1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title:
Novel graphene/sirolimus coating devoted to nitinol stents during tracheal collapse in dogs

1.1. Project goals

- Development of a two-component biocompatible coating consisting of two components - graphene and sirolimus.

- Validation of the obtained coatings using Raman spectroscopy and scanning electron microscopy

- Evaluation of antimicrobial properties in selected strains of gram (+) bacteria and gram (-) bacteria

- Evaluation of cytotoxic properties on mouse BALB/3T3 fibroblasts and vascular endothelial and vascular smooth muscle cells.

1.2. Outline

Tracheal inflammation is a common disease in dogs. Small and miniature breed dogs are particularly predisposed. It is a progressive disease, which, despite the implementation of pharmacological treatment, often leads to almost complete closure of the tracheal lumen. Clinically, this is manifested by increasing dyspnea, cyanosis and even fainting. In severe cases, the first-line therapy is the implantation of a stent to support the airway. This procedure provides immediate relief but is still associated with many complications and side effects, especially in long-term follow-up. The most mentioned is excessive swelling (excessive proliferation) of the tracheal epithelium caused by an inflammatory response. This leads to secondary airway obstruction, as well as secondary bacterial infections which is often classified as a life-threatening condition. One way to counteract adverse reactions is to cover the stent with a coating that will reduce overreaction to the implant material. To date, a coating that could be applied to commercially available stents dedicated to tracheal collapse in dogs has not yet been obtained in veterinary medicine. Therefore, the project will be a preliminary study in which a two-component, biocompatible coating will be produced to apply on commercially available nitinol stents. The first component is graphene, which has well-documented antimicrobial properties. It will be enriched with a polymer of sirolimus, which exhibits immunosuppressive and antiproliferative effects. It seems that this combination will be able to reduce the severity of the most common adverse reactions observed after implantation of drugless stents. However, it is necessary to test this hypothesis. Developing a way to manufacture and validating such a coating is a necessary step before attempting to use the stent to treat diseased animals.

1.3. Work plan

- Obtaining coatings based on graphene and polymer enriched in sirolimus. Spectroscopic and microscopic evaluation of the obtained coatings. 1-20 months.
  - Obtaining graphene coating - modified cold-wall chemical vapor deposition (CW-CVD) procedure. The obtained barrier coating consisting of several graphene layers will be validated by Raman spectroscopy.
  - After applying graphene to the base material, the next step will be to coat it with a polymer enriched with the antiproliferative drug sirolimus.

-Scanning electron microscopy and other spectroscopic techniques will be used to evaluate the quality of the obtained coating

- Evaluation of cytotoxicity of the developed coatings on selected cell lines. 20-30 months. - As part of this activity, appropriate samples will be prepared for testing on cell lines (mouse BALB/3T3 fibroblasts, vascular endothelial cells and vascular smooth muscle cells). - Samples for direct and indirect cytotoxicity tests will be prepared in accordance with the current ISO 10993-12 standard. - Phase contrast microscopy and fluorescence microscopy will be used for qualitative evaluation of cytotoxicity in accordance with ISO 10993-5.

- Evaluation of antimicrobial properties of the obtained coatings. 30-36 months. - The ability of selected G(+) and G(-) bacterial strains to adhere to the biomaterial surface with simultaneous fluorescent staining of live and dead cells (including propidium iodide and bis-benzamidine staining) and resusarin staining will be evaluated.

1.4. **Literature** *(max. 7 listed, as a suggestion for a PhD candidate preliminary study)*

- Wasyluk, Ł., Hreniak, D., Boiko, V., Sobieszczańska, B., Bologna, E., Zingales, M., ... & Wawrzyńska, M. (2024). Functional Mechanical Behavior and Biocompatible Characteristics of Graphene-Coated Cardiovascular Stents. International Journal of Molecular Sciences, 25(24), 13345.

- Delgoffe, G. M., Kole, T. P., Zheng, Y., Zarek, P. E., Matthews, K. L., Xiao, B., ... & Powell, J. D. (2009). The mTOR kinase differentially regulates effector and regulatory T cell lineage

commitment. *Immunity*, *30*(6), 832-844.

- Robin, T., Robin, E., & Le Boedec, K. (2024). A systematic review and meta-analysis of prevalence of complications after tracheal stenting in dogs. Journal of Veterinary Internal Medicine, 38(4), 2034-2044.

- Congiusta, M. et al. (2021). Comparison of short-, intermediate-, and long-term results between dogs with tracheal collapse that underwent multimodal medical management alone and those that underwent tracheal endoluminal stent placement. J Am Vet Med. Assoc, 258(3), 279-28.

1.5. Required initial knowledge and skills of the PhD candidate

- Diploma in veterinary medicine

- Interest in scientific research, e.g., work in SKN, publications during the study period, participation in the implementation of research projects, gaining funding for own research projects, scientific internships during the study period.

- Communication skills. Knowledge of oral and written English, at least at the B2 level.

- Ability to work independently and in a team.

- Knowledge of the use of stents in veterinary medicine and coatings dedicated to stents.

1.6. Expected development of the PhD candidate's knowledge and skills

- Knowledge of stent applications in medicine and veterinary medicine,

- Conducting in vitro cell culture studies

- Conducting research with bacterial cultures

- Ability to analyze and read the results of advanced spectroscopic technique

- Mastery of statistical analysis techniques

- Planning a scientific project, including the ability to formulate a research hypothesis, select an appropriate methodology, including correct estimation of the risk of failure, the ability to develop a cost estimate and control the experiment during its duration, develop the results (including methods of statistical analysis of the results), the ability to critically evaluate the results obtained and correctly draw conclusions.