

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

Project title:

Regenerative collagen material elaboration for medicine, stomatology and cosmetology purposes

1.1. Project goals

1. Invention of regenerative material that significantly accelerate the process of healing
2. Determination of proper physical and chemical properties of the material
3. Evaluation of the most compatible active substance for material incorporation
4. Possibility of patenting new technology using for obtaining collagen-based material and production of innovatory invention useful in numerous industry branches, including stomatology, regenerative medicine, cosmetology and tissue engineering, which is still looking for new solutions.

1.2. Outline

Collagen constitutes a material with significant tissue regenerative potential.^{1,2,3,4} However, on the market still is not sufficient stock of collagen materials certified for biomedical applications.

Using collagen as a structural material and combination of peptides of appropriate origin it is possible to influence activation processes of heat shock proteins, also known as *chaperone proteins*.⁵

Heat shock proteins genes transcription is induced under the influence of numerous factors, such as: environmental stress or pathophysiological conditions.⁶ Heat shock proteins performs a valid role in maintaining homeostasis, thereby accelerating reparative processes of soft and hard tissues.^{7,8,9,10}

In view of continuous exposition to damaging factors skin comprises organ that is eminently vulnerable to it and demands exceptional treatment.

Assorting proper collagen origin it is possible to initiate regeneration processes and effectively improve its appearance in significantly shorter pace.^{11,12}

Collagen materials have attracted significant academic interest due to its biological properties in native state. However, in many cases the mechanical properties and degradation rate should be tailored to especial biomedical and cosmetic applications.

Collagen-based biomaterials are widely used in laboratory and clinical practice because of their excellent biocompatibility^{13,14,15}, appropriate mechanical strength¹⁶, flexibility^{17,18} and ability to delivery of nutrients or drugs^{19,20,21}. Last decades abounded in numerous innovations in the field of collagen-based biomaterials^{22,23}, which ensures enormous development in tissue engineering and regenerative medicine²⁴. Usually, these materials are generated through cross-linking reaction due to the collagen instability. There are three types of polymerization techniques that reinforce the collagen structure: physical, chemical and enzymatic cross-linking.²⁵ The set of cross-linking methods of collagen is neither a closed nor a completed topic. Future perspectives for cross-linking of collagen materials will be focused on dual cross-linking using two different chemical cross-linkers or combine chemical cross-linkers with physical ones. Novel cross-linking processes may lead to new collagen-based materials for biomedical applications. Appropriate selection of cross-linked collagen material incorporated with active substances with high reparative potential is remarkably promising issue in regenerative medicine. Industry do not offer materials that exhibits excellent biocompatibility, mechanical strength and flexibility containing substances with incredible reparative potential, which are essential for instance in extensively conceived medicine and stomatology.

Patenting new technology using for obtaining collagen-based materials and production of innovatory invention essential in numerous industry branches, including tissue engineering, which is constantly looking for new solutions, makes an eminently promising issue and will be an indisputable contribution in science and industry field.

1.3. Work plan

1st year: Determination of relevant physical and chemical features of the collagen used as a matrix:

- a) Determination of thermodynamic stability by microcalorimetry
- b) Designation of UV light influence on collagen structure by circular dichroism
- c) Investigation of amino acid composition using SDS-page and HPLC amino acids analysis.
- d) Denotation of rheological properties by viscosimetry and rheometry.

2nd year: Determination of the most accurate cross-linking factor or factors for the collagen matrix by examination physical and chemical properties of cross-liked material.

- a) FTIR and Raman spectroscopy
- b) Swelling properties
- c) Mechanical properties
- d) SEM and AFM microscopy

3rd year:

Incorporation of active substance or substances to the cross-linked matrix. Methods will be selected accurately to the chosen material.

4th year:

Selection of the most firm, flexible material containing the satisfactory dosage of active healing substances.

- a) Determination of thermodynamic stability by microcalorimetry
- b) Determination of surface structure of final materials
- c) Determination of mechanical properties of final materials
- d) Biocompatibility tests.

1.4. Literature

1. Zeltz C., Gullberg D. *The integrin-collagen connection-a glue for tissue repair?* *J Cell Sci* (2016): 15;129(4):653-64.
2. Mostaço-Guidolin L, Rosin NL. *Imaging Collagen in Scar Tissue: Developments in Second Harmonic Generation Microscopy for Biomedical Applications.* *Int J Mol Sci* (2017): 15;18(8).
3. Scott A., Sell M., McClure J., Koyal P., Wolfe G. and Bowlin L. *Electrospinning of collagen/biopolymers for regenerative medicine and cardiovascular tissue engineering.* *Adv. Drug Deliv.* (2009): Vol. 61, 12, 5: 1007-1019.
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5. Kazuhiro Nagata. *Expression and function of heat shock protein 47: A collagen-specific molecular chaperone in the endoplasmic reticulum,* *Matrix Biology* (1998): Vol. 16, 7, 379-386.
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7. Qing Shi¹, Zhongjun Dong¹ and Haiming Wei. *The Involvement of Heat Shock Proteins in Murine Liver Regeneration.* *Cell Mol Immunol* (2007): Vol. 4, 1.
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13. Song E., Yeon S., Taehoon C., Hyun-Jung B., MooLee, *Collagen scaffolds derived from a marine source and their biocompatibility. Biomaterials (2006): Vol. 27, 15, 2951-2961.*
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25. Weadock K., Olson R., Silver F. *Evaluation of Collagen Crosslinking Techniques. Biomaterials, Medical Devices, and Artificial Organs (1983): vol. 11, 4.*

1.5. Required initial knowledge and skills of the PhD candidate: basic knowledge of chemistry or biotechnology, basic skills in laboratory work, knowledge of speaking and writing English.

1.6. Expected development of the PhD candidate’s knowledge and skills: It is expected that the PhD candidate will learn new techniques of collagen purification and characterization. The knowledge of the PhD candidate will be developed base on international workshops and tutorials.