj rezonanci (SPR). Kao dokaz koncepta, ovi pristupi primenjeni su na glikoprofilisanje i komparativni skrining nekoliko tipova EV. Lektini različite specifičnosti vezivanja ugljenih hidrata omogućavaju procenu odabranih glikanskih obeležja i poređenje obrazaca glikozilacije među uzorcima EV. Metode su još u razvoju, a aktuelni rad usmeren je na optimizaciju testa i analitičke performanse. Ovi pristupi mogu predstavljati komplementarna oruđa za karakterizaciju EV i istraživanje biomarkera zasnovanih na glikanima.

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Glycosylation profiling of extracellular vesicles by lectin-based microarray and SPR

Jaroslav Katrlík*, Lucia Pažitná, Patrícia Dudoňová, Muhammad Umair Khan, Jana Žilová

Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia;*(katrlík@yahoo.com)

Extracellular vesicles (EVs) carry complex molecular information, including characteristic surface glycosylation patterns that may reflect their cellular origin and physiological or pathological state. We are developing affinity-based approaches employing lectins for EV glycosylation profiling using two complementary platforms: lectin-based microarrays and surface plasmon resonance (SPR). As a proof of concept, these approaches have been applied to glycoprofiling and comparative screening of several types of EVs. Lectins with different carbohydrate-binding specificities enable the assessment of selected glycan features and comparison of glycosylation patterns among EV samples. The methods are still under development, with ongoing work focused on assay optimisation and analytical performance. These approaches may provide complementary tools for EV characterisation and glycan-based biomarker research.

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Molekularna i morfološka karakterizacija ekstracelularnih vezikula iz krvne plazme korišćenjem super-rezolucione mikroskopije

Mihajlo D. Radmilović¹, Yingchao Liang¹, Nan Jiang¹, Benjamin Purnell¹, Diana Alexandra Tanase¹, Stephen J. Gould², Kendall Van Keuren-Jensen³, Lisa Feldman⁴, Tijana Jovanović-Talisman¹

¹Department of Cancer Biology and Molecular Medicine, Beckman Research Institute, City of Hope Comprehensive Cancer Center, Duarte, CA, USA, *(mradmilovic@coh.org); ²Department of Cell Biology, Johns Hopkins University, Baltimore, MD, USA; ³Translational Genomics Research Institute, Phoenix, AZ, USA; ⁴Department of Surgery, City of Hope, Duarte, CA, USA

Ekstracelularne vezikule (EV) predstavljaju značajnu komponentu bioloških tečnosti, uključujući krvnu plazmu, cerebrospinalnu tečnost i suze. Zahvaljujući svojoj zastupljenosti, molekularnom sastavu i ulozi u međucelijskoj komunikaciji, imaju značajan biološki i dijagnostički potencijal. Njihove dimenzije nalaze se tipično ispod granice rezolucije konvencionalnih mikroskopskih metoda (250 nm), što otežava njihovu karakterizaciju konfokalnom i standardnom fluorescentnom mikroskopijom. Tehnike super-rezolucione mikroskopije, pre svega mikroskopija lokalizacije pojedinačnih molekula (Single-Molecule Localization Microscopy, SMLM), u kombinaciji sa protokolom SEVEN (Single Extracellular Vesicle Nanoscopy), omogućavaju kvantifikaciju morfoloških svojstava EV, kao i detekciju i određivanje zastupljenosti njihovih molekularnih markera.

U ovom radu EV su imobilisane na pokrovnim staklima funkcionalizovanim polimerom MCP-4 i odgovarajućim antitelima za vezivanje. Njihova detekcija omogućena je korišćenjem antitela usmerenih prema tetraspaninima CD9, CD81 i CD63 (EV markeri), prethodno obeleženih fluoroforom Alexa Fluor 647. Analizirani su broj, molekularni profil i morfološka svojstva vezikula koje sadrže različite tetraspanine, proteine koji organizuju mikrodomene na ćelijskoj membrani i time regulišu ključne ćelularne procese. U krvnoj plazmi zdravih donora najzastupljenije su bile vezikule pozitivne na konvencionalne tetraspaninske markere CD9, CD81 i CD63. Pored njih, identifikovane su i EV koje nose 1) tetraspanine specifične za imune ćelije, CD37 i CD53; 2) tetraspanine često obogaćene u mozgu, Tspan7 i Tspan3; i 3) tetraspanine koji su često povezani sa razvojem tumora, CD82, CD151, Tspan1 i Tspan8. Dobijeni rezultati ukazuju na molekularnu i morfološku heterogenost EV koje su pozitivne na različite tetraspanine i pružaju osnovu za njihovu dalju analizu u različitim patološkim stanjima. Jednako važno je da korišćenje specifičnih tetraspanina pruža mogućnost da ukaže na poreklo EV iz ćelija.

Zahvalnica: Layne Foudation, Concern Foundation, Circle 1500.

Molecular and morphological characterization of extracellular vesicles from plasma using super-resolution microscopy

Mihajlo D. Radmilović¹, Yingchao Liang¹, Nan Jiang¹, Benjamin Purnell¹, Diana Alexandra Tanase¹, Stephen J. Gould², Kendall Van Keuren-Jensen³, Lisa Feldman⁴, Tijana Jovanović-Talisman¹

¹Department of Cancer Biology and Molecular Medicine, Beckman Research Institute, City of Hope Comprehensive Cancer Center, Duarte, CA, USA *(mradmilovic@coh.org); ²Department of Cell Biology, Johns Hopkins University, Baltimore, MD, USA; ³Translational Genomics Research Institute, Phoenix, AZ, USA; ⁴Department of Surgery, City of Hope, Duarte, CA, USA

Extracellular vesicles (EVs) represent a major component of biofluids, including plasma, cerebrospinal fluid, and tears. Due to their abundance, molecular composition, and role in intercellular communication, EVs have significant biological and diagnostic potential. Their size is typically below the resolution limit of conventional microscopy (250 nm), which makes their characterization by confocal and standard fluorescence microscopy challenging. Super-resolution microscopy techniques, particularly Single-Molecule Localization Microscopy (SMLM), combined with the SEVEN (Single Extracellular VEicle Nanoscopy) protocol, enable the quantification of EV morphological properties, as well as the detection and determination of the abundance of their molecular markers.

In this study, EVs were immobilized on coverslips functionalized with MCP-4 polymer and appropriate capture antibodies. Their detection was enabled using antibodies targeting tetraspanins CD9, CD81, and CD63 (EV markers) conjugated with Alexa Fluor 647. The abundance, molecular marker content, and morphological properties of EVs expressing different tetraspanins were analyzed. Tetraspanins are proteins that organize membrane microdomains and thereby regulate key cellular processes. In the plasma of healthy donors, vesicles positive for the conventional EV markers CD9, CD81, and CD63 were the most abundant. In addition, EVs carrying 1) immune cell-associated tetraspanins, CD37 and CD53; 2) tetraspanins frequently enriched in the brain, Tspan7 and Tspan3; and 3) tetraspanins commonly associated with tumor development, CD82, CD151, Tspan1, and Tspan8, were quantified. The results demonstrate the molecular and morphological heterogeneity of EVs positive for different tetraspanins and provide a basis for their further investigation in various pathological conditions. Equally importantly, the use of specific tetraspanins may provide insights into the cellular origin of EVs.

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Poređenje metoda za izolaciju vezikula iz malignog pleuralnog izliva kod pacijenata sa NSCLC

Miodrag Vuković¹, Lidija Filipović², Nina Petrović^{1,3}, Andrej Zečević⁴, Maja Kosanović⁵, Miljana Tanić¹, Radmila Janković¹, Tatjana Stanojković¹, Milica Popović², Aleksandra Korać⁶, Sanja Stevanović⁷, Milena Čavić¹

¹Odeljenje za eksperimentalnu onkologiju, Institut za onkologiju i radiologiju Srbije, Beograd, Srbija; Katedra za biohemiju, Hemijski fakultet, Univerzitet u Beogradu, Beograd, Srbija; ³Klinika za pulmologiju, Univerzitetski klinički centar Srbije, Beograd, Srbija; ⁴Institut za primenu nuklearne energije, INEP, Univerzitet u Beogradu, Beograd, Srbija; ⁵Biološki fakultet, Centar za elektronsku mikroskopiju, Univerzitet u Beogradu, Beograd, Srbija; ⁶Institut za hemiju, tehnologiju i metalurgiju, Centar za elektrohemiju, Univerzitet u Beogradu, Beograd, Srbija

Maligni pleuralni izliv (MPE) čest je klinički znak uznapredovalog nesitnoćelijskog karcinoma pluća (NSCLC) i predstavlja dragocen izvor komponenti tumorskog porekla, uključujući ekstracelularne vezikule (EV). Cilj ovog istraživanja bio je da se uporede tri različite metode izolacije EV sa potencijalnom primenom u kliničkoj praksi: komercijalni Norgen kit (NOR), imunoafinitetna izolacija (IA) i ultracentrifugiranje (UC). Nakon karakterizacije EV, IA je pokazala najbolje ukupne performanse u pogledu prinosa i čistoće EV, NOR je pokazao srednju efikasnost, dok je UC bila najmanje delotvorna metoda. Metoda IA pokazala je svojstva pogodna za moguću kliničku primenu, dok bi NOR i UC zahtevali kombinovanje sa dodatnim metodama izolacije. Napredovanje tečne biopsije zasnovane na pleuralnom izlivu ka kliničkoj primeni zavisice od razvoja robusnih, skalabilnih i jednostavnih protokola za izolaciju EV, kao i od usklađenih smernica za prikupljanje uzoraka, njihovu pripremu i izveštavanje o dobijenim rezultatima. Uspostavljanje takvih standarda unaprediće ponovljivost, omogućiti smisljena poređenja između studija i ubrzati uključivanje biomarkera zasnovanih na EV u preciznu onkologiju.

