## ABSTRACT

### "Radiation-initiated radical processes in collagen and their macroscopic consequences"

Purpose and scope of work:

The skin has a protective function that maintains the body's homeostasis. Many times, skin injuries (e.g., extensive burns) or genetic diseases (e.g., Epidermolysis Bullosa) can pose an immediate threat of loss of life. In such situations, the only salvation is a skin transplant.

A number of works are currently in the research stage to create biocompatible dressings that could mimic human skin as closely as possible. Increased interest in the properties of biopolymer materials and their manufacture show the need to develop methods for processing collagen used in medicine. This bioresorbable material is an excellent base for creating medical dressings.

One of the main steps in preparing grafts for medical use is sterilization, to ensure effective elimination of pathogens. For bioresorbable materials, ionizing radiation is the recommended method of sterilization, eliminating fungi, viruses and bacteria.

The radiation sterilization process, which is used to disinfect biomedical products such as bones, grafts, tissues, can affect the physicochemical properties of the collagen matrix. Exposure to ionizing radiation carries the risk of damaging the collagen structure.

The main goal of my work was to deepen our understanding of how ionizing radiation affects the physicochemical properties of collagen preparations, including cell-free collagen matrices derived from human skin, which are used for tissue engineering. Understanding these changes can help improve the efficacy and safety of skin grafts and implants, which is particularly important for patients who require skin grafts as a result of burns, injuries or disease.

As part of this objective, I undertook to study the radical processes occurring in collagen preparations as a result of ionizing radiation and to evaluate what changes in the physicochemical properties of collagen occur during the sterilization process.

I undertook the following tasks:

- To study the effects of ionizing radiation on individual amino acids that are components of collagen,

- To investigate and compare the effects of ionizing radiation on model collagen type I and cell-free collagen matrices obtained from human skin.

- To evaluate the suitability of type I collagen from bovine tendons as a model material for cell-free matrices of human skin, in physicochemical studies.

Methodology:

For this study, I used amino acids, commercial purified type I collagen extracted from bovine tendons, and cell-free collagen matrices derived from human skin from bariatric surgery. The methodology for obtaining these matrices was developed as part of the Bioopa project STRATEGMED2/269807/14/NCBR/2015), under which the research contained in this dissertation was performed.

In the study, I used spectroscopic methods: EPR, UV-VIS diffuse reflectance spectroscopy, total attenuated reflectance infrared (ATR-FTIR), separation methods: gas chromatography (GC) and gel electrophoresis (SDS-PAGE), thermal analysis methods: thermogravimetry (TGA) and differential scanning calorimetry (DSC). I used scanning electron microscopy (SEM) to visualize the structure of the preparations. Within the study, I determined the effect of 25, 35 and 50 kGy doses on some physicochemical and mechanical properties of collagen. I analyzed the effect of sample humidity and the presence of oxygen in the irradiation atmosphere on the test material during the sterilization process.

Results obtained:

Studying the basic components of collagen, i.e., three selected amino acids: glycine, proline and hydroxyproline, I identified radicals initiated by ionizing radiation using the EPR method. The EPR spectra of the irradiated amino acids are complex, due to the formation of several radicals characterized by similar stability. The presence of four radicals in irradiated glycine, one radical in irradiated proline and four radicals in irradiated hydroxyproline is confirmed. Analogs of these radicals are not present in collagen. This is probably due to the chain structure of the protein, its different mobility or the presence of other components of the collagen chain that are sensitive to radiation. TGA studies have shown that the higher the radiation dose, the lower the temperature of amino acid decomposition. Presumably, irradiation of amino acids in an air atmosphere

leads to the formation of oxygen-containing, heat-sensitive functional groups, the amount of which increases with the increasing radiation dose.

Gas chromatography made it possible to observe different radiation yields of hydrogen release for different amino acids. The highest efficiency was observed for proline. Comparatively, it was almost 8-fold higher compared to glycine and 4-fold to hydroxyproline.

