

Opis raziskovalnega dela (Research work description)

1. Članica UL (UL member):

Veterinarska fakulteta (Veterinary Faculty)

2. Ime, priimek in elektronski naslov mentorja/ice (Mentor's name, surname and email):

Ana Nemec; ana.nemec@vf.uni-lj.si

3. Raziskovalno področje (Research field):

Veterina; Morfologija, fiziologija in reprodukcija živali (Veterinary medicine; Morphology, physiology, animal reproduction)

4. Opis raziskovalnega dela (Research work description):

Vključuje morebitne dodatne pogoje, ki jih mora izpolnjevati kandidat/ka za mladega raziskovalca/ko, ki niso navedeni v razpisu za mlade raziskovalce (*It includes any additional conditions that the candidate for a young researcher must meet, which are not listed in the call to tender for young researchers.*).

Slov.:

Raziskava bo temeljila na proučevanju značilnosti pasjih in konjskih matičnih celic in izvenceličnih veziklov, vzpostavitvi analize zunajceličnih veziklov s pretočno citometrijo, primerjavi terapevtskega potenciala matičnih celic s terapevtskim potencialom veziklov samih in preverjanju možnosti enkapsulacije izvenceličnih veziklov v biomateriale. Namen raziskave bo razvoj celičnega produkta za potencialno klinično uporabo v regenerativni veterinarski medicini.

V okviru programa usposabljanja bo mladi raziskovalec proučeval značilnosti pasjih in konjskih matičnih celic in njihovih zunajceličnih veziklov. Poleg uveljavljenih metod za karakterizacijo matičnih celic in veziklov (ki vključujejo Nanoparticle Tracking Analysis in elektronsko mikroskopijo), ki jih bomo izvedli na Veterinarski fakulteti v sodelovanju z Medicinsko fakulteto in Institutom Jozefa Stefana, bomo vzpostavili analizo zunajceličnih veziklov matičnih celic živali s pretočno citometrijo, kar bo omogočalo bolj natančno določanje fizikalnih in bioloških lastnosti veziklov ter prispevalo k standardizaciji postopkov za njihovo karakterizacijo. Terapevtski potencial matičnih celic in njihovih veziklov bomo analizirali z ugotavljanjem njihovega vpliva na celjenje z uporabo *in vitro* metode »scratch assay« ter na preverjanjem njihovega terapevtskega učinka z vidika imunomodulacije. Pri tem se bomo poslužili tehnike kokulture z imunskimi celicami ter ugotavljali kakšen vpliv imajo matične celice in vezikli na proliferacijo imunskih celic ter tako preverili sposobnost uravnavanja mehanizmov vnetnega odziva. Če bodo finančna sredstva to omogočala in če se bo izkazalo, da je terapevtski učinek izvenceličnih veziklov podoben ali boljši kot pri samih celicah, bomo vezikle enkapsulirali v različne biomateriale z namenom počasnega sproščanja in podaljšanja njihovega terapevtskega učinka, kar bo omogočilo premostitev ovire hitre eliminacije veziklov ob njihovi klinični aplikaciji.

Raziskava bo predstavljala pomemben napredek na področju regenerativnega zdravljenja v veterinarski medicini. Optimizacija metode pretočne citometrije bo omogočila bolj natančno določanje lastnosti veziklov ter prispevala k standardizaciji karakterizacije veziklov. Analiza veziklov s pretočno citometrijo bo na dolgi rok omogočila detekcijo različnih površinskih markerjev, kar nam omogoča iskanje kliničnih markerjev bolezni. Rezultati analize terapevtskega potenciala skupaj z enkapsulacijo veziklov v ustrezni biomaterial pa bo vodila k razvoju brezceličnega celičnega produkta, ki bi lahko nadomestil uporabo avtolognih matičnih celic. S tem bomo naslovili potrebo po razvoju stabilnejših oblik zdravljenja, ki bodo vodile do dostopnejših in rutinskih terapij.

Eng.:

The research will be based on studying the properties of stem cells and extracellular vesicles from dogs and horses, establishing the analysis of extracellular vesicles by flow cytometry, comparing the therapeutic potential of stem cells with the therapeutic potential of the vesicles themselves and investigating the possibility of encapsulating extracellular vesicles in biomaterials. The aim of the research is to develop a cell product for potential clinical use in regenerative veterinary medicine.

In addition to the established methods for the characterization of stem cells and vesicles (including nanoparticle tracking analysis and electron microscopy) performed at the Faculty of Veterinary Medicine and in collaboration with the Faculty of Medicine and the Jozef Stefan Institute, we will establish the analysis of extracellular vesicles of animal stem cells by flow cytometry, which will allow a more precise determination of the physical and biological

properties of the vesicles and contribute to the standardisation of characterization procedures. The therapeutic potential of stem cells and their vesicles will be analysed by determining their effect on healing using the in vitro scratch assay method. In addition, we will test their therapeutic effect in terms of immunomodulation by creating a coculture with immune cells to determine the effect of stem cells and vesicles on immune cell proliferation and thus their ability to regulate the mechanisms of the inflammatory response. If funding permits and the therapeutic effect of the extracellular vesicles proves to be similar to that of the cells themselves, we will encapsulate the vesicles in different biomaterials to slow their release and prolong their therapeutic effect, thus overcoming the barrier of rapid elimination of the vesicles at the time of their clinical application.

The research will represent an important advance in the field of regenerative therapies in veterinary medicine. The optimization of the flow cytometric method will allow a more precise characterization of the vesicles and contribute to the standardisation of vesicle characterization. The results of the analysis of the therapeutic potential, together with the encapsulation of the vesicles in a suitable biomaterial, will lead to the development of a cell-free cell product that could replace the use of autologous stem cells and address the need to develop more stable forms of treatment leading to more accessible and routine therapies.

