"POLYMERIC BIOMATERIALS WITH SPECIAL APPLICATIONS"

- Summary -

The doctoral thesis includes the following main chapters: 1. Presentation of the doctoral theme. Concepts and methods; 2. Published research articles in extenso; 3. General conclusions; 4. Original contributions; 5. Dissemination; 6. References.

Keywords: type II collagen, biomaterials, drug delivery systems

The first chapter of the thesis presents the doctoral theme and a critical study of the literature in the field in order to expose the properties of the collagen and type II collagen biomaterials. In the continuation of this chapter the objectives of this doctoral thesis are defined as well as the specific objectives of the original research:

Objective 1. Development of new type II collagen extraction technology and the stabilization of the collagen extracts through chemical crosslinking using different crosslinking agents.

Objective 2. Design of the new biomaterials based on type I and type II collagen as drug delivery systems and composites with natural polymers and antibiotics.

Objective 3. Characterization of the collagen extracts and the obtained biomaterials using modern structural, morphological, thermic biological, goniometric and mechanical methods.

Objective 4. Establishing the directions for the use of obtained biomaterials in medicine, pharmacy and the food industry.

In order to achieve the main goals, strategic and specific objectives were set:

Specific objective 1. To obtain and characterize type II collagen from bovine cartilage as a potential biomaterial that can be used in cartilage regeneration.

Specific objective 2. To study the synthesis of type II collagen biomaterials with and without crosslinking agents.

Specific objective 3. To design and characterize some collagen/chondroitin sulfate supports with doxycycline for cartilage tissue regeneration.

Specific objective 4. To obtain, characterize, and optimize the microcapsules formulation based on type II collagen, sodium alginate, and sodium carboxymethyl cellulose loaded with doxycycline as an antibiotic model drug that could be incorporated further in hydrogels to improve the localized therapy of septic arthritis.

Specific objective 5. To obtain new composite samples using type II collagen and different types of mineral clays. For the investigation of the influence of nanoclay type on the drug release efficiency, the obtained composite samples were additional encapsulated with gentamicin.

Specific objective 6. To obtain and characterize biomaterials for the regeneration of the soft tissue of the digestive system.

In the second chapter of the doctoral thesis are presented the published articles in extenso as a result of the original research:

1. **M. M. Marin**, M.G. Albu Kaya, A. Ficai, M.V. Ghica, L. Popa, R. Tutuianu, *Collagen hydrolysate-based ingestible bioproducts for the treatment of gastric disorders*, Romanian Journal of Materials, 2018, 48 (1), 121-126. (**IF: 0.563**)
2. **M. M. Marin**, M. G. Albu-Kaya, G.M. Vlasceanu, J. Ghitman, I. C. Radu, H. Iovu, *The effect of crosslinking agents on the properties of type II collagen biomaterials*, Plastic Materials, 2020, 57 (4), 166-180. (**IF: 0.593**)
3. **M. M. Marin**, M. G. Albu-Kaya, H. Iovu, C. E. Stavarache, C. CHELARU, R. R. Constantinescu, C. DINU-PÎRVU, M. V. Ghica, *Obtaining, evaluation, and optimization of doxycycline-loaded microparticles intended for the local treatment of infectious arthritis*, Coatings, 2020, 10 (10), 990. (**IF: 3.236**)
4. **M.M. Marin**, M. G. Albu-Kaya, M.V. Ghica, E. Dănilă, G. Coară, L. Popa, C. Chelaru, D.A. Kaya, V. Anuța, C.E. Dinu Pîrvu, I. Cristescu, *Design and evaluation of doxycycline/collagen/chondroitin sulfate delivery systems used for cartilage regeneration*, Proceedings of ICAMS 2020 – 8th International Conference on Advanced Materials and Systems, 2020, 201-206.
5. **M. M. Marin**, M. G. Albu-Kaya, C. E. Stavarache, R. R. Constantinescu, C. CHELARU, J. Ghitman, H. Iovu, *Extraction and studies on the properties of type II collagen as potential biomaterial in cartilage repair*, University Politehnica of Bucharest Scientific Bulletin Series B, 2021, 83 (1), 229-238.
6. **M. M. Marin**, R. Ianchis, R. Leu Alexa, I. C. Gifu, M. G. Albu-Kaya, D. I. Savu, R. C. Popescu, E. Alexandrescu, C. M. Ninciuleanu, S. Preda, M. Ignat, R. Constantinescu, H. Iovu, *Development of new collagen/clay composite biomaterials*, International Journal of Molecular Sciences, 2022, 23 (1), 401. (**IF: 6.208**)