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Comparison of EV enrichment methods from malignant pleural effusion in patients with NSCLC

Miodrag Vuković¹, Lidija Filipović², Nina Petrović^{1,3}, Andrej Zečević⁴, Maja Kosanović⁵, Miljana Tanić¹, Radmila Janković¹, Tatjana Stanojković¹, Milica Popović², Aleksandra Korać⁶, Sanja Stevanović⁷, Milena Čavić¹

¹Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia; ²Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Belgrade, Serbia; ³Clinic for Pulmonology, University Clinical Center of Serbia, Belgrade, Serbia; ⁴Institute for the Application of Nuclear Energy, INEP, University of Belgrade, Belgrade, Serbia; ⁵Faculty of Biology, Center for Electron Microscopy, University of Belgrade, Belgrade, Serbia; ⁶Institute of Chemistry, Technology and Metallurgy, Department of Electrochemistry, University of Belgrade, Belgrade, Serbia

Malignant pleural effusion (MPE) is a common clinical manifestation of advanced non-small cell lung cancer (NSCLC) and represents a valuable source of tumor-derived components, including extracellular vesicles (EVs). The aim of this study was to compare three different EV enrichment methods with potential applicability in clinical practice: a commercial Norgen kit (NOR), immunoaffinity capture (IA), and ultracentrifugation (UC). Following EV characterization, IA demonstrated the best overall performance in terms of EV yield and purity, NOR showed intermediate efficiency, while UC was the least effective method. The IA method exhibited characteristics suitable for potential clinical implementation, whereas NOR and UC may require combination with additional enrichment approaches. Advancing pleural effusion-based liquid biopsy toward clinical application will depend on the development of robust, scalable, and user-friendly EV enrichment workflows, along with harmonized guidelines for sample collection, preprocessing, and data reporting. Establishing such standards will enhance reproducibility, enable meaningful cross-study comparisons, and accelerate the integration of EV-based biomarkers into precision oncology.

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Terapeutski potencijal EV
Therapeutic potential of EVs

Kombinovanje probiotika i egzozoma poreklom iz matičnih ćelija u cilju poboljšanja regeneracije kosti

Miloš Lazarević¹, Dijana Mitić¹, Jelena Čarkić¹, Milica Jakšić Karišik¹, Tamara Vlajić Tovilović², Jelena Milašin³

¹Centar za istraživanje implanata, Stomatološki fakultet, Univerzitet u Beogradu, Beograd, Srbija; Katedra za mikrobiologiju, Stomatološki fakultet, Univerzitet u Beogradu, Beograd, Srbija; ³Katedra za humanu genetiku, Stomatološki fakultet, Univerzitet u Beogradu, Beograd, Srbija

Uvod: Egzozomi poreklom iz mezenhimalnih matičnih ćelija i probiotici su nezavisno pokazali obećavajuća regenerativna svojstva; međutim, njihovi kombinovani efekti na osteogenu diferencijaciju još nisu ispitivani. U ovoj studiji je procenjeno da li prekondicioniranje *Lactobacillus salivarius* i egzozomi poreklom iz matičnih ćelija apikalne papile (SCAP) sinergistički pojačavaju osteogenu diferencijaciju SCAP u dvodimenzionalnim (2D) kulturama i trodimenzionalnim (3D) modelima sa nano-hidroksiapatitnim skafoldom.

Metode: Egzozomi poreklom iz SCAP izolovani su i karakterisani pomoću transmisione elektronske mikroskopije, analize praćenja nanočestica, kvantifikacije proteina i profilisanja mikroRNK. SCAP su tretirane sa *L. salivarius*, egzozomima ili njihovom kombinacijom. Vijabilnost ćelija procenjena je MTT testom, dok je osteogena diferencijacija ocenjena pomoću aktivnosti alkalne fosfataze (ALP), bojenja Alizarin Red S, imunofluorescencije za BMP2 i osteokalcin, kao i qPCR analize osteogenih markera i signalnih puteva PI3K/Akt/mTOR, Wnt/ β -catenin, PTEN i MAPK.

Rezultati: Nijedan od tretmana nije uticao na vijabilnost ćelija. Kombinovani tretman je proizveo najveći porast osteogene diferencijacije u obe 2D i 3D kulture. Egzozomi su značajno podstakli osteogeno sazrevanje, dok je prekondicioniranje probioticima primarno stimulisalo ranu osteogenu determinaciju kroz povećanu ekspresiju ALP, RUNX2, BMP2 i BMP4. Kombinovani tretman je dodatno povećao ekspresiju osteogenih markera i aktivirao signalne puteve PI3K/Akt/mTOR, MAPK i Wnt/ β -catenin, ukazujući na komplementarne biološke mehanizme.

Zaključak: U ovoj studiji, kombinacija prekondicioniranja *Lactobacillus salivarius* i egzozoma poreklom iz SCAP predstavlja novu strategiju za pojačavanje osteogeneze zasnovane na skafoldu. Ovi nalazi podržavaju razvoj bioaktivnih zamena za kost nove generacije koje uključuju probiotike i ekstracelularne vezikule za regenerativnu stomatologiju i inženjering koštanog tkiva.

Combining probiotics and stem cell-derived exosomes to improve bone regeneration

Miloš Lazarević¹, Dijana Mitić¹, Jelena Čarkić¹, Milica Jakšić Karišik¹, Tamara Vlajić Tovilović², Jelena Milašin³

¹Implant-Research Center, Faculty of Dental Medicine, University of Belgrade, Belgrade, Serbia; ²Department of Microbiology, Faculty of Dental Medicine, University of Belgrade, Belgrade, Serbia; ³Department of Human Genetics, Faculty of Dental Medicine, University of Belgrade, Belgrade, Serbia

Background: Mesenchymal stem cell-derived exosomes and probiotics have independently demonstrated promising regenerative properties; however, their combined effects on osteogenic differentiation have not yet been investigated. This study evaluated whether *Lactobacillus salivarius* preconditioning and stem cells from the apical papilla (SCAP)-derived exosomes synergistically enhance osteogenic differentiation of SCAPs in both two-dimensional (2D) cultures and three-dimensional (3D) nano-hydroxyapatite scaffold models.

Methods: SCAP-derived exosomes were isolated and characterized using transmission electron microscopy, nanoparticle tracking analysis, protein quantification, and microRNA profiling. SCAPs were treated with *L. salivarius*, exosomes, or their combination. Cell viability was assessed by MTT assay, while osteogenic differentiation was evaluated using alkaline phosphatase (ALP) activity, Alizarin Red S staining, immunofluorescence for BMP2 and osteocalcin, and qPCR analysis of osteogenic markers and PI3K/Akt/mTOR, Wnt/ β -catenin, PTEN, and MAPK signaling pathways.

Results: None of the treatments affected cell viability. The combined treatment produced the greatest enhancement of osteogenic differentiation in both 2D and 3D cultures. Exosomes markedly promoted osteogenic maturation, whereas probiotic preconditioning primarily stimulated early osteogenic commitment through increased expression of ALP, RUNX2, BMP2, and BMP4. Combined treatment further increased osteogenic marker expression and activated PI3K/Akt/mTOR, MAPK, and Wnt/ β -catenin signaling pathways, indicating complementary biological mechanisms.

Conclusion: In this study, the combination of *Lactobacillus salivarius* preconditioning and SCAP-derived exosomes represents a novel strategy for enhancing scaffold-based osteogenesis. These findings support the development of next-generation bioactive bone substitutes incorporating probiotics and extracellular vesicles for regenerative dentistry and bone tissue engineering.

Parazitske ekstracelularne vezikule kao prirodni imunomodulatori: mehanizmi delovanja i terapijski potencijal u respiratornim alergijama

Sofija Glamočija Jekić¹, Anna Schmid², Nataša Radulović³, Alisa Gruden-Movsesijan¹, Ljiljana Sabljic¹, Sergej Tomić¹, Jelena Đokić⁴, Irma Schabussova², [Maja Kosanović¹](#)

¹Institut za primenu nuklearne energije – INEP, Univerzitet u Beogradu, Beograd, Srbija; ²Institut za specifičnu profilaksu i tropsku medicinu, Medicinski univerzitet u Beču, Beč, Austrija; ³Institut za biološka istraživanja „Siniša Stanković“, Univerzitet u Beogradu, Beograd, Srbija; ⁴Institut za molekularnu genetiku i genetičko inženjerstvo, IMGGI, Univerzitet u Beogradu, Beograd, Srbija

Paraziti imaju sposobnost da modulišu imunski odgovor domaćina. Poznato je da su ključni nosioci ove sposobnosti ekstracelularne vezikule (EV) parazita. Cilj ove studije je bio da se utvrde karakteristike EV iz mišićnih larvi *Trichinella spiralis* (TsEVs) i da se ispita njihov uticaj na imunski odgovor, kako u in vitro modelu tj. na humane dendritske ćelije (DC), tako i in vivo modelu tj. modelu respiratorne alergije kod miša.