5. Priloge, ki jih kandidat priloži k prijavi (*Documents that the candidate submits with the application*):

- ☒ **diplomska listina/potrdilo o zaključku študijskega programa** (*diploma certificate for study programme, with which the candidate has enrolled/ will enroll in a doctoral degree programme*)
- ☒ **priloga k diplomii/ potrdilo o opravljenih obveznostih** (*official transcript of all the grades for study programme, with which the candidate has enrolled/will enroll in a doctoral degree programme*)
- ☒ **potrdilo o do sedaj opravljenih obveznostih z ocenami študijskega programa, s katerim se bo kandidat prijavil na študij** (*official transcript of all the grades the candidate has received so far for the study programme, with which the candidate will enroll to a doctoral degree programme*)
- ☒ **nagrade** (*awards (e.g. Prešeren Prize of the University of Ljubljana, Prešeren Prize of a University of Ljubljana member and/or another equivalent award)*)
- ☒ **bibliografija** (*bibliography*)
- ☒ **življenjepis** (*CV*)
- ☒ **motivacijsko pismo** (*motivation letter*)
- ☒ **opis dosedanjega sodelovanja pri raziskovalnem delu** (*description of the candidate's research work*)
- ☐ **osnutek idejne zasnove raziskovalnega dela** (*preliminary research proposal*)
- ☒ **priporočilno pismo** (*letter of recommendation*)
- ☐ **druge priloge** (*other attachments*)

Opis raziskovalnega dela (Research work description)

1. Članica UL (UL member):

Univerza v Ljubljani, Veterinarska fakulteta (Inštitut za mikrobiologijo in parazitologijo)
University of Ljubljana, Veterinary Faculty (Institute of Microbiology and Parasitology)

2. Ime, priimek in elektronski naslov mentorja/ice (Mentor's name, surname and email):

Znan. sod. dr. Bojan Papić, bojan.papic@vf.uni-lj.si
Res. Assoc. Bojan Papić, Ph.D., bojan.papic@vf.uni-lj.si

3. Raziskovalno področje (Research field):

4.04 Veterina
4.04 Veterinarian medicine

4. Opis raziskovalnega dela (Research work description):

Vključuje morebitne dodatne pogoje, ki jih mora izpolnjevati kandidat/ka za mladega raziskovalca/ko, ki niso navedeni v razpisu za mlade raziskovalce (*It includes any additional conditions that the candidate for a young researcher must meet, which are not listed in the call to tender for young researchers.*).

Slov.:

Mladi raziskovalec se bo usposabljal na Inštitutu za mikrobiologijo in parazitologijo, Laboratorij za molekularno bakteriologijo. Pri delu bo uporabljal najnovejše metode v molekularni mikrobiologiji s poudarkom na sekvenciranju naslednje generacije (NGS). Z metodo NGS bo sekvenciral celokupno DNA iz kompleksnih vzorcev za namen opredelitve sestave mikrobne združbe (mikrobiom) in genov za odpornost proti protimikrobnim zdravilom (rezistom). S sekvenciranjem celotnih genomov bo opredelil genetske značilnosti izbranih klinično relevantnih bakterijskih izolatov.

Mladi raziskovalec bo predvidoma z metagenomskim pristopom v kompleksnih vzorcih iz različnih živinorejskih panog ugotavljal prisotnost potencialnih bakterijskih patogenov in njihovih genov za odpornost. Vzporedno bo prisotnost odpornih bakterij in njihovih genov za odpornost opredelil tudi s klasičnimi gojitvenim pristopom in sekvenciranjem pridobljenih izolatov. Na osnovi zbranih rezultatov bo opredelil tveganje različnih živinorejskih panog kot rezervoarja odpornih bakterij in njihovih genov za odpornost.

Zaželeno je veselje do dela z bioinformatičnimi orodji za analizo podatkov NGS (predznanje zaželeno, ni pa nujno, ključna je pripravljenost za učenje in uporabo teh orodij). Zaželene so tudi izkušnje z delom v mikrobiološkem laboratoriju na področju molekularne mikrobiologije.

Eng.:

The young researcher will be trained at the Institute of Microbiology and Parasitology, Laboratory of Molecular Bacteriology. In his/her work, he/she will apply state-of-the-art methods in molecular microbiology, with a particular focus on next-generation sequencing (NGS). Using NGS, the young researcher will sequence total DNA from complex samples to determine the composition of the microbial community (microbiome) and identify genes associated with antimicrobial resistance (resistome). In addition, he/she will characterize the genetic features of selected clinically relevant bacterial isolates by performing whole-genome sequencing (WGS).

The young researcher is intended to apply a metagenomic approach to analyze complex samples from various livestock sectors, identifying potential bacterial pathogens and their resistance genes. In parallel, he will use conventional culture methods, followed by sequencing of the obtained isolates to detect resistant bacteria and their resistance genes. Based on the data collected, he/she will assess the risk posed by different livestock sectors as potential reservoirs of resistant bacteria and resistance genes.

An interest in working with bioinformatics tools for NGS data analysis is highly desirable. Previous knowledge of these tools is an advantage but it is not essential – what is most important is a willingness to learn and apply them. Experience in a microbiology laboratory, especially in the field of molecular microbiology, is also an advantage.

5. Priloge, ki jih kandidat priloži k prijavi (Documents that the candidate submits with the application):

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☒ **bibliografija** (*bibliography*)

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