In the third chapter are presented the general conclusions of the doctoral thesis on the synthesis and characterization of type II collagen from bovine cartilage and biomaterials based on type I and type II collagen that can be used in medical applications. Experimental results published in the aforementioned research articles have shown that new obtained type II collagen represent a promising biomaterial that can be used for medical field. The stabilization of new

type II collagen was successfully achieved using three types of crosslinking agents: TA, GE and EDC/NHS. To enhance type II collagen properties, crosslinked collagen scaffolds with different cross-linking agents were prepared by lyophilization process. All these results suggest that this study could be useful for the development of a new collagen-based biomaterial for tissue engineering applications having suitable properties and reduced antigenicity with all crosslinking agents. Further, collagen/chondroitin sulfate supports with doxycycline for cartilage tissue regeneration were designed and characterized. Collagen, chondroitin sulfate and doxycycline samples were cross-linked with various concentrations of glutaraldehyde and then lyophilized for the purpose of obtaining collagen scaffolds. The obtained results recommend these new scaffolds based on doxycycline/collagen/chondroitin sulfate as a promising approach for the treatment of cartilage problems. Finding favorable results by stabilizing the extracted collagen using a new patented method, further microcapsules formulation based on type II collagen, sodium alginate, and sodium carboxymethyl cellulose loaded with doxycycline were obtained. A new microcapsule formulation as an antibiotic model drug was obtained, characterized, and optimized. The obtained results recommend these new optimized microcapsules as promising drug systems to be further incorporated in type II collagen hydrogels used for septic arthritis. Also, extracted type II collagen was used in order to develop new collagen/clay composite biomaterials. By using 5 typologies of clays, modern composite biomaterials were synthesized. Using these results as basis for further research, the influence of loading collagen/mineral clays composites with different concentration of mineral nanopowders on the viability and the potential antibiotic releasing characteristics will be studied. The results obtained in the study suggests that the composites based on collagen and nanoclays are promising candidates for new materials developed in the medical branch. Finally, another direction was to obtain and characterize biomaterials for the regeneration of the soft tissue of the digestive system. Powders with different concentrations of type I collagen hydrolysate, zeolite and propolis were obtained by lyophilization. The best results for gastric mucosal regeneration were presented by the P6 sample containing high concentrations of collagen hydrolysate and propolis. Further studies are necessary to clearly and completely establish the physico-chemical changes of the zeolites after ingestions and to assess the biological influence. The research results presented inside the thesis exhibit a high degree of originality targeting the synthesis of new biomaterials based on type I and type II collagen. The results will significantly influence the knowledge concerning the technologies for collagen extraction and characterization.

The fourth chapter presents the original contributions of the thesis in the field of biomaterials based on collagen and type II collagen. The personal contributions made to these subjects are structured as follows:

1. Extraction and studies on the properties of type II collagen as potential biomaterial in cartilage repair

The experimental studies of this thesis have started with the obtaining of the type II collagen using a new extraction method which was, also, registered to OSIM (patent application no. A 00840/26.10.2018). The application of the new extracted collagen leads to the following advantages:

- the process, according to the new technology, allows the preparation of collagen extracts from cartilaginous tissues only by acid and alkaline hydrolysis, without the use of enzymes that are difficult to inactivate;
- the process according to the invention is simple, takes place at room temperature: 20 ... 25 ° C, with low energy consumption and with simple apparatus, allowing the preparation of extracts with high purity, specific of the materials for medical applications.

The originality of this study is related to the new type II collagen extracted from bovine cartilage and is based on the fact that the promoted technology does not use enzymes that can affect the collagen's triple helix structure.

2. The effect of crosslinking agents on the properties of type II collagen biomaterials

This study presents an original concept for investigation of the influence of different crosslinking agents such as genipin, tannic acid and EDC/NHS system on the performances of a type II collagen-based scaffold to be used in cartilage tissue regeneration. The type II collagen-based biomaterials were crosslinked with two natural (GE, TA) and a synthetic (EDC/NHS) agent and the effect of these crosslinkers upon the morphology, mechanical and thermal properties was investigated. Furthermore, the impact of the selected crosslinking agents on the secondary conformation of type II collagen, which is responsible for the therapeutic activity of the protein, using circular dichroism, was investigated. Also, the swelling behavior and *in vitro* enzymatic degradation of the obtained crosslinked scaffold with natural or synthetic agents were studied.

This novel idea has never been reported in the literature, comparing these three types of crosslinking agents on type II collagen and has given new directions for the enhancement of the collagen mechanical and thermal behavior.