TsEVs su izolovane iz parazitskih ekskretorno-sekretornih produkata diferencijalnim centrifugiranjem/ultrafiltracijom i okarakterisane.

Tretman DC sa TsEVs je doveo do razvoja populacije tolerogenih DC, koje usmeravaju polarizaciju T ćelijskog odgovora u regulatornom i Th2 pravcu. Ovaj fenotipski pomak bio je praćen aktivacijom ključnih regulatornih signalnih kaskada i rezultirao je efikasnom indukcijom kako konvencionalnih FoxP3⁺ regulatornih T ćelija tako i FoxP3-negativnih IL-10-produktivnih Tr1 ćelija. Tolerogene osobine DC zadržane su čak i u prisustvu inflamatornog stimulusa, što naglašava robustnost TsEV-posredovane imunomodulacije.

Efikasnost imunomodulacije posredstvom TsEVs in vivo ispitana je u modelu ovalbuminom indukovane alergijske upale disajnih puteva BALB/c miševa. Intranazalna primena TsEVs je smanjila regrutaciju eozinofila u disajne puteve i pluća, smanjila produkciju OVA-specifičnog IgE i suprimirala sekreciju Th2 citokina (IL-4, IL-5, IL-13). TsEVs su istovremeno promovisale ekspanziju populacija regulatornih T ćelija (CD4⁺FoxP3⁺ i CD4⁺FoxP3⁻IL-10⁺) u plućima i slezini i pojačale lokalnu i sistemsku produkciju IL-10. Ex vivo analize ćelija iz pluća i slezine miševa sa alergijom potvrdile su TsEVs posredovanu modulaciju Imunskog odgovora pokrenutog alergenom.

Dobijeni rezultati pozicioniraju TsEVs kao prirodne, evoluciono usavršene imunomodulatorne nanopartikule sposobne da indukuju toleranciju i obuzdaju inflamaciju, čije dalje istraživanje može dovesti do razvoja inovativnih bioloških lekova za lečenje respiratornih alergijskih oboljenja.

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Parasite-derived extracellular vesicles as natural immunomodulators: mechanisms of action and therapeutic potential in respiratory allergies

Sofija Glamočija Jekić¹, Anna Schmid², Nataša Radulović³, Alisa Gruden-Movsesijan¹, Ljiljana Sabljic¹, Sergej Tomić¹, Jelena Đokić⁴, Irma Schabussova², [Maja Kosanović¹](#)

¹Institute for the Application of Nuclear Energy – INEP, University of Belgrade, Belgrade, Serbia; ²Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria; ³Institute for Biological Research “Siniša Stanković”, University of Belgrade, Belgrade, Serbia; ⁴Institute of Molecular Genetics and Genetic Engineering, IMGGI, University of Belgrade, Belgrade, Serbia

Parasites are capable of modulating the host immune response. Extracellular vesicles (EVs) of parasites are recognized as key mediators of this capacity. The aim of this study was to determine the characteristics of EVs derived from muscle larvae of *Trichinella spiralis* (TsEVs) and to examine their effects on the immune response, both in an in vitro model using human dendritic cells (DCs) and in an in vivo model of respiratory allergy in mice.

TsEVs were isolated from parasite excretory–secretory products by differential centrifugation/ultrafiltration and characterized.

Treatment of DCs with TsEVs led to the development of a tolerogenic DC population that directed T-cell polarization toward regulatory and Th2 responses. This phenotypic shift was accompanied by activation of key regulatory signaling cascades and resulted in efficient induction of both conventional FoxP3⁺ regulatory T cells and FoxP3-negative IL-10–producing Tr1 cells. The tolerogenic properties of DCs were retained even in the presence of an inflammatory stimulus, underscoring the robustness of TsEV-mediated immunomodulation.

The in vivo efficacy of TsEV-mediated immunomodulation was assessed in a model of ovalbumin-induced allergic airway inflammation in BALB/c mice. Intranasal administration of TsEVs reduced eosinophil recruitment to the airways and lungs, decreased production of OVA-specific IgE, and suppressed secretion of Th2 cytokines (IL-4, IL-5, IL-13). Concurrently, TsEVs promoted expansion of regulatory T-cell populations (CD4⁺FoxP3⁺ and CD4⁺FoxP3⁻IL-10⁺) in the lungs and spleen and enhanced local and systemic IL-10 production. Ex vivo analyses of lung and spleen cells from allergic mice confirmed TsEV-mediated modulation of the allergen-driven immune response.

These findings position TsEVs as naturally occurring, evolutionarily refined immunomodulatory nanoparticles capable of inducing tolerance and restraining inflammation, whose further investigation may lead to the development of innovative biologics for the treatment of respiratory allergic diseases.

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Kinetika oslobađanja ADSC-EVs iz hibridnog PMAA/PEGDA/želatin/kolagen sistema

Marija Milivojević¹, Maja Kosanović², Teodora Jakovljević¹, Đorđe Janačković³, Đorđe Veljović³

¹Inovacioni centar Tehnološko-metalurškog fakulteta u Beogradu d.o.o., Beograd, Srbija; (mmilivojevic@tmf.bg.ac.rs);

²Institut za primenu nuklearne energije, Univerzitet u Beogradu, Beograd, Srbija; ³Tehnološko-metalurški fakultet, Univerzitet u Beogradu, Beograd, Srbija

Primena ekstracelularnih vezikula (EVs) u regeneraciji koštanog tkiva ograničena je njihovim brzim uklanjanjem sa mesta koštanog defekta, što skraćuje trajanje njihovog lokalnog delovanja. Stoga razvoj nosača koji omogućavaju njihovu retenciju i kontrolisano oslobađanje predstavlja važan izazov u ovoj oblasti. U ovom istraživanju razvijen je hibridni makroporozni nosač koji predstavlja kombinaciju 3D štampanog hidrogela na bazi PMAA/PEGDA/želatina i hidrogela na bazi kolagena, koji je nosač EVs, sa ciljem da se omogući njihovo produženo lokalno oslobađanje.

EVs su izolovane iz kondicioniranog medijuma mezenhimskih stromalnih ćelija poreklom iz masnog tkiva pacova (rADSC) diferencijalnim centrifugiranjem, a njihova koncentracija, raspodela veličine i morfologija analizirane su analizom praćenja nanočestica (NTA) i elektronskom mikroskopijom. Makroporozni nosači na bazi PMAA/PEGDA/želatina proizvedeni su mSLA 3D štampom i odlikovali su se međusobno povezanim porama prosečnog prečnika 835 ± 58 μm . Inkorporacija EVs u prethodno izrađenu 3D strukturu vrši se neinvazivnim postupkom inkorporacije u nepolimerizovani kolagen, kojim se zatim ispunjavaju pore nosača i vrši geliranje. Za praćenje oslobađanja, EVs su fluorescentno obeležene PKH67 bojom i inkorporirane u kolagen tipa I u finalnoj koncentraciji od $1,6 \times 10^9$ EVs/mL. Oslobađanje EVs praćeno je tokom 14 dana u PBS-u na 37 °C primenom fluorescentne NTA.

Dobijeni rezultati pokazali su početnu bržu fazu oslobađanja, nakon koje je sledilo postepeno i produženo oslobađanje EVs tokom perioda praćenja. Približno polovina inkorporiranih EVs oslobođena je tokom prvih nekoliko dana, dok je do 14. dana kumulativno oslobođeno oko 80% početno inkorporiranih vezikula. Analiza kinetike profila oslobađanja pokazalo je najbolje slaganje sa Higuchi modelom, ukazujući na dominantan doprinos difuzije oslobađanju EVs iz nosača.

Predstavljeni hibridni sistem omogućava naknadnu inkorporaciju EVs u prethodno izrađenu 3D štampanu strukturu, čime se izbegava njihovo direktno izlaganje uslovima fotopolimerizacije, uz istovremeno omogućavanje lokalizovanog i produženog oslobađanja. Dobijeni rezultati ukazuju na potencijal ovog pristupa kao platforme za lokalnu isporuku EVs u regeneraciji koštanog tkiva.

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Release kinetics of ADSC-EVs from a hybrid PMAA/PEGDA/gelatin/collagen system

Marija Milivojević¹, Maja Kosanović², Teodora Jakovljević¹, Đorđe Janačković³, Đorđe Veljović³

¹Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia (mmilivojevic@tmf.bg.ac.rs);

²Institute for the Application of Nuclear Energy, University of Belgrade, Belgrade, Serbia; ³Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia

The application of extracellular vesicles (EVs) in bone tissue regeneration is limited by their rapid clearance from the site of the bone defect, which reduces the duration of their local activity. Therefore, the development of delivery systems capable of retaining EVs and enabling their controlled release represents an important challenge in this field. In this study, a hybrid macroporous scaffold combining a 3D-printed PMAA/PEGDA/gelatin-based hydrogel with an EV-loaded collagen hydrogel was developed to enable prolonged local EV release.

EVs were isolated from the conditioned medium of rat adipose-derived mesenchymal stromal cells (rADSCs) by differential centrifugation, and their concentration, size distribution, and morphology were analyzed by nanoparticle tracking analysis (NTA) and electron microscopy. Macroporous PMAA/PEGDA/gelatin-based scaffolds were fabricated by mSLA 3D printing and exhibited interconnected pores with an average diameter of $835 \pm 58 \mu\text{m}$. EVs were incorporated into the pre-fabricated 3D structure using a non-invasive approach in which they were first dispersed in non-polymerized collagen, which was subsequently used to fill the scaffold pores and allowed to gel. For release studies, EVs were fluorescently labeled with PKH67 and incorporated into type I collagen at a final concentration of 1.6×10^9 EVs/mL. EV release was monitored over 14 days in PBS at 37 °C using fluorescence NTA.

