PHD PROJECT DESCRIPTION
(4000 characters max., including the aims and work plan to de published online)

Project title: Design and characterization of bio-colloidal formulations of bioactive milk proteins for biomedical applications

1.1. Project goals

The aim of the project is to develop and characterize bio-colloidal systems based on bioactive milk proteins, especially lactoferrin, its peptides and metal-modified derivatives, for biomedical (especially dermatological) applications. Native and modified proteins will be incorporated into selected semi-solid or hydrogel-based formulations and evaluated for physicochemical stability, bioactivity and application potential. Particular emphasis will be placed on *in vitro* 3D cell culture models to assess biological responses under skin-like conditions. The project will address skin protection, regeneration and pathological conditions such as atopic dermatitis, psoriasis, impaired wound healing and microbial infections.

1.2. Outline

Milk proteins are promising natural bioactive compounds for biomedical and dermocosmetic formulations [1-3]. The project will focus mainly on lactoferrin, its hydrolysates, peptides and metal-modified derivatives, but may also include lactoperoxidase and whey proteins such as β-lactoglobulin or α-lactalbumin. These proteins show antimicrobial, antioxidant, immunomodulatory and regenerative properties, and some may also serve as structural components of protein-based hydrogels [2,4].

The project will involve the rational design of creams, gels, ointments and hydrogel-based systems containing bioactive milk proteins. The formulation type will be selected according to the intended application, such as skin care, management of inflammatory or barrier-related skin disorders, wound healing, controlled release or tissue-regeneration-oriented use. A key challenge will be to preserve protein stability, accessibility and biological activity after incorporation into the formulation, since technological processing and interactions with excipients, polymers, emulsifiers or metal ions may reduce the activity of the active substance.

The developed formulations are expected to support skin protection and regeneration, especially in conditions such as atopic dermatitis, psoriasis, irritation, impaired wound healing and microbial infections. They may combine antibacterial protection, modulation of local inflammatory responses, support of epidermal repair and improvement of the skin barrier. In hydrogel systems, milk proteins may additionally act as biomaterial-forming components, providing three-dimensional networks with suitable mechanical, swelling and release properties.

The systems will be characterized using physicochemical, microbiological and biological methods, including stability, rheology, protein incorporation, release profile, antimicrobial activity, cytotoxicity and regenerative potential. Special emphasis will be placed on cell culture models, including 3D skin models, to determine the incorporated milk proteins ability to address inflammation, microbial colonization, impaired barrier function or delayed tissue repair.

1.3. Work plan

The first stage will involve the development of stable bio-colloidal systems containing bioactive milk proteins and their derivatives, among others lactoferrin, lactoferrin-derived peptides and other whey proteins. The systems will be designed as creams, gels, ointments or hydrogels, with optimized excipients such as emollients, emulsifiers, thickeners, stabilizers, non-toxic cross-linking agents and humectants.

The formulations will be characterized in terms of rheology, dispersion stability, pH, water content, viscosity, texture, protein stability and the content of biologically relevant metals, such as silver, zinc, ruthenium, etc. Protein integrity and formulation structure will be assessed using spectroscopic, chromatographic, spectrometric, electrophoretic and microscopic techniques.

The next stages will include microbiological evaluation of antibacterial activity, inhibition of bacterial growth and biofilm formation, using tests like resazurin-based viability assays and fluorescence microscopy with live/dead staining. Biocompatibility studies (according to ISO 10993-5), will be performed using murine L929 fibroblasts, human keratinocytes and fibroblasts, harnessing MTT/XTT, LDH assays, microscopy and scratch assays to assess cytotoxicity, membrane integrity, cell morphology and regenerative potential.

An important task will be to develop an in-house protocol for 3D skin models to evaluate selected formulations under skin mimicking conditions, in terms of tissue integrity, oxidative stress, inflammatory response and regeneration. The most promising systems may be further tested in murine wound-healing models or preliminary volunteer studies assessing skin tolerance, moisturizing effects and supportive care potential in atopic-prone skin.

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

1. Takayama, Y. Lactoferrin and Its Role in Wound Healing; Springer Netherlands: Dordrecht, 2012; ISBN 978-94-007-2466-2.
2. Pryshchepa, O.; et al. Synthesis, Physicochemical Characterization, and Antibacterial Performance of Silver-Lactoferrin Complexes. Int J Mol Sci 2022, 23, 7112, doi:10.3390/ijms23137112.
3. Dyrda-Terniuk, and T. Pomastowski, P. The Multifaceted Roles of Bovine Lactoferrin: Molecular Structure, Isolation Methods, Analytical Characteristics, and Biological Properties. J. of Agric. and Food Chem. 2023, 71(51), 20500-20531, doi: 10.1021/acs.jafc.3c06887
4. Rodzik, A. et al. Enhancing wound healing with zinc and silver nanocomposites synthesized with β-lactoglobulin: antimicrobial properties, collagen deposition, and systemic effects in a C57BL/6J mouse model. Discover Nano 2024, 19(1), 150, doi: 10.1186/s11671-024-04091-9

1.5. Required initial knowledge and skills of the PhD candidate

The PhD candidate should be creative, scientifically curious and able to work independently and in a team. The strongest background is expected in mammalian cell culture and molecular biology, including cell line maintenance, cytotoxicity testing and biological evaluation of bioactive compounds or biomaterials. Experience with skin-related cell models, such as keratinocytes and fibroblasts, will be particularly valuable. Basic knowledge of microbiology, protein separation and analytical techniques, including SDS-PAGE, LC-MS, GC-MS, ICP-OES, UV-Vis spectroscopy and microscopy, is also expected. Skills in sample preparation, data processing and English sufficient for scientific literature reading are required.

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

During the PhD project, the candidate will gain interdisciplinary knowledge and practical skills in the development of bioactive preparations and formulations for skin care, wound healing and dermatological applications. The candidate will develop competences in microbiology, cell biology, analytical chemistry and dairy science, with emphasis on milk-protein-based systems, including lactoferrin, its peptides, whey proteins and metal-modified protein complexes. Practical experience in formulation design, physicochemical characterization, microbiological testing, cytotoxicity and biocompatibility assessment using skin-related cell models will be developed. The candidate will also improve analytical and statistical data processing skills. The results will be presented in scientific publications and at domestic and international conferences, and may contribute to patent-protected technological solutions.