3. Design and evaluation of doxycycline/collagen/chondroitin sulfate delivery systems used for cartilage regeneration

Novel type II collagen/chondroitin sulfate supports with doxycycline for cartilage tissue regeneration have been developed and characterized in this study. The scaffolds loaded with doxycycline should prevent the development of potential infections. Collagen, chondroitin sulfate and doxycycline samples were cross-linked with various concentrations of glutaraldehyde and then lyophilized for the development of the collagen sponges. All the characteristics for the new synthesized biomaterials were firstly assessed using modern investigation methods. The obtained results recommend these new scaffolds based on doxycycline/collagen/chondroitin sulfate as an encouraging approach for the regeneration of cartilage problems.

This concept has never been published to date, this being the only research study exploiting the idea of chondroitin sulfate addition into collagen matrix with doxycycline in order to obtain systems for cartilage regeneration.

4. *Obtaining, evaluation, and optimization of doxycycline-loaded microparticle intended for the local treatment of infectious arthritis*

In this study were designed, characterized and optimized original microcapsules formulations based on type II collagen, sodium alginate, and sodium carboxymethyl cellulose loaded with doxycycline as an antibiotic model drug, that could be used further in hydrogels to improve the localized therapy of septic arthritis. The novel synthesized microcapsules were assessed by spectral (FT-IR), morphological (optical microscopy), and biological analysis (enzymatic biodegradation, antimicrobial activity). The size distribution of the obtained microcapsules was determined using optical microscopy. The drug encapsulation efficiency was also determined. To optimize the microcapsules' composition, some physical-chemical and biological analyses were optimized using a technique based on an experimental plan, response surface methodology, and the Taguchi technique, and the adequate formulations were selected. The obtained results recommend these new microcapsules as promising drug systems to be further incorporated in type II collagen hydrogels used for the treatment of arthritis.

5. *Development of new collagen/clay composite biomaterials*

The research studies presented in this chapter were focused to create new collagen/clay composite scaffolds. Modern composite biomaterials were created utilizing five types of clays. For the investigation of the influence of nanoclay type on the drug release efficiency, the obtained composite samples were additional encapsulated with gentamicin.

This study includes an evaluation of the effect of different mineral clay nanoparticles on the properties of type II collagen–clay biomaterials encapsulated with gentamicin. As per current understanding, our study represents the first systematic research investigations on the addition of natural or functionalized nanoclays into the polymeric matrix that can be used for cartilaginous tissue repair, representing a first for the composites family of research.

The presence of the nanopowders gave the samples a porous structure with a variety of pore sizes. An intercalated collagen-clay structure was discovered using X-ray Diffraction techniques, with the mineral layers being evenly dispersed in the organic portion. Fourier-transform infrared spectroscopy (FTIR) revealed that the addition of the clays did not cause the collagen triple helix structure to denaturate. The composites created by including the mineral nanoparticles exhibited higher mechanical properties when compared to the basic collagen samples and reduced biodegradability with lower ratios of PBS uptake in swelling studies. The *in vitro* experiments on antibiotic release showed a latent period followed by a large release, which is consistent with the kinetics of composites based on organo-clays. All of the investigated pathogenic aerobic bacteria experienced a biological growth inhibition due to the inclusion of the mineral nanoparticles. Three composite materials tested for cellular viability showed adequate biocompatibility. The impact of loading collagen/mineral clay composites with various concentrations of mineral nanopowders on the viability and possible antibiotic releasing features will be further investigated using these results as the foundation for future studies.

According to the study's findings, composites made of type II collagen and nanoclays represent excellent candidates as novel materials produced for the medical industry.

6. Collagen hydrolysate-based ingestible bioproducts for the treatment of gastric disorders

Gastric ulcer disease is a common problem of the gastro-intestinal tract with its increasing incidence and prevalence attributed to the loss of balance between aggressive and protective factors. The purpose of this study was to obtain and characterize new biomaterials for the regeneration of the soft tissue of the digestive system. Powders with different concentrations of collagen hydrolysate, zeolite and propolis were obtained by lyophilization. The morphology

(SEM), the spectral characteristics (FTIR, XRD), the goniometry (contact angle), in vitro assessment to simulated gastric acid and the biocompatibility (viability tests) of the designed powders were studied. The novel samples based on collagen hydrolysate, zeolite and propolis biomaterials exhibited characteristics that make them potential candidates for use in the treatment of gastric ulcer.

The doctoral thesis *POLYMERIC BIOMATERIALS WITH SPECIAL APPLICATIONS* presents a high degree of originality by developing new strategies for the synthesis type II collagen. Several strategies for the synthesis of biomaterials for medical applications have been developed, and the obtained results will greatly influence the methods of obtaining biomaterials based on collagen and type II collagen.

The fifth chapter of the doctoral thesis presents the publications resulting from this paper and the dissemination of results at national and international conferences.