The results showed an initial phase of faster release, followed by gradual and sustained EV release throughout the monitoring period. Approximately half of the incorporated EVs were released during the first few days, while by day 14, approximately 80% of the initially incorporated vesicles had been cumulatively released. Kinetic analysis of the release profile showed the best fit to the Higuchi model, indicating that diffusion was the predominant mechanism governing EV release from the scaffold.

The presented hybrid system enables the post-fabrication incorporation of EVs into a pre-formed 3D-printed structure, thereby avoiding their direct exposure to photopolymerization conditions while simultaneously providing localized and sustained release. These findings indicate the potential of this approach as a platform for local EV delivery in bone tissue regeneration.

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Ekstracelularne vezikule u osteoartritisu: terapija nove generacije bez primene ćelija

Vesna Spasovski

Institut za molekularnu genetiku i genetičko inženjerstvo, Univerzitet u Beogradu, Beograd, Srbija

Jedan od glavnih trendova u regenerativnoj medicini je usmeren na razvoj terapija bez upotrebe živih ćelija, pri čemu se ekstracelularne vezikule (EV) poreklom iz matičnih ćelija sve više prepoznaju kao perspektivni terapeutici nove generacije. Male ekstracelularne vezikule (sEV) koje oslobađaju mezenhimske matične ćelije (MMĆ) nose u svom lumenu proteine, lipide i regulatorne RNK koje mogu da moduliraju inflamaciju, lučenje proteina vanćelijskom matriksa i popravku tkiva.

Osteoartritis (OA) je degenerativno oboljenje hrskavice, koje se javlja u kasnijem životnom dobu, ali i ranije kod osoba sa predisponirćim faktorima kao što je gojaznost, ili intenzivno habanje usled preopterećenja, kao što je slučaj kod sportista. U terapiji osteoartritisa (OA) pokazano je povoljno dejstvo MMĆ, a sve više dokaza ukazuje da se povoljni efekti ostvaruju pretežno putem njihove parakrine aktivnosti, naročito preko EV i njihovog bioaktivnog sadržaja. Njihova sposobnost da utiču na inflamatorne puteve, funkciju hondrocita i tkivnu homeostazu čini EV atraktivnim kandidatima za terapijske strategije bez ćelija.

Važan rastući pravac u regenerativnoj medicini zasnovanoj na EV jeste prelazak sa proučavanja naivnih EV ka EV poreklom iz prekondicioniranih ili stimulisanih ćelija, sa ciljem da se pojača njihov terapijski potencijal i prilagodi njihov molekularni teret specifičnim patološkim stanjima.

U tom kontekstu ispitivali smo karakteristike EV poreklom iz matičnih ćelija i procenili njihove biološke efekte u in vitro modelu OA. Proučavan je njihov efekat na inflamatorne puteve i signalne puteve sinteze proteina vanćelijskog matriksa hrskavice. Ovaj pristup pruža uvid u interakciju između EV i OA ćelija i omogućava poređenje terapijskog potencijala naivnih i EV dobijenih stimulacijom.

Extracellular vesicles in osteoarthritis: new generation of biological cell-free therapy

Vesna Spasovski

Institute of Molecular Genetics and Genetic engineering, University of Belgrade, Belgrade, Serbia

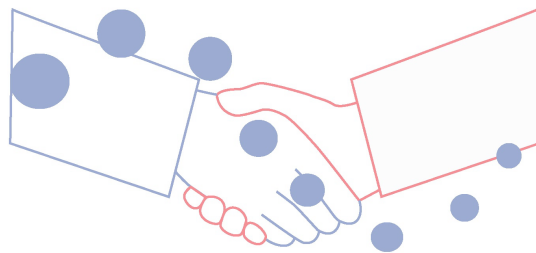
A major emerging trend in regenerative medicine is the shift from cell-based therapies toward cell-free approaches, with stem cell-derived extracellular vesicles (EVs) increasingly recognized as promising next-generation therapeutic agents. Small extracellular vesicles (sEVs) released by mesenchymal stem cells (MSCs) carry a complex cargo of proteins, lipids, and regulatory RNAs that can modulate inflammation, cellular responses, and tissue repair.

In osteoarthritis (OA), the beneficial effects of MSCs have been demonstrated, with increasing evidence suggesting that these effects are mediated predominantly through their paracrine activity, particularly via EVs and their bioactive cargo. Their ability to influence inflammatory pathways, chondrocyte function, and tissue homeostasis makes EVs attractive candidates for cell-free therapeutic strategies.

An important emerging direction in EV-based regenerative medicine is the transition from studying naïve EVs toward EVs derived from preconditioned or stimulated cells, with the aim of enhancing their therapeutic potential and tailoring their molecular cargo to specific pathological conditions.

In this context, we investigated the characteristics of stem cell-derived EVs and evaluated their biological effects in an in vitro cell culture model of OA. EVs were applied to OA- cell culture model to assess their ability to modulate disease-associated responses, particularly inflammation and cartilage-related functions. This approach provides insight into the interaction between EVs and the OA cells and allows comparison of the therapeutic potential of naïve and stimulation-derived EVs.

Poster prezentacije | Poster presentations



Fundamentalna istraživanja EV

Fundamental EV research

Od sekvestracije metala do oslobađanja nanočestica: divergentni odgovori *Chlorella sorokiniana* na mangan i bakar

Jelena Danilović Luković^{1*}, Anna Romolo², Matej Hočevár³, Darja Feizpour³, Barbara Šetina Batič³, Tina Sever³, Isidora Santrač¹, Marija Tanović¹, Valentina Ćurić⁴, Ana Obaha⁵, Jan Dolinar⁶, Andrej Race⁷, Aleš Iglič⁸, Vankata Subba Rao Jampani⁹, Marko Novinec⁵, Ivan Jerman⁷, Veronika Kralj-Iglič²

¹Institut za multidisciplinarna istraživanja, Univerzitet u Beogradu, Beograd, Srbija; ²Univerzitet u Ljubljani, Fakultet zdravstvenih nauka, Ljubljana, Slovenija; ³Institut za metale i tehnologiju, Ljubljana, Slovenija; ⁴Univerzitet u Beogradu, Biološki fakultet, Beograd, Srbija; ⁵Univerzitet u Ljubljani, Fakultet za hemiju i hemijsku tehnologiju, Ljubljana, Slovenija; ⁶Univerzitet u Ljubljani, Fakultet fizike, Ljubljana, Slovenija; ⁷Nacionalni institut za hemiju, Ljubljana, Slovenija; ⁸Univerzitet u Ljubljani, Fakultet elektrotehnike, Ljubljana, Slovenija; ⁹Institut Jožef Stefan, Ljubljana, Slovenija; *(ognjana@imsi.bg.ac.rs)

Izlaganje zelene mikroalge *Chlorella sorokiniana* Mn i Cu izaziva različite ćelijske odgovore. Dok se oba metala inicijalno zadržavaju na površini ćelije, Mn se postepeno redistribuira u intracelularne fosfatne komplekse i klastere bogate Mn. Cu, s druge strane, uglavnom ostaje povezan sa ćelijskim omotačem putem detoksikacije posredovane fosfatima. Budući da je nedavno pokazano da se izbacivanje teških metala putem formiranja nanočestica (engl. nanoparticles, NPs) može odvijati u ćelijama sisara *in vitro*, ovim istraživanjem je ispitano da li se sličan mehanizam javlja kod mikroalgi. Kulture *C. sorokiniana* bile su izložene 1 mM MnCl₂ ili CuCl₂ tokom 72 h, nakon čega su prebačene u svež hranjivi medijum i tokom narednog sata praćene u pogledu produkcije NPs primenom transmisione elektronske mikroskopije (TEM), u kombinaciji sa spektroskopijom X-zraka (EDS). EDS analiza pokazala je različitu raspodelu elemenata među NPs, precipitatima i regionima površine ćelije nakon tretmana Mn i Cu. U grupi tretiranoj Mn, male NPs (100–300 nm) bile su obogaćene Mn, dok su velike NPs (>300 nm) sadržale veće količine fosfora, što ukazuje na raspodelu elemenata zavisno od veličine NPs. Nasuprot tome, Cu je bio ravnomernije raspoređen između NPs i različitih regiona na površini ćelije. NPs nastale nakon tretmana Cu su pokazivale niži sadržaj fosfora u poređenju sa NPs nakon tretmana Mn. Ovo ukazuje na razlike u raspodeli elemenata i sastavu mineralne faze. Elementalni profil NPs povezanih sa Cu u skladu je sa prethodno opisanim putem detoksikacije u *C. sorokiniana*, pri kojem se Cu prvenstveno imobilizuje na površini ćelije vezujući se za fosfate. Nasuprot tome, poznato je da se Mn nakuplja u intracelularnim kompartmentima koji su bogati fosfatima, nakon 72 h od tretmana, formirajući stabilne depozite bogate Mn. Naši preliminarni rezultati ukazuju na mogućnost da se male količine Mn takođe izbacuju iz ćelije u obliku NPs nakon prebacivanja u svež medijum. Stoga bi sinteza NPs mogla predstavljati dodatni mehanizam eliminacije teških metala kod mikroalgi, što je potrebno potvrditi daljim istraživanjima.

From metal sequestration to nanoparticle release: divergent manganese and copper responses in *Chlorella sorokiniana*

Jelena Danilović Luković^{1*}, Anna Romolo², Matej Hočevár³, Darja Feizpour³, Barbara Šetina Batič³, Tina Sever³, Isidora Santrač¹, Marija Tanović¹, Valentina Ćurić⁴, Ana Obaha⁵, Jan Dolinar⁶, Andrej Race⁷, Aleš Iglič⁸, Vankata Subba Rao Jampani⁹, Marko Novinec⁵, Ivan Jerman⁷, Veronika Kralj-Iglič²

¹Institute of Multidisciplinary Research, University of Belgrade, Belgrade, Serbia; ²University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia; ³Institute of Metals and Technology, Ljubljana, Slovenia; ⁴University of Belgrade, Faculty of Biology, Belgrade, Serbia; ⁵University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia; ⁶University of Ljubljana, Faculty of Physics, Ljubljana, Slovenia; ⁷National Institute of Chemistry, Ljubljana, Slovenia; ⁸University of Ljubljana, Faculty of Electrical Engineering, Ljubljana, Slovenia; ⁹J. Stefan Institute, Ljubljana, Slovenia; *(ognjana@imsi.bg.ac.rs)

Exposure of the green microalga *Chlorella sorokiniana* to Mn and Cu elicits distinct cellular responses. While both metals are initially retained at the cell surface, Mn is progressively redistributed into intracellular phosphate complexes and Mn-rich clusters. Cu remains primarily associated with the cell envelope through phosphate-mediated detoxification. As heavy metal extrusion via nanoparticles (NPs) formation has recently been reported in mammalian cells *in vitro*, we investigated whether a similar mechanism occurs in microalgae. *C. sorokiniana* cultures were exposed to 1 mM MnCl₂ or CuCl₂ for 72 h, transferred to fresh growth medium, and monitored for NPs production during the following hour using transmission electron microscopy (TEM) coupled with energy-dispersive X-ray spectroscopy (EDS). EDS analysis revealed distinct elemental distributions among NPs, precipitates, and cell surface regions following Mn and Cu treatments. In the Mn-treated group, small NPs (100–300 nm) were enriched in Mn, whereas large NPs (> 300 nm) contained higher phosphorus levels, indicating size-dependent elemental partitioning. In contrast, Cu was more uniformly distributed among NPs and cell surface regions, while Cu-treated NPs exhibited lower phosphorus contents than Mn-treated NPs, suggesting differences in elemental incorporation and mineral phase composition. The elemental signatures of Cu-associated nanoparticles are consistent with the previously described detoxification pathway in *C. sorokiniana*, in which Cu is preferentially immobilized at the cell surface through phosphate-mediated binding. In contrast, Mn is known to be sequestered into intracellular phosphate-rich intermediates before forming stable Mn-rich deposits after 72 h of exposure. Our preliminary findings suggest that small amounts of Mn may also be expelled as NPs following transfer to fresh medium. Together, these observations suggest that NP production may represent an additional mechanism of heavy metal elimination in microalgae, although further investigation is needed.

Uticaj leta u svemir na ekstracelularne čestice u kulturi *Chlorella sorokiniana*, humanoj plazmi i hibridozomima jabuke

Anna Romolo¹, Matej Hočevár², Rok Podlipec³, Silvio Greco⁴, Rok Dolinar³, Eylul Gokce Acar¹, Yelena Istileulova¹, Jelena Danilović Luković⁵, Petra Knaus^{6,7}, Bence Matyas⁷, Roman Štukelj^{1,7}, Venkata SR Jampani³, Aleš Iglič⁸, Monika Jenko⁹, Veronika Kralj-Iglič¹

¹Univerzitet u Ljubljani, Fakultet zdravstvenih nauk, Ljubljana, Slovenija; ²Institut za metale i tehnologiju, Ljubljana, Slovenija; ³Institut Jožef Stefan, Ljubljana, Slovenija; ⁴Schaefer SEE, Trst, Italija; ⁵Institut za multidisciplinarna istraživanja, Univerzitet u Beogradu, Beograd, Srbija; ⁶Genoplant, Murski Črnci, Slovenija; ⁷Genesis Space Flight Laboratories, Čakovec, Hrvatska; ⁸Fakultet elektrotehnike, Ljubljana, Slovenija; ⁹MD-RI Istraživački institut za materijale u medicini

Ispitivali smo uticaj leta u svemir na ekstracelularne partikule (EP) u izolatu iz humane plazme, u kulturi *Chlorella sorokiniana* i u hibridozomima sastavljenim od sojinog lecitina, supernatanta iz postupka izolacije EP iz homogenata jabuke i glicerola. Uzorci u hermetički zatvorenim epruvetama od 1 mL lansirani su u svemir sa Esrange Space Center (Kiruna, Švedska) 31. maja 2026. kapsulom za povratak GEN-1P. Kapsula je dostigla visinu od 260 km, čime su uzorci bili izloženi mikrogravitaciji i atmosferskom povratku brzinom od 7 Maha, a istraživačima su vraćeni 16. juna 2026. Aliquoti uzoraka čuvani su u istom tipu zatvorenih epruveta u laboratoriji na Zemlji na 18 °C. Izolaciju EP vršili smo u termostatisanoj centrifugi pri $17.570 \times g$ i u ultracentrifugi pri $180.000 \times g$. U svim frakcijama je testirana koncentracija EP interferometrijskom svetlosnom mikroskopijom (ILM) i odabrana je frakcija sa najvišom vrednošću. Soj *Chlorella sorokiniana* CCAP 211/8K dobijen je iz Culture Collection of Algae and Protozoa (CCAP) (Oban, Škotska). Mikroalge su gajene u BBM medijumu u Erlenmajerovim tikvicama od 400 mL na orbitalnoj mućkalici (120 o/min) na 18 °C pri dnevnom svetlu. Hibridozomi su pripremljeni mešanjem jednakih masenih udela liofilizovanih granula sojinog lecitina sa supernatantom izolacije EP iz jabuka i glicerolom. Uzorak je čuvan 10 meseci na sobnoj temperaturi. Po povratku, hibridozomi su rastvoreni u fiziološkom rastvoru. Uzorke smo analizirali svetlosnom mikroskopijom, skenirajućom elektronskom mikroskopijom, laserskom fluorescentnom mikroskopijom, interferometrijskom svetlosnom mikroskopijom i UV-vis spektroskopijom. Uticaj hibridozoma testiran je in vivo na rast kulture *Chlorella sorokiniana*. Utvrđeno je da su svemirski uzorci plazme imali znatno veću koncentraciju EP (n) od zemaljskih uzoraka plazme ($(471 \pm 45) \times 10^9/\text{mL}$ i $(149 \pm 9) \times 10^9/\text{mL}$). Sadržaj nukleinskih kiselina i proteina u uzorcima EP iz plazme bio je, u proseku, jednak u oba tipa uzoraka. U svemirskom uzorku mikroalgi n je bio ispod granice detekcije, a u zemaljskom uzorku $(1,62 \pm 0,26) \times 10^9/\text{mL}$. Sadržaj nukleinskih kiselina i proteina u svemirskom uzorku bio je manji od 10% vrednosti zemaljskog uzorka. Dok su zemaljski uzorci sadržavali vitalne mikroalge, svemirski uzorci sadržavali su izdvojene ćelije sa smanjenom količinom hlorofila. Svemirski uzorak hibridozoma predstavljao je materijal manje sklon stvaranju vezikula pri razblaživanju; n uzorka lipozoma bio je $(380 \pm 91) \times 10^9/\text{mL}$ u poređenju sa $(1,740 \pm 681) \times 10^9/\text{mL}$ u zemaljskom uzorku, pri čemu je sadržaj nukleinskih kiselina i proteina bio veći u svemirskom nego u zemaljskom uzorku (absorbanca na 260 nm: $(4,67 \pm 0,0) \times 10^7$ arbitrarnih jedinica u svemirskom i $(2,96 \pm 0,0) \times 10^7$ u zemaljskom uzorku). Međutim, razlika in vivo efekta razblaženih svemirskih i zemaljskih hibridozoma na mikroalge nije se razlikovala unutar standardne devijacije. Ovi nalazi ukazuju da je svemirski let uticao na agregaciju i stabilnost EP, što ističe potrebu da se ovi efekti uzmu u obzir u budućim istraživanjima svemirske biologije. Koliko nam je poznato, ovo je prva studija svemirskog leta usmerena na EP.

Effect of space flight on extracellular particles in *Chlorellae sorokiniana* culture, human plasma and apple hybridosomes

Anna Romolo¹, Matej Hočevár², Rok Podlipec³, Silvio Greco⁴, Rok Dolinar³, Eylul Gokce Acar¹, Yelena Istileulova¹, Jelena Danilović Luković⁵, Petra Knaus^{6,7}, Bence Matyas⁷, Roman Štukelj^{1,7}, Venkata SR Jampani³, Aleš Iglič⁸, Monika Jenko⁹, Veronika Kralj-Iglič¹

¹University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia, ²Institute for Metals and Technology, Ljubljana, Slovenia, ³J Stefan Institute, Ljubljana, Slovenia, ⁴Schaefer SEE, Trieste, Italy, ⁵Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia, ⁶Genoplant, Murski Črnci, Slovenia, ⁷Genesis Space Flight Laboratories, Čakovec, Croatia, ⁸Faculty of Electrical Engineering, Ljubljana, Slovenia, ⁹MD-RI Research Institute for Materials in Medicine

We studied the effect of spaceflight on extracellular particles (EPs) in isolate from human plasma, *Chlorella sorokiniana* culture, and in hybridosomes composed of soya lecithin, supernatant of isolation procedure of EPs from apple homogenate, and glycerol. Samples in 1 mL sealed vials were launched into space from the Esrange Space Center (Kiruna, Sweden) on May 31, 2026, aboard the GEN-1P reentry capsule. The capsule reached an altitude of 260 km, exposing the samples to microgravity and atmospheric reentry at a speed of Mach 7, and the samples were returned to the researchers on June 16, 2026. The aliquots of the samples were kept in the same type of sealed vials in the laboratory on Earth at 18 °C. We performed isolation of EPs in a thermostated centrifuge at 17570xg, and in an ultracentrifuge at 180,000 × g. We tested all the fractions for the number density of EPs by Interferometric Light Microscopy (ILM) and chose the fraction that scored the highest. The *Chlorella sorokiniana* strain CCAP 211/8K was from the Culture Collection of Algae and Protozoa (CCAP) (Oban, Scotland). Microalgae grew in BBM medium in 400 mL Erlenmeyer flasks placed on the orbital shaker (120 rpm) at 18 °C at daylight. Hybridosomes were prepared by mixing equal weight proportions of lyophilized granules of soya lecithin with the supernatant of the isolation of EPs from apples and glycerol. The sample was kept for 10 months at room temperature. Upon return, hybridosomes were dissolved in saline. We assessed the samples with light microscopy, scanning electron microscopy, laser fluorescence microscopy, interferometric light microscopy, and UV-vis spectroscopy. The effect of hybridosomes was tested in vivo on the *Chlorella sorokiniana* culture growth. We found that the plasma space samples had a considerably higher number density of EPs (n) than the plasma Earth samples ($(471 \pm 45) \times 10^9/\text{mL}$, and $(149 \pm 9) \times 10^9/\text{mL}$, respectively). The nucleic acid and protein content of plasma EP samples was, on average, equal in both types. In the microalgae space sample, n was below the detection limit, and it was $(1.62 \pm 0.26) \times 10^9/\text{mL}$ in the Earth sample. The nucleic acid and protein content of the space sample was less than 10% of the Earth sample. While the Earth samples contained vital microalgae, the space samples contained segregated cells with a decreased amount of chlorophyll. Hybridosome space sample presented material that was less prone to produce vesicles when diluted; n of liposome sample was $(380 \pm 91) \times 10^9/\text{mL}$ comparing to $(1.740 \pm 681) \times 10^9/\text{mL}$ in the Earth sample with nucleic acid and protein content higher in the space sample than in the Earth sample (absorbance at 260 nm was $(4.67 \pm 0.0) \times 10^7$ arbitrary units in the space sample and $(2.96 \pm 0.0) \times 10^7$ in the Earth sample). However, the difference between the in vivo effect on microalgae of the diluted space and Earth hybridosomes did not differ within the standard deviation. These findings indicate that spaceflight impacted EP aggregation and stability which outlines the necessity to consider these effects in the future studies of space biology. To our best knowledge this is the first EP-directed spaceflight study.

Biomarkerski potencijal EV
Biomarker potential of EVs

Poređenje površinskih sijalo-glikana EV iz semene plazme normospermičnih i oligoastenospermičnih muškaraca

Filip Janjić, Miroslava Janković, Tamara Janković, Sanja Goč, [Ninoslav Mitić](#)

Institut za primenu nuklearne energije, Univerzitet u Beogradu, Beograd, Srbija

Semena plazma (sp) čoveka predstavlja bogat izvor ekstracelularnih vezikula (EV), pri čemu njihov najveći deo potiče od ćelija prostate. Ranija istraživanja pokazala su prisustvo sijalinizovanih i manozilovanih glikana na površini spEV kod normospermičnih (normalni broj spermatozoida; NspEV) muškaraca, dok podaci ukazuju da se površinska glikozilacija može menjati u različitim (pato)fiziološkim stanjima. Pored toga, metoda izolacije EV takođe može uticati na dostupnost ovih glikana i time menjati vezivanje lektina za EV populacije različitog dijametra. Cilj istraživanja bila je komparativna analiza površinske sijalinizacije spEV iz semene plazme normospermičnih i oligoastenospermičnih (smanjen broj i pokretljivost spermatozoida; OAspEV) muškaraca primenom F-NTA (fluorescent nanoparticle tracking analysis).

EV su izolovane primenom diferencijalnog ultracentrifugiranja i gel filtracije na Sepharose™ CL-2B koloni. Površinski glikani analizirani su korišćenjem fluorescentno obeleženih lektina: WGA (wheat germ agglutinin) iz pšeničnih klica i SNA (Sambucus nigra agglutinin) iz zove, specifični za različite sijalinizovane glikanske strukture. Metodom NTA/F-NTA određene su koncentracije ukupnih i lektin-pozitivnih čestica, i analizirana je njihova veličina.

Distribucija veličina se između NspEV i OAspEV nije razlikovala. Oko 80% vezikula u oba izolata pripadalo je populaciji malih EV (<200 nm), sa sličnom medijanom dijametra (142 nm za NspEV i 140 nm za OAspEV). Udeo SNA-pozitivnih EV bio je 38% niži u NspEV, dok je udeo WGA-pozitivnih EV bio približno tri puta veći nego u OAspEV. Kod WGA-pozitivnih EV, udeo malih vezikula bio je 25% niži u OAspEV, dok je udeo velikih vezikula (>200 nm) bio 30% veći. Udeo malih i velikih SNA-pozitivnih EV nije se znatno razlikovao između ispitivanih EV izolata. Medijana dijametra SNA-pozitivnih EV bila je slična ukupnoj EV populaciji (za NspEV 143 i OAspEV 146 nm), dok su WGA-pozitivne EV bile veće (173 i 178 nm, redom).

Uprkos sličnoj distribuciji veličina EV kod N i OA uzoraka, one su pokazale različite površinske sijalo-strukture. OAspEV su imale manju zastupljenost WGA-pozitivnih EV, pri čemu je među njima bio veći udeo vezikula >200 nm, dok su SNA-pozitivne EV pokazale manje izražene razlike između izolata. Kombinacija fluorescentnih lektina i NTA omogućava detekciju ovih razlika i pruža dodatni uvid u promene površinske sijalinizacije povezane sa smanjenom pokretljivošću spermatozoida.

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Comparison of surface sialoglycans of EVs from seminal plasma of normozoospermic and oligoasthenozoospermic men

Filip Janjić, Miroslava Janković, Tamara Janković, Sanja Goč, [Ninoslav Mitić](#)

Institute for the Application of Nuclear Energy, University of Belgrade, Belgrade, Serbia

Human seminal plasma (sp) is a rich source of extracellular vesicles (EVs), with the majority originating from prostate cells. Previous studies have demonstrated the presence of sialylated and mannosylated glycans on the surface of spEVs from normozoospermic men (normal sperm count; NspEV), while available evidence indicates that surface glycosylation may change under different (patho)physiological conditions. In addition, the EV isolation method may affect glycan accessibility and consequently alter lectin binding to EV populations of different diameters. The aim of this study was to comparatively analyze the surface sialylation of spEVs from the seminal plasma of normozoospermic and oligoasthenozoospermic men (reduced sperm count and motility; OAspEV) using F-NTA (fluorescent nanoparticle tracking analysis).

EVs were isolated by differential ultracentrifugation followed by gel filtration on a Sepharose™ CL-2B column. Surface glycans were analyzed using fluorescently labeled lectins: wheat germ agglutinin (WGA) and Sambucus nigra agglutinin (SNA), which recognize distinct sialylated glycan structures. NTA/F-NTA was used to determine the concentrations of total and lectin-positive particles and to analyze their size distribution.

The size distribution did not differ between NspEVs and OAspEVs. Approximately 80% of vesicles in both isolates belonged to the small EV population (<200 nm), with similar median diameters (142 nm for NspEVs and 140 nm for OAspEVs). The share of SNA-positive EVs was 38% lower in NspEVs, whereas the share of WGA-positive EVs was approximately threefold higher than in OAspEVs. Among WGA-positive EVs, the share of small vesicles was 25% lower in OAspEVs, whereas the share of large vesicles (>200 nm) was 30% higher. The shares of small and large SNA-positive EVs did not differ substantially between the EV isolates. The median diameter of SNA-positive EVs was similar to that of the total EV population (143 nm for NspEVs and 146 nm for OAspEVs), whereas WGA-positive EVs were larger (173 and 178 nm, respectively).

Despite the similar EV size distributions in N and OA samples, the isolates exhibited distinct surface sialo-structures. OAspEVs showed a lower share of WGA-positive EVs, with a higher share of vesicles >200 nm within this population, whereas SNA-positive EVs showed less pronounced differences between the isolates. The combination of fluorescent lectins and NTA enables the detection of these differences and provides additional insight into alterations in EV surface sialylation associated with reduced sperm motility.

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Nivo ekspresije miR-146a-5p u serumskim ekstraćelijskim vezikulama trudnica sa gestacijskim dijabetesom negativno je korelisan sa ekspresijom gena IL1B, CXCL8 i TNF u mononuklearnim ćelijama periferne krvi

Jovana Stevanović¹, Ninoslav Mitić¹, Milica Mandić², Dragana Maglić², Vesna Mandić-Marković^{2,3}, Iva Vasiljević⁴, Miloš Brkušanin⁴, Olgica Nedić¹, Zorana Dobrijević¹

¹Institut za primenu nuklearne energije, Univerzitet u Beogradu, Beograd, Srbija; ²Ginekološko-akušerska klinika "Narodni front", Beograd, Srbija; ³Medicinski fakultet, Univerzitet u Beogradu, Beograd, Srbija; ⁴Univerzitet u Beogradu, Biološki fakultet, Beograd, Srbija

Gestacijski dijabetes (GD) karakteriše izmenjeni obrazac ekspresije molekula mikro RNK u odnosu na normoglikemičnu trudnoću. Takođe, GD je asociran sa hroničnom inflamacijom niskog intenziteta, a koja je u osnovi razvoja različitih komplikacija trudnoće i porođaja vezanih za GD. Naše ranije istraživanje pokazalo je povišen nivo miR-146a-5p poreklom iz serumskih ekstraćelijskih vezikula (EV) kod trudnica sa GD, a koja je jedna od ključnih mikro RNK regulatora inflamacije. S obzirom na funkcionalna svojstva navedene mikro RNK, cilj istraživanja bio je da se ispita potencijalna korelacija između nivoa miR-146a-5p u EV trudnica sa GD i ekspresije proinflamatornih citokina u mononuklearnim ćelijama periferne krvi (MČPK). Analiza je uključivala uzorke 36 trudnica sa GD i 32 normoglikemične trudnice sličnog gestacijskog doba koje su činile kontrolnu grupu. EV su izolovane diferencijalnim ultracentrifugiranjem iz uzoraka seruma i karakterisane pomoću analize praćenja nanopartikula, transmisione elektronske mikroskopije i Western blot-a. Analiza ekspresije miR-146a-5p, IL6, IL1B, CXCL8 i TNF sprovedena je pomoću qPCR-a. Rezultati su pokazali negativnu korelaciju između nivoa ekspresije miR-146a-5p i IL1B ($r = -0.455$), CXCL8 ($r = -0.356$) i TNF ($r = -0.380$). U slučaju IL6, koji je bio i jedini gen za citokine sa značajno izmenjenom ekspresijom u GD, nije ustanovljena korelacija sa nivoom ekspresije miR-146a-5p iz EV. Navedene korelacije ustanovljene su isključivo u grupi trudnica sa GD, dok su izostale u kontrolnoj grupi. Rezultati ukazuju na potencijalni značaj miR-146a-5p iz EV u regulaciji ekspresije gena za proinflamatorne citokine u MČPK u hiperglikemičnoj trudnoći, a koji je neophodno potvrditi kroz mehanističke studije.

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The expression level of miR-146a-5p in serum extracellular vesicles of pregnant women with gestational diabetes is negatively correlated with the expression of the IL1B, CXCL8, and TNF genes in peripheral blood mononuclear cells

Jovana Stevanović¹, Ninoslav Mitić¹, Milica Mandić², Dragana Maglić², Vesna Mandić-Marković^{2,3}, Iva Vasiljević⁴, Miloš Brkušanin⁴, Olgica Nedić¹, Zorana Dobrijević¹

¹Institute for the Application of Nuclear Energy, University of Belgrade, Belgrade, Serbia; ²Clinic of Gynecology and Obstetrics "Narodni front", Belgrade, Serbia; ³Faculty of Medicine, University of Belgrade, Belgrade, Serbia;

⁴University of Belgrade, Faculty of Biology, Belgrade, Serbia

Gestational diabetes (GD) is characterized by an altered pattern of microRNA expression relative to normoglycemic pregnancy. GD is also associated with low-grade chronic inflammation, which underlies the development of various pregnancy and delivery complications related to GD. Our previous study showed an elevated level of miR-146a-5p originating from serum extracellular vesicles (EVs) in pregnant women with GD, a microRNA that is a key regulator of inflammation. Given the functional properties of this microRNA, the aim of the study was to examine a potential correlation between the level of miR-146a-5p in EVs of pregnant women with GD and the expression of proinflammatory cytokines in peripheral blood mononuclear cells (PBMCs). The analysis included samples from 36 pregnant women with GD and 32 normoglycemic pregnant women of similar gestational age who constituted the control group. EVs were isolated from serum samples by differential ultracentrifugation and characterized by nanoparticle tracking analysis, transmission electron microscopy, and Western blot. Expression of miR-146a-5p, IL6, IL1B, CXCL8, and TNF was analyzed by qPCR. The results showed a negative correlation between the expression level of miR-146a-5p and IL1B ($r = -0.455$), CXCL8 ($r = -0.356$), and TNF ($r = -0.380$). For IL6, which was also the only cytokine gene with significantly altered expression in GD, no correlation with the EV-derived miR-146a-5p expression level was found. These correlations were observed exclusively in the GD group and were absent in the control group. The results indicate a potential role of EV-derived miR-146a-5p in regulating the expression of proinflammatory cytokine genes in PBMCs in hyperglycemic pregnancy, which needs to be confirmed by mechanistic studies.

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Metode u istraživanju EV

Methods in EV research

Kvalitet plazme za regeneraciju. Uticaj parametara centrifugiranja kod psa, konja i čoveka

Yelena Istileulova¹, Adrienn Molnar², Sara Michelini³, Anna Romolo¹, Matevž Arko¹, Maxence Berry¹, Boštjan Korenjak¹, Matej Hočevár⁴, Kaja Troha⁵, Aleš Iglič⁶, Damjana Drobne³, Saba Battelino⁵, Vladimira Erjavec⁷, Alenka Nemec Svete⁷, Gitta Schlosser², Veronika Kralj-Iglič¹

¹Univerzitet u Ljubljani, Fakultet zdravstvenih nauka, Ljubljana, Slovenija; ²MTA-ELTE Lendület (Momentum) istraživačka grupa za jonsko-mobilnu masenu spektrometriju, Prirodno-matematički fakultet, Institut za hemiju;

⁴Univerzitet u Ljubljani, Biotehnički fakultet, Ljubljana, Slovenija; ⁵Institut za metale i tehnologiju, Ljubljana, Slovenija; ⁶Klinika za otorinolaringologiju i cervikofacijalnu hirurgiju, Univerziteti klinički centar Ljubljana, Ljubljana, Slovenija; ⁷Fakultet elektrotehnike, Ljubljana, Slovenija; ⁸Univerzitet u Ljubljani, Veterinarski fakultet, Ljubljana, Slovenija

Autologna plazma priprema se iz sopstvene krvi pacijenta postupcima koji su jednostavni, jeftini i lako izvodljivi. Pri autolognoj primeni rizik od infekcija ili imunskog odbacivanja nizak je. Ovi postupci već se koriste u kliničkoj praksi u mnogim oblastima medicine; međutim, mehanizmi zarastanja još nisu u potpunosti razjašnjeni. Novija istraživanja ističu ekstracelularne vezikule (EV) u preparatima iz krvi kao vektore zarastanja. Ovde smo pripremili uzorke plazme iz krvi psa, konja i čoveka primenom različitih podešavanja centrifuge u jednostepenom centrifugiranju krvi. U plazmi smo odredili koncentraciju trombocita protočnom citometrijom i koncentraciju ekstracelularnih čestica interferometrijskom svetlosnom mikroskopijom (ILM). U odabranim uzorcima procenili smo markere inflamacije i faktore rasta ELISA testovima, markere oksidativnog stresa spektrofotometrijom i sadržaj lipida masenom spektrometrijom. Konstruisali smo matematički model sedimentacije eritrocita radi procene fizičko-hemijskih svojstava pojedinačne plazme. Izveli smo parametar srazmeran proizvodu vremena centrifugiranja i centripetalne akceleracije rotora centrifuge kao objedinjujući parametar za sve analizirane uzorke krvi. Utvrđeno je da su prinos trombocita, koncentracija faktora rasta i sadržaj lipida osetljivi na τ . Premalo veliko τ činilo je plazmu siromašnom trombocitima i faktorima rasta. Koncentracije EV bile su u opsegu 1010/mL, a njihova veličina nije zavisila od τ . Zaključujemo da priprema plazme značajno utiče na njen sastav. Posebno, predugo ili presnažno centrifugiranje može dovesti do osiromašenja plazme lipidima, trombocitima i faktorima rasta, ali ne i EV. Podešavanja centrifuge treba prilagoditi pojedinačnom uzorku na osnovu matematičkog modela

Zahvalnica: Zahvaljujemo se Slovenačkoj agenciji za istraživanje i inovacije na grantovima P1-0099, P2-0132, IO-0006, P2-0232, P3-0388, J2-60051, J3-60063 i N1-0435.

Quality of plasma for regeneration. Effect of the centrifugation parameters in dog, horse and human

Yelena Istileulova¹, Adrienn Molnar², Sara Michelini³, Anna Romolo¹, Matevž Arko¹, Maxence Berry¹, Boštjan Korenjak¹, Matej Hočevár⁴, Kaja Troha⁵, Aleš Iglič⁶, Damjana Drobne³, Saba Battelino⁵, Vladimira Erjavec⁷, Alenka Nemec Svete⁷, Gitta Schlosser², Veronika Kralj-Iglič¹

¹University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia, ²MTA-ELTE Lendület (Momentum) Ion Mobility Mass Spectrometry Research Group, Faculty of Science, Institute of Chemistry, ³University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia, ⁴Institute for Metals and Technology, Ljubljana, Slovenia, ⁵Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana, Ljubljana, Slovenia, ⁶Faculty of Electrical Engineering, Ljubljana, Slovenia, ⁷University of Ljubljana, Veterinary Faculty, Ljubljana, Slovenia

Autologous plasma is prepared from the patient's own blood following procedures that are simple, low cost, and easy to perform. With autologous application, there is a low risk of infections or immune rejection. Moreover, the procedures are already being used in clinical practice in many fields of medicine; however, the healing mechanisms are not yet completely understood. Recent developments have outlined extracellular vesicles (EVs) in preparations from blood as the healing vectors. Here, we have prepared plasma samples from canine, equine, and human blood by applying different centrifuge settings to one-step centrifugation of blood. In plasma, we have determined platelet number density by flow cytometry and extracellular particle number density by interferometric light microscopy (ILM). In selected samples, we have assessed inflammation markers and growth factors by ELISA tests, oxidative stress markers by spectrophotometry, and lipid content by mass spectrometry. We have constructed a mathematical model of erythrocyte sedimentation to assess the physicochemical properties of individual plasma. We outlined a parameter that is proportional to the product of the time of centrifugation and centripetal acceleration of the centrifuge rotor as a unifying parameter for all blood samples analyzed. We found that platelet yield, concentration of growth factors, and lipid content were sensitive to τ . Too large τ rendered plasma poor in platelets and growth factors. EV number densities were in the range of 1010/mL, and their size was insensitive to τ . To conclude, plasma preparation significantly affects its composition. In particular, too long or too strong centrifugation may result in the impoverishment of plasma with lipids, platelets, and growth factors, but not with EVs. The settings of the centrifuge should be made specific for the individual sample based on the mathematical model.

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Uticaj različitih metoda za uklanjanje kazeina na izolaciju ekstracelularnih vezikula iz kozjeg mleka

Filip Janjić, Ninoslav Mitić, Sanja Goč, Tamara Janković, Miroslava Janković

Institut za primenu nuklearne energije - INEP, Univerzitet u Beogradu, Beograd, Srbija

Uvod: Ekstracelularne vezikule (EV) iz mleka privlače posebnu pažnju zbog visoke biokompatibilnosti kao i otpornosti na kiselu sredinu želuca, digestivne enzime i žučne soli, što ih čini potencijalno pogodnim nanonosačima za oralnu isporuku lekova i nukleinskih kiselina. U istraživanjima se, najčešće, koriste EV iz kravljeg mleka, dok su EV iz kozjeg mleka znatno manje proučavane. Izolacija EV iz mleka, generalno, predstavlja metodološki izazov zbog visokog sadržaja kazeina koji se koizoluje kao kontaminant. Razlike u sastavu kravljeg i kozjeg mleka, uključujući zastupljenost kazeinskih frakcija, mogle bi biti od značaja za izolaciju EV i uticati na biološku aktivnost izolata. U cilju procene uticaja na prinos i biofizička svojstva izolovanih EV, u ovom radu je vršena komparativna analiza izolata iz kozjeg mleka u kome je sadržaj kazeina smanjen primenom dva različita postupka.

Materijal i metode: EV su izolovane iz kozjeg mleka nabavljenog u maloprodaji. Nakon centrifugiranja ($2000 \times g$, 20 minuta na 4°C) radi uklanjanja mlečne masti, somatskih ćelija i ćelijskih ostataka, sadržaj kazeina u mleku je smanjen metodom zakišeljavanja ili heliranjem jona kalcijuma. Precipitacija kazeina vršena je dodavanjem 6 M HCl do vrednosti pH 4,6, dok je disocijacija kazeinskih micela vršena dodavanjem 0,25 M EDTA (1:1), uz inkubaciju od 15 minuta na 4°C . Nakon toga, kazein je uklonjen centrifugiranjem na $10000 \times g$, 30 minuta na 4°C . Dobijeni supernatant je, zatim, centrifugiran na $17000 \times g$, 30 minuta na 4°C i na $100000 \times g$, 1 h na 4°C (dva puta). Izdvojeni talog koji sadrži EV je resuspendovan u 50 mM fosfatnom puferu, pH 7,4 i izolati su analizirani transmisijom elektronskom mikroskopijom (TEM) i NTA metodom (*nanoparticle tracking analysis*).

Rezultati: U EV-EDTA izolatu kao i u EV-HCl izolatu, primenom TEM je detektovano prisustvo i svetlih i tamnih čestica koje su imale morfologiju karakterističnu za EV. Razlika je uočena u pogledu agregacije EV i prisustva amorfne proteinske supstance koji su bili izraženiji u EV-EDTA nego u EV-HCl. Koncentracija čestica, određena NTA metodom, je iznosila $2,6 \times 10^{13}$ čestica/mL u EV-EDTA i $4,5 \times 10^{11}$ čestica/mL u EV-HCl. Čestice veličine 50–200 nm su činile 85,9% svih detektovanih čestica u EV-EDTA i 88,2% u EV-HCl. Medijana veličine čestica bila je 126 nm za EV-EDTA i 124 nm za EV-HCl.

Zaključak: EV-EDTA i EV-HCl se nisu, znatno, razlikovale po distribuciji i medijani veličine čestica. Prinos izolovanih EV-EDTA čestica je, međutim, bio oko 57 puta veći nego prinos EV-HCl čestica. Pored toga, amorfni materijal koji bi mogao poticati od kontaminanata je bio izrazito uočljiv u EV-EDTA. Bez obzira na manji prinos, EV-HCl su imale bolju čistoću i integritet čestica, zbog čega bi zakišeljavanje moglo predstavljati metodu izbora.

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The effect of different casein removal methods on the isolation of extracellular vesicles from goat milk

Filip Janjić, Ninoslav Mitić, Sanja Goč, Tamara Janković, Miroslava Janković

Institute for the application of nuclear energy – INEP, University of Belgrade, Belgrade, Serbia

Introduction: Milk-derived extracellular vesicles (EVs) are of particular interest due to their high biocompatibility and resistance to the acidic gastric environment, digestive enzymes, and bile salts, making them potentially suitable nanocarriers for the oral delivery of drugs and nucleic acids. Research most commonly utilizes EVs from bovine milk, whereas EVs from goat milk have been considerably less studied. The isolation of EVs from milk generally presents a methodological challenge due to the high casein content, which is co-isolated as a contaminant. Differences in the composition of bovine and goat milk, including the relative abundance of casein fractions, could be relevant for EV isolation and affect the biological activity of the isolates. To assess the impact on the yield and biophysical properties of isolated EVs, this study performed a comparative analysis of isolates from goat milk in which the casein content was reduced using two different procedures.

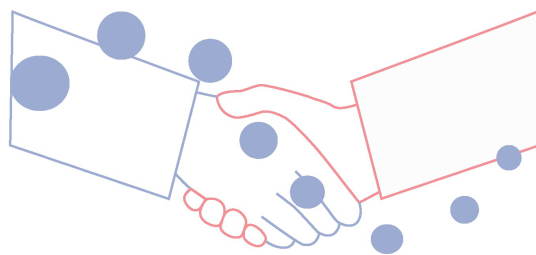
Materials and Methods: EVs were isolated from commercially available goat milk. Following centrifugation ($2,000 \times g$, 20 min at 4 °C) to remove milk fat, somatic cells, and cellular debris, the casein content in the milk was reduced either by acidification or by calcium ion chelation. Casein precipitation was performed by adding 6 M HCl to a pH of 4.6, whereas casein micelle dissociation was performed by adding 0.25 M EDTA (1:1) with incubation for 15 min at 4 °C. Subsequently, casein was removed by centrifugation at $10,000 \times g$ for 30 min at 4 °C. The resulting supernatant was then centrifuged at $17,000 \times g$ for 30 min at 4 °C and at $100,000 \times g$ for 1 h at 4 °C (twice). The isolated EV pellet was resuspended in 50 mM phosphate buffer, pH 7.4, and the isolates were analyzed by transmission electron microscopy (TEM) and *nanoparticle tracking analysis* (NTA).

Results: In both the EV-EDTA and EV-HCl isolates, TEM revealed the presence of both light and dark particles with morphology characteristic of EVs. Differences were observed in terms of EV aggregation and the presence of amorphous proteinaceous material, which were more pronounced in EV-EDTA than in EV-HCl. The particle concentration determined by NTA was 2.6×10^{13} particles/mL in EV-EDTA and 4.5×10^{11} particles/mL in EV-HCl. Particles ranging from 50–200 nm in size accounted for 85.9% of all detected particles in EV-EDTA and 88.2% in EV-HCl. The median particle size was 126 nm for EV-EDTA and 124 nm for EV-HCl.

Conclusion: EV-EDTA and EV-HCl did not differ substantially in particle size distribution or median particle size. However, the yield of isolated EV-EDTA particles was approximately 57-fold higher than that of EV-HCl particles. In addition, amorphous material that may have originated from contaminants was prominent in EV-EDTA. Despite the lower yield, EV-HCl exhibited greater purity and particle integrity, suggesting that acidification could be considered the method of choice.

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Milica Jovanović Krivokuća

Zorana Dobrijević

Jovana Kuveljić

Dragana Nikolić

Vesna Spasovski

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