Comparison of model type I collagen and cell-free dermal matrices leads to the following conclusions:

- using TGA and DSC methods, it was confirmed that the thermal properties of collagen-based materials, do not change significantly after irradiation.

- using EPR confirmed, the formation of identical paramagnetic products in both collagen materials. Radicals are formed, which are precursors of small changes in the structure of the materials studied. EPR studies have shown that the following processes take place in the biopolymer: C-H bond breakage (-$CH^{\bullet}$-), damage to amino acid side chains (-$CH_2CH_2^{\bullet}$) and oxidation (-$COO^{\bullet}$). At ambient temperature, the radicals are not stable, and their decay rate increases with the increase of collagen humidity. Similar observations have been made on the basis of DRS UV-VIS spectroscopy, where the formation of transient absorption bands is observed in the 264 and 326 nm range, which disappear at a given temperature with a humidity-dependent rate, and which can be attributed to transient radiolysis products. The resulting modifications observed by spectral methods, do not disrupt the collagen structure, which remains stable. This is probably due to the unique intra- and intermolecular structure of collagen materials, containing numerous cross-links.

- Based on the images obtained at different SEM magnifications, there were no significant differences in in the surface morphology formed by the collagen fibers of the two materials.

- using GC, significant differences were found in the ratio of oxygen-to-hydrogen radiative yields for the two materials. Cell-free human skin matrices have a 5-fold higher ratio of oxygen-to-hydrogen production radiation efficiency, while for model type I collagen the ratio is as much as 60-fold higher. The reason may be the difference in the active surface area of the material. Model type I collagen exists in the form of "fluff," with a much more developed surface area compared to cell-free human skin matrices, resulting in more extensive oxidation.

Despite differences in oxidation intensity, exposure to ionizing radiation of model collagen type I and cell-free human skin matrices yields a similar radiation response, in terms of free radicals formed. At the same time, both materials maintain stable thermal properties to a similar degree, with the results for the collagen I preparation having greater reproducibility, confirming the suitability of type I collagen as a model material for cell-free human skin matrices.

The effect of irradiation on the fragmentation of collagen chains in cell-free skin matrices was also investigated using gel electrophoresis. The study observed fragmentation of collagen chains, which, however, did not translate into collagen stability when tested by thermal methods. It is noteworthy that the fragmentation effect was observed in preparations digested with pepsin (which makes it possible to study collagen by electrophoresis), suggesting that the particular cross-linked structure of natural collagen provides it with remarkable stability, even in the presence of numerous defects in individual collagen chains.

Electron beam irradiation of model collagen type I and cell-free human skin matrices with doses of 25, 35 kGy and 50 kGy allowed us to prove that the dose in this wide range has little effect on the physicochemical and thermal properties of collagen. This confirms that both the 25 kGy dose recommended by the IAEA and the 35 kGy dose often used in the practice of Polish tissue banks are safe in terms of preserving the physicochemical properties of the material under study.

Based on TGA, DSC, EPR, UV-VIS, FTIR, SEM and GC studies, there was no significant effect of moisture content on changes in physicochemical properties during the sterilization process of the materials, in the wide range of moisture content applied, from 4% to 22.5% of the water content of the sample. The only differences observed relate to the rate of free radical recombination processes. Higher moisture content promotes faster radical decay, as observed by EPR and UVVIS spectroscopic methods.

Despite the small changes observed in the physicochemical properties of the studied samples and the overall good stability of collagen in the process of radiation sterilization, the observed oxidation processes, as well as the dependence of the efficiency of reaction with oxygen during irradiation on the active surface of the material, indicate the validity of introducing a protective atmosphere in the sterilization process of collagen-based dressing materials.

In summary, the results of the study made it possible to determine the mechanisms of radiation damage occurring in the cell-free matrix of human skin and to determine the degree of fragmentation of the material in a dose-dependent manner. The applicational goal of selecting optimal conditions during the sterilization process of cell-free human skin matrices for further laboratory studies and clinical proceedings was